BIKEN JOURNAL Vol. 8, 1-5, 1965

STUDIES ON THE FINE STRUCTURE OF *CANDIDA ALBICANS* CELL, WITH SPECIAL REFERENCE TO THE NUCLEAR SITE

TSUNESABURO FUJINO, YOSHIYA AKITA and MASASHI OKADA

Department of Bacteriology and Serology, Research Institute for Microbial Diseases, Osaka University, Osaka

(Received March 10, 1965)

SUMMARY The nuclear site of *Candida albicans* was studied morphologically. The results obtained were as follows:

1) In cells treated with desoxyribonuclease (DNAse) at 37°C for 12 hrs., the Feulgen positive sites seen in untreated cells could no longer be distinguished.

2) Electron microscopical examination of ultrathin section of DNAse treated cells showed that nuclear units were no longer visible and only some string-like structures could still be seen.

The structure which was destroyed by DNAse treatment is thought to be identical with the site surrounded by the nuclear membrane in intact cell preparations.

INTRODUCTION

In recent years, there have been many studies of the structure of yeast and yeast-like fungi by light or electron microscope. The fine structure of the nucleus and other cell components of *Saccharomyces species* especially have been described in detail by several authors. (AGAR and DOUGLAS, 1957; YOTSUYANAGI, 1959, 1960)

Some authors have reported on the fine structure of *Candida albicans*. (YOSHIZAWA, 1958; YUDA, 1959; KIMURA, 1959; AKAGI, 1961; FUJINO, 1961)

The problem of whether the whole structure surrounded by a fine nuclear membrane, or only the electron transparent area within it, corresponds to the Feulgen positive site has not yet solved. Thus, in this study, attempts were made to determine the nuclear site by DNAse treatment.

However, the intact cells of *Candida albicans* were found to be resistant to the action of DNAse. Thus, studies were made on cells treated with acetone. By this technique the electron-dense DNA in the nuclear site of *Candida albicans* could be effectively eliminated.

MATERIALS AND METHODS

1. *Organism*

Candida albicans FIA 1048, which had been isolated from a case of human candidiasis and stored in our Institute's Type Culture Collection was used for the present study.

2. *Cell preparation*

C. albicans FIA 1048 was cultured in Sabouraud's glucose broth on a reciprocating shaker (120 strokes per minute) for 24 hrs. at 37°C.

3. *Treatment of the organism with DNAse for morphological studies.*

The intact cells could not be treated with DNAse, so an acetone powder was prepared from them, as shown in the accompanying table.

Preparation of acetone powder

Candida albicans FIA 1048

Cells harvested from Sabouraud's glucose broth culture shaken at 37°C for 24 hrs.

↓ Washed 3 times with distilled water

Cell paste dropped into approximately 20 volumes of cold acetone

↓ Filtered, precipitate washed with cold ether

Dried in desiccator

The cell acetone powder was washed twice with Gomori's Tris-buffer (pH 5.7) (GOMORI, 1948), and incubated for 12 hrs. at 37°C in DNAse solution—0.05 mg crystallized DNAse (N.B.C.) per ml of Gomori's Tris-buffer containing 0.2 M $MgSO_4 \cdot 7H_2O$ (KORSON, 1951 : AMANO, 1962). The pH of the solution was 5.7. Control cells were treated with the same solution containing no DNAse.

Effect of DNAse on the cell structure of Candida albicans

Acetone powder of *C. albicans* FIA 1048

↓

Washed twice with Gomori's Tris-buffer (pH 5.7)

(DNAse Treatment)	(Control)
0.05 mg crystallized DNAse (N.B.C.) per 1 ml of Gomori's Tris buffer (pH 5.7) containing 0.2 M $MgSO_4$	Suspended in Gomori's Tris buffer containing 0.2 M $MgSO_4$

↓

Incubated at 37°C for 12 hrs.

↓

Feulgen staining

Electron microscopical observations

4. *Cell staining*

The Feulgen technique was carried out by the method of De TOMASI (1936) with a slight modification.

5. *Observations by electron microscopy*

Cells were fixed for 2 hrs. at room temperature with 4 per cent formaldehyde bufferd with 0.1 M phosphate buffer solution (pH 7.4), prepared according to the method of SABATINI (1963) and then refixed for 2 hrs. at room temperature in 1 per cent osmium tetraoxide in 0.34 M isotonic veronal acetate buffer solution (pH 7.2), prepared according to the method of MICHAELIS. Specimens were dehydrated by serial passage through graded concentrations of ethyl alcohol. Epon 812 was used for embedding. The specimens were placed in gelatine capsules and polymerized at 60°C for 36 to 48 hrs. Ultra-thin sections were cut with a glass knife using a LKB ultramicrotome. Sections were placed upon 150 mesh copper grids coated with collodion film reinforced with carbon, and examined with a Hitachi H S-6 type (Hitachi Ltd.) electron microscope.

RESULTS

Cytochemical studies on DNAse treated cells

1) *Observation of the nuclear site by the Feulgen Technique*

The Feulgen reaction in intact cells showed that the nuclear site was located laterally in the cells (Fig. 1).

In cells treated with DNAse at 37°C for 12 hrs., the Feulgen positive site could no longer be seen (Fig. 2).

2) *Electron microscopical observation of DNAse treated cells*

In intact cells, the cell wall and mitchondria-like structure could be distinguished.

The nucleus surrounded by a definite membrane (width: 100 to 300 Å) could be seen and this seems to correspond to the Feulgen positive site (Fig. 3).

In DNAse treated cells, the cytoplasm was coarser than in intact cells.

No mitochondria-like structure could be seen. Nuclear units were no longer visible and only some string-like structures could still be seen (Fig. 4).

These string-like structures could not be stained by any of the methods tested.

DISCUSSION

Morphological findings on DNAse treated fungal cells have not previously been reported.

AGAR and DOUGLAS (1957) were the first to demonstrate the nucleus of yeast (*Saccharomyces cerevisiae*) surrounded by a double membrane just as in the cells of higher organisms.

On the other hand, less electron dense intranuclear areas (nucleoli?) have beeh seen in electron micrographs of *S. cerevisiae* by YOTSUYANAGI (1959) and he considered that these corresponded to Feulgen positive sites.

AKAGI (1961) suggested that the electron-transparent intranuclear areas of *C. albicans* might correspond to Feulgen positive sites.

FUJINO (1961) proposed that the whole structure surrounded by a fine membrane or the electron-transparent areas within it might be identical with the Feulgen positive site.

TAKAGI (1962) thought that the nucleus surrounded by a fine membrane probably corresponded to the Feulgen positive site.

In this study, the structure which was destroyed by DNAse treatment was thought to be identical with the whole site surrounded by the nuclear membrane in intact cells.

ACKNOWLEDGEMENT

We are greatly indebted to Dr. M. AMANO of the National Cancer Center, and to Drs. K. FUKAI, T. MIWATANI and M. KONDO of the Research Institute for Microbial Diseases, Osaka University.

REFERENCES

AGAR, H. D. and Douglas, D. C. (1957). Studies on the cytological structure of yeast: Electron microscopy of thin sections. *J. Bacteriol.* **73**, 365–375.

AKAGI, M. (1961). Morphological studies on the cell structure of *Candida albicans*. *Osaka Daigaku Igaku Zasshi* **13**, 369–374.

AMANO, M. (1962). Improved technique for the enzymatic extraction of nucleic acids from tissue section. *J. Histochem. Cytochem.* **10**, 204–212.

De TOMASI, J. A. (1963). Improving technique of the Feulgen stain. *Stain Technol.* **11**, 134–144.

FUJINO, T., KAMIKI, T., AKITA, Y. and AKAGI, M. (1962). Morphological studies on the mode of action of Nystatin on *Candida albicans*. *Biken Journal* **5**, 233–238.

GOMORI, G. (1948). Histochemical demonstration of site of choline esterase activity. *Proc. Soc. Exptl. Biol. Med.* **68**, 354–358.

KIMURA, K. (1959). On the Chlamydospore of *Candida albicans*. *Osaka Daigaku Igaku Zasshi* **11**, 3051–3060.

KORSON, R. (1951). A differential stain for nucleic acids. *Stain Technol.* **26**, 265–270.

MICHAELIS, L. (1931). Der Acetat-Veronal- Puffer. *Biochem. Z.* **234**, 139–141.

SABATINI, D. D., BENSH, K. and BARREWETT, R. J. (1963). The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell. Biol.* **17**, 19–58.

TAKAGI, A. and NAGATA, A. (1962). Studies on the fine structure of *Candida albicans*, with special reference to intracytoplasmic membrane system. *Japan. J. Microb.* **6**, 95–111.

YOSHIZAWA, S. (1958). Morphological studies on *Candida albicans* and true yeast with special reference to nuclear apparatus. *Yonago Med. J.* **9**, 329–338.

YOTSUYANAGI, Y. (1959). Cytologie-Étude au microscope électronique des coupes ultra-fines de la levure. *Compt. Rend. Acad. Sci.* **248**, 274–277.

YOTSUYANAGI, Y. (1960). Cytologie-Mise en évidence au microscope électronique des chromosomes, de la levure par une coloration spécifique. *Compt. Rend. Acad. Sci.* **250**, 1522–1524.

YUDA, Y. (1959). Cellular studies of *Candida albicans*. *Japan. J. Obs. Gynecol.* **10**, 1407–1410.

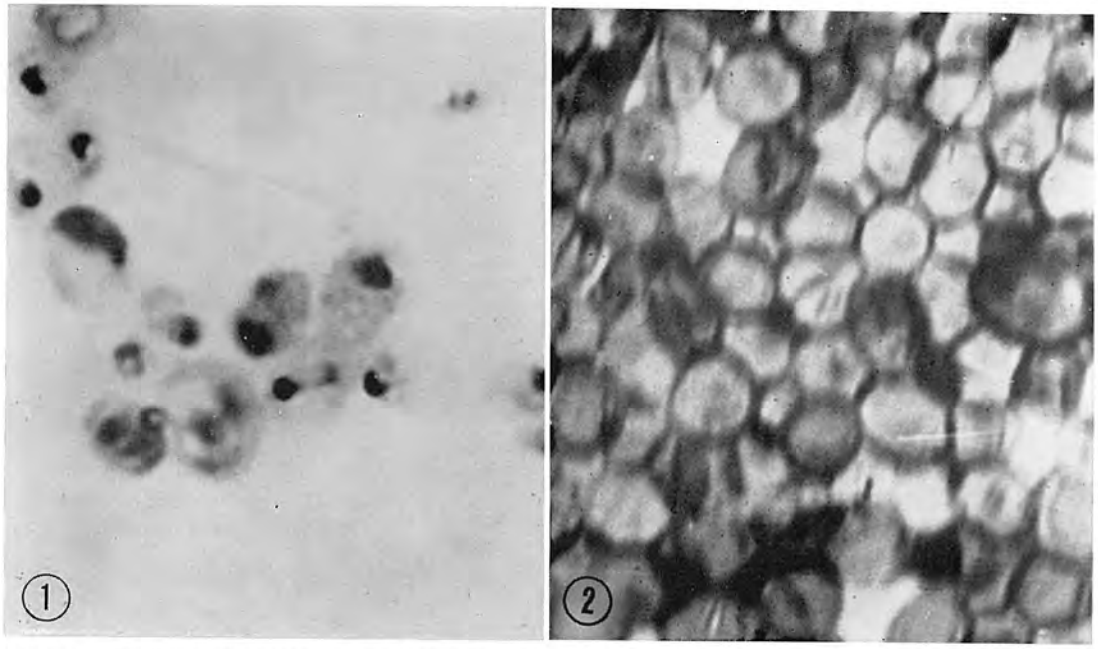
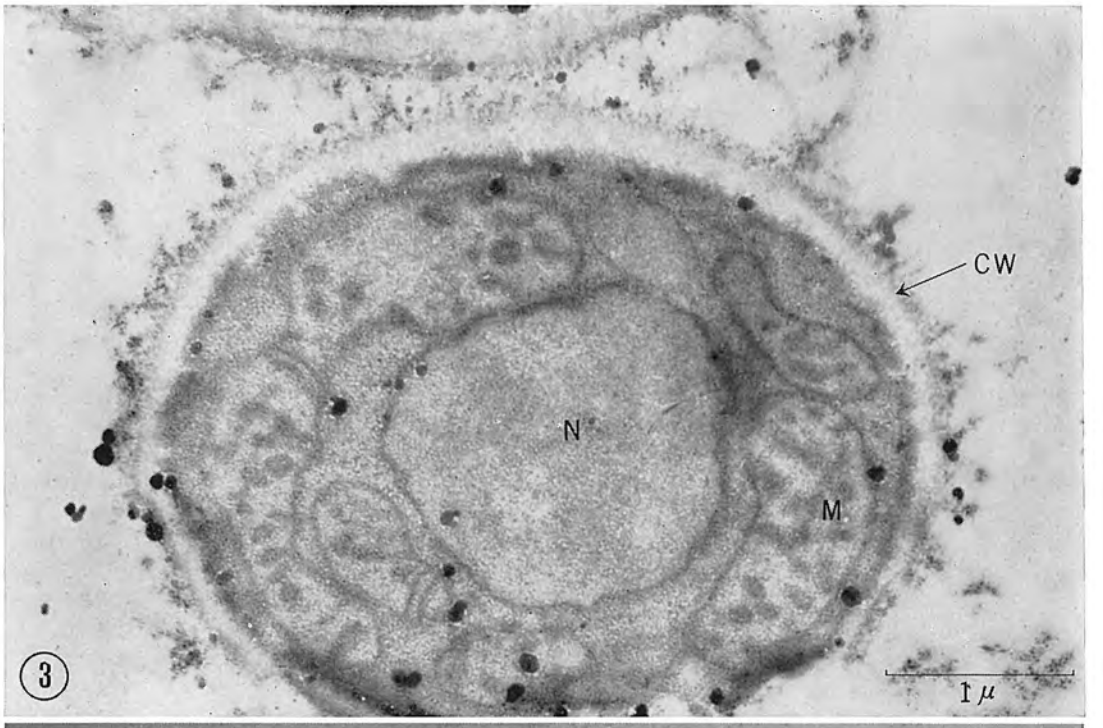


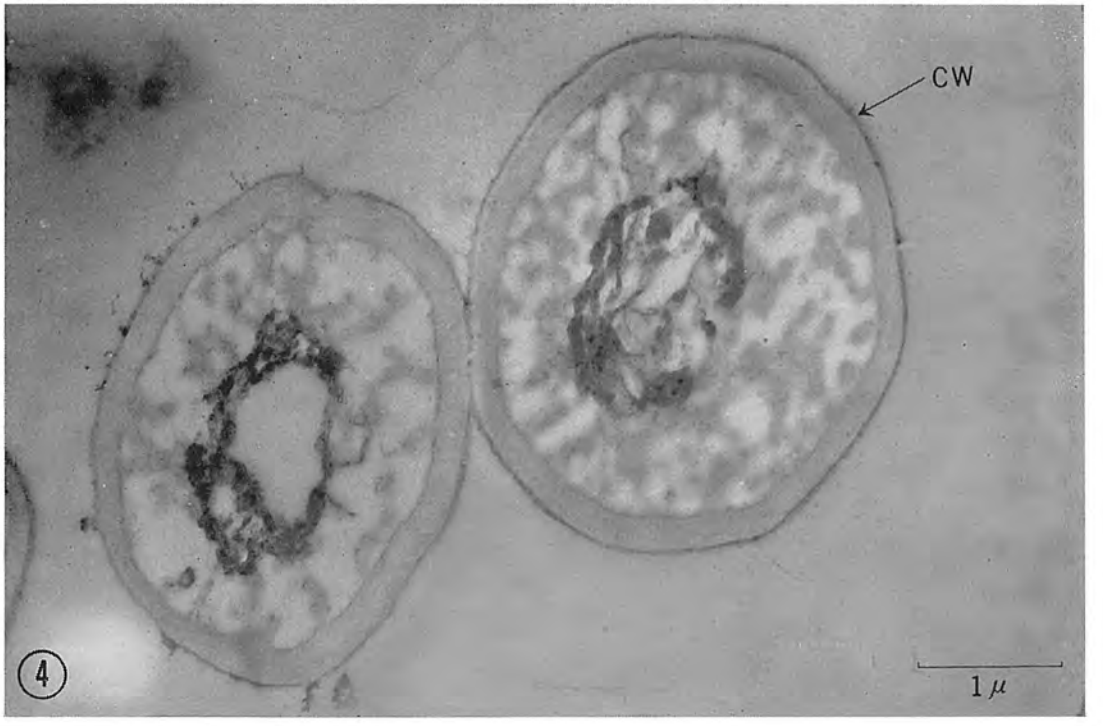
FIGURE 1 Feulgen stain of intact cells of *Candida albicans* (×2,000) showing Feulgen positive stainability.

FIGURE 2 Feulgen stain of DNAse treated cells (×2,000) showing Feulgen negative stainability.

FIGURE 3 Electron micrograph of intact cells showing nucleus, cell wall and mitochondria.
CW : cell wall, N : nucleus, M : mitochondria

FIGURE 4 Electron micrograph of DNAse treated cells showing destruction of subcellular structures, especially in the nucleus.


CW
N
M
3
1 μ


CW
4
1 μ