THE ORGANIZATION OF YEAST NUCLEOHISTONE FIBERS

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INTRODUCTION

Previous studies have indicated that yeast histones are considerably less basic than those found in higher organisms and that the very lysine-rich histone fraction is absent (1, 2, 3). Since yeast chromosomes do not condense completely at mitotic and meiotic metaphase (4, 5), it has been suggested (1, 2) that the very lysine-rich histone fraction occurs only in organisms exhibiting condensed chromosomes at metaphase. It has also been postulated that this fraction plays an essential role in the structural organization of metaphase chromosomes (1, 2).

The purpose of this communication is to report electron microscope studies on chromatin fibers of the yeast Saccharomyces cerevisiae. The study was initiated to determine whether the basic nucleohistone structure of yeast, a rather primitive eucaryote, differed from that of multicellular organisms exhibiting condensed chromosomes and containing the very lysine-rich histone fraction (6–10).

MATERIALS AND METHODS

The diploid strains (S41 and Y35) of Saccharomyces cerevisiae were kindly supplied by Dr. H. O. Halvorson (Brandeis University). Cells were grown according to
In order to measure the diameters of yeast nucleohistone fibers, the method of Esposito, et al. (11) was used. Log phase cultures were harvested by centrifugation at 3000 g for 5 min. The cells were then washed twice with water and the volume of cells was determined. The washed cells were suspended for 15 min at 30°C in 2.5 vol of medium A consisting of 0.1 M 0-mercaptoethanol and 0.02 M piperazine-N,N'-bis(2-ethane sulfonic acid) monosodium monohydrate (PIPES) buffer finally adjusted to pH 9.0. This buffer was chosen because of its nonchelating properties (12). Cells were then centrifuged at 3000 g for 5 min and washed once in 5 vol of medium B: 0.9 M sorbitol and 0.025 M PIPES finally adjusted to pH 5.9-6.0.

Digestion of the yeast cell walls to form spheroplasts was accomplished by suspending 1 vol of washed cells in 1.3 vol of medium B and 0.25 vol of snail gut enzyme, glusulase (Endo Laboratories, Garden City, N. Y.). The mixture was incubated at 30°C until digestion was complete. The progress of the digestion was monitored by making turbidity readings (OD 650) of 1:500 dilutions of the digestion mixture. Digestion was considered to be complete when turbidity decreased to 35-40% of the zero time value, which occurred after 15-20 min. The spheroplasts were washed twice with medium B at 2°C and finally sedimented for 5 min at 3000 g. Additional portions of the washed log phase cells were taken to form spheroplasts at room temperature in a mixture of glusulase and sorbitol according to the method of Hutchison and Hartwell (13).

An adaptation of the Kleinschmidt monolayer technique (14) was used to prepare nucleohistone fibers from spheroplasts. Small droplets of the spheroplast pellet were placed on the air-water or air-sodium citrate (5 mM) interface as described by Ris (6) for Triturus viridescens erythrocytes. Nucleohistone fibers were picked up on 200 or 400 mesh grids coated with 0.25% Formvar and carbon. The fibers were fixed for 30 s-5 min in 4% paraformaldehyde, washed briefly in water, stained for 30 min in 1% uranyl magnesium acetate (15), dehydrated in ethanol followed by amyl acetate, and dried by the critical point method of Anderson (16).

The preparations were examined with a Siemens Elmiskop I electron microscope using double condenser illumination and a 50 µm objective aperture.

Fiber measurements were made according to the method reported by Zirkin (10). A set of lines was scored on an acetate sheet at 0.25 inch intervals. Another set of lines similarly spaced was scored perpendicular to the first. The resulting transparent grid was then placed on top of the prints and fiber diameters were measured wherever a fiber crossed a line. Such measurements included areas of stretching as well as those with pronounced side protuberances. If the outline of a fiber where it crossed a line was not clear, measurements were not made at that position.

### RESULTS

Nucleohistone fibers from nuclei of yeast spheroplasts spread on an air-water interface are shown in Figs. 1 a and 1 b. Individual fibers of the complex network have a knobby appearance. Due to this knobby structure, the thickness of the nucleohistone fibers is somewhat variable. Fibers spread on six separate occasions were measured and the average diameter of the fibers in these preparations ranged from 165 to 185 Å (Table I). Fiber diameters can range as high as 370 Å and as low as 40 Å but these infrequent extremes generally are attributable to regions of irregularly spaced, more highly pronounced side protuberances and regions of pronounced stretching, respectively. Fiber diameter is the same with either method used to produce spheroplasts.

Portions of spheroplasts were also spread on the surface of 5 mM sodium citrate. Nucleohistone fibers prepared by this method are shown in Fig. 1 c. These fibers are considerably thinner than those spread on water. The diameter of the sodium citrate-spread nucleohistone fibers averages about 100 Å (Table I).

Some areas of water-spread chromatin frequently appear more condensed than the rest of the chromatin (Figs. 2 a and 2 b). Profiles of these regions exhibit extensive knobbiness and side pro-

### Table I

|                  | Mean diameter | Standard deviation | Range  |
|------------------|---------------|--------------------|-------|
|                  | Å             | Å                  | Å     |
| Spheroplasts lysed on water |               |                    |       |
| Experiment 1     | 163.4         | 32.4               | 70-370|
| 2                | 171.3         | 42.8               | 65-250|
| 3                | 175.0         | 52.7               | 65-300|
| 4                | 185.5         | 48.7               | 80-330|
| 5                | 175.6         | 54.6               | 65-320|
| 6                | 171.2         | 52.7               | 40-300|
| Spheroplasts lysed on 5 mM sodium citrate |               |                    |       |
| Experiment 1     | 95.2          | 50.8               | 70-140|
| 2                | 106.3         | 44.6               | 80-230|
FIGURES 1a and 1b Network of nucleohistone fibers from nuclei of yeast spheroplasts spread on water. × 72,000.

FIGURE 1c Nucleohistone fibers from yeast spread on 5 mM sodium citrate. Calibration lines represent 0.1 μm. × 72,000.

tuberances of variable length. Such regions of the chromatin are suggestive of heterochromatin seen in sections. Aggregates of ribosomes which mimic the morphology of these areas are also observed. They look like branched chains composed of roughly spherical particles (Fig. 2c).
DISCUSSION

Yeast nucleohistone fibers spread on an air-water interface appear morphologically similar to those of other eucaryotes spread in the same way. There is, however, a greater variability in fiber diameter due to the greater knobbiness of yeast chromatin.

Measurements of the yeast fibers indicated that their average diameter is about 175 Å. This is within the range of reported fiber diameters for water-spread chromatin of other eucaryotes, including those eucaryotes possessing the full complement of histones (7, 8, 9, 10).

The average diameter of the basic chromatin fibers spread on 5 mM sodium citrate is about 100 Å and is thus very similar to that reported for other eucaryotes despite the absence of a major histone fraction. As in other eucaryotes the fibers spread in the absence of chelating agents is considerably thicker, though it is at present not clear how the thin fiber is derived from the thicker one through the action of chelating agents.

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