Fractionation of *Saccharomyces cerevisiae* Cell Populations by Centrifugal Elutriation

C. N. Gordon* and S. G. Elliott

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

Received for publication 24 September 1976

An exponential population of *Saccharomyces cerevisiae* cells was fractionated by centrifugal elutriation, using water as the elutriating liquid. Evidence that the population had been fractionated according to age in the cell cycle was obtained by examining the fractions for their size distribution, their microscopic appearance after Giemsa staining, and their ability to initiate synchronous growth.

A number of procedures have been employed for obtaining synchronous cultures of the yeast *Saccharomyces cerevisiae* for use in studies of the cell cycle (3). Generally, synchronization procedures are subject to the criticism that the synchronization process may introduce distortions in the normal sequence of events in the cell cycle. In addition, some techniques use only a small percentage of the original cell population in growing the synchronous culture and, consequently, the yield of cells at any particular stage of the cell cycle is low.

Sebastian et al. carried out large-scale fractionations of exponential cultures of *S. cerevisiae* by velocity sedimentation through sucrose gradients in a rotating-seal zonal rotor (7). Similar fractionations of the fission yeast *Schizosaccharomyces pombe* were carried out on nonliving cells by Wells and James in a reorienting gradient zonal rotor (8). These methods have an important advantage over synchronous growth in that cell cycle measurements are made on cells that have been fractionated from a condition of balanced growth, thus avoiding possible artifacts induced by the synchronization process.

Centrifugal elutriation ("counterflow centrifugation") was used as a method of separating biological particles nearly 30 years ago (5), but a rotor suitable for general use has only recently become commercially available. In this procedure, the force tending to sediment particles in a centrifugal field is counterbalanced by a liquid flowing in the opposite direction. A cell population can be fractionated into discrete size classes by incrementally increasing the flow rate while maintaining the centrifuge rotor at a constant speed (6). Centrifugal elutriation has two important advantages over zonal centrifugation. First, the rotor has a much longer effective path length, and the number of size classes obtained can be greatly increased by choosing smaller incremental increases in flow rate. Second, the fractionation can be carried out in virtually any aqueous solution of uniform density.

In this paper we describe the fractionation of an exponential yeast population by centrifugal elutriation with water as the elutriating liquid. Our results indicate that elutriation separates the yeast cell population into fractions representing successive stages of the cell cycle, and is an effective method of obtaining large numbers of cells for cell cycle studies.

MATERIALS AND METHODS

Organism. *S. cerevisiae* SKQ2n, a prototrophic diploid with the genotype *a/a, ade1*/+, *ade2*/+, *his1* was used. It was obtained from Brian Cox, Botany School, South Parks Road, Oxford, OX1 3RA, United Kingdom.

Growth and Harvest of Cells. Cells were grown in YM-1 medium (1) to a cell density of 1.5 × 10⁷ to 3.0 × 10⁷ cells/ml at 23°C on a rotary shaker. Growth was retarded by chilling to 0°C, and the cells were centrifuged and washed with water at 4°C. The cells (3 × 10⁹) were suspended in 10 ml of ice water and while jacketed in an ice bath were sonically treated for 10 s in a Biosonik Sonifier (Bromwill Scientific Inc., Rochester, N.Y.) at half the maximal setting. The sonically treated cell suspension was then kept in ice until it was loaded into the elutriator rotor.

Fractionation by centrifugal elutriation. A Beckman JE-6 elutriator rotor was driven in a Beckman J-21 centrifuge. The centrifuge was modified for elutriation and equipped with a stroboscope assembly by the manufacturer (Beckman Instruments, Inc., Palo Alto, Calif.). The pump used in the system was a Masterflex 745 equipped with a model 7014 pump head (Cole-Parmer Instrument Co., Chicago, Ill.). To allow greater sensitivity in determining the pump flow rate, the one-turn potentiometer speed control was removed and substituted with a 10-turn control (Beckman helipot 7276R50L.25) and a sepa-
rate on-off switch. The flow system was set up in the following way. The receptacle of a 100-ml "Yale Luer-Lok" syringe (Becton-Dickinson and Co., Rutherford, N.J.) was interposed between the pump inlet and the water reservoir by means of a three-way bypass valve and Silastic tubing. The syringe contained the cell suspension to be loaded into the rotor. With the pump running continuously, manipulation of the bypass valve allowed direct flow from the reservoir into the rotor or, alternatively, loading of the cell suspension into the rotor.

Elutriation was done at a centrifuge well temperature of 4°C while the water reservoir and collection flasks were maintained in ice baths. The length of connecting tubing was kept to a minimum. Water flow through the rotor was started and the rotor was brought to a speed of 3,000 rpm. Care was taken to remove all air bubbles in the system before the cells were loaded. The chilled and sonically treated cell suspension was placed in the loading syringe, and then the flow rate was brought to 9 ml/min. The cells were loaded at this flow rate and then flushed with 200 ml of water. Next, 200-ml fractions were collected. The first fraction was collected at 11 ml/min, and successive fractions were obtained with increments of 1 ml/min. At a flow rate of 27 ml/min, virtually all of the single cells were removed from the rotor.

Analysis of fractions. To each fraction, NaCl was added to 0.1 M and formaldehyde was added to 0.4%. The cells were collected by centrifugation and suspended in 0.9% NaCl at a cell density of 10^5 cells/ml. The size distribution of the cells in each fraction was determined in a Coulter counter that had been calibrated with latex spheres of average particle diameter 3.49 μm. Samples of the fractions were prepared for microscopic observation by a modified Giemsa staining procedure (2).

To demonstrate synchronous growth in fractions elutriated at 11 and 25 ml/min, formaldehyde was omitted and the centrifuged fractions were suspended in YM-1 medium at a cell density of 3 x 10^7/ml. The cell suspensions were incubated at 23°C, and 0.1-ml portions were removed at the indicated times and placed in 0.9% NaCl containing 0.5% formaldehyde. After sonic dispersion for 10 s, cell numbers were determined in a model F Coulter counter.

**RESULTS**

The size distributions of four fractions obtained from the elutriator rotor and then fixed with formaldehyde are shown in Fig. 1. It is evident that there is an increase in the diameter of the cells elutriated with increasing flow rate. Evidence that the elutriation is effecting a fractionation of the population according to age in the cell cycle was obtained from microscopic examination of the fractions after Giemsa staining. In Giemsa-stained preparations, cells can be classified into one of four groups, shown schematically in the inset to Fig. 2. The distribution of the four basic cell types in the fractions obtained from the elutriator rotor is also shown. At the lower flow rates (11 to 13 ml/min), the population consists predominantly of unbudded cells. Budded cells, in which the nucleus has not yet migrated into the bud neck, constitute the major component in fractions elutriated at flow rates of 16 to 18 ml/min. Cells in which the nucleus is in the act of migration reach a peak in the fraction elutriated at 21 ml/min. Finally, the fractions obtained at flow rates of 23 to 26 ml/min consist primarily of cells in which partition of the nucleus between mother and daughter cells has been completed. Remaining in the rotor were clumped cells representing less than 1% of the cells loaded into the rotor. These clumps may have been formed during growth, harvesting, and washing of the cells, and they were apparently not dispersed by the sonic treatment.

Figure 3 shows growth curves obtained with fractions elutriating at 11 and 25 ml/min. The growth curve of the unfractionated culture is shown for comparison. It is evident that the fractions obtained by elutriation behave like synchronously growing cultures.
DISCUSSION

Our results show that centrifugal elutriation is an effective method of separating an exponential yeast population into fractions representing successive stages of the cell cycle. This conclusion is based on the following lines of evidence. (i) Successive fractions from the elutriator rotor that were examined in the Coulter counter showed an increase in average cell size with increasing flow rate. (ii) Microscopic examination of the fractions showed that the increase in average size of the cell population within each fraction was due to an enrichment of the fraction by cells in comparable stages of the cell cycle. (iii) Fractions obtained from the elutriator rotor showed synchronous growth.

An important advantage of this method over zonal centrifugation or other selection techniques is the wide choice of media available to carry out the fractionation. Although water was used in our experiments, the separation can be effected in any medium of uniform density, including growth medium. In addition, the effective path length of the rotor can be increased by using smaller incremental increases in flow rate. Under our conditions, flow rate could be controlled to within 0.1 ml/min; however, smaller increments could be realized with more elaborate pumping systems.

The synchrony shown here (Fig. 3) is admitted not as good as that obtained by Sebastian et al. for cells fractionated by zonal centrifuga-
tion (7). However, Sebastian et al. fractionated the cells at room temperature, whereas our experiment was carried out in the cold and nonuniform recovery from cold shock may have worsened the degree of synchrony. Low-temperature fractionation was chosen because we wished to fractionate the entire population of cells. This process takes 2 to 3 h under our conditions of flow rate and centrifuge speed, and a low temperature was used to minimize growth changes during this period. However, elutriation of a particular size class can be performed rapidly at room temperature. For example, suppose one wished to obtain the fraction of cells that elutriates at 21 ml/min. After the cells were loaded, the flow rate would be immediately raised to 20 ml/min, and all cells elutriating at flow rates of 20 ml/min or less would be flushed out of the rotor. The flow rate would be raised to 21 ml/min, and the cells in this size class would be obtained as a discrete size class. In theory, the fractionation process could be shortened by collecting smaller volumes and by the use of higher centrifuge speeds and faster flow rates. However, we have not determined what, if any, effect these variables would have on the quality of the separation.

Cell cycle studies were performed on cells that were harvested during balanced growth and then fixed with trichloracetic acid prior to fractionation by zonal centrifugation (8). In this case, the length of time required to carry out the separation does not affect the resolution of the system because the cells cannot exhibit growth changes during the fractionation process. Elutriation would be ideally suited for this purpose because of the fine degree of separation obtained by using small incremental increases in flow rate. Thus, events occupying relatively short periods in the cell cycle can be studied.

Since both zonal centrifugation and centrifugal elutriation separate cells on the basis of sedimentation rate, some comparisons of the two systems are in order. Intrinsic variation in the basic parent cell size among the members of a yeast population would result in a poorer fractionation with both methods. Size heterogeneity among a yeast population arises because of divisional age—as cells get older (i.e., acquire more bud scars), they become larger (4). Thus, any exponential yeast cell population will show some degree of size heterogeneity, and this places a basic limitation on the extent to which a population can be fractionated according to cell cycle age. Since size heterogeneity is also a strain-dependent phenomenon, optimum separation on the basis of cell cycle age will be obtained for strains that show high degrees of size uniformity.

Both methods have additional physical characteristics that further impair the efficiency of fractionation of a yeast cell population. In elutriation, a Coriolis or streaming effect tends to make the removal of small particles from the rotor less efficient than the removal of larger particles (6). In zonal centrifugation, resolution is impaired by diffusion of the starting zone (8) and possibly by the pushing of small cells into the gradient by the larger cells as they begin migration (7). Because of these limitations, neither method can achieve a perfect separation of cells on the basis of cell cycle age. Elutriation, because of its advantages of long path length and wide choice of solvents, is a useful addition to existing methods of obtaining cell cycle separations of yeast by size selection.

ACKNOWLEDGMENTS

We wish to thank C. McLaughlin for his comments and suggestions, James Cladek for assistance with some of the fractionations, and O. M. Griffith, Bob Lopes, and Bill Cassel of Beckman Instruments for aid and advice in setting up and operating the elutriation system. This work was supported by grant BMS 73-06847 A01 from the National Science Foundation. S.G.E. is supported by Public Health Service grant CA 10628 (awarded to C. McLaughlin) from the National Cancer Institute.

LITERATURE CITED