POLYRIBOSOMES FROM *TETRAHYMENA*

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INTRODUCTION

The free-living, ciliated protist *Tetrahymena*, which can be grown in synchronized cultures, lends itself to studies of protein synthesis and organelle morphogenesis in eukaryotic cells. The isolation of polyribosomes, the sites of protein synthesis, from *Tetrahymena* was reported by Whitson et al. (5). After lysis of the cells by indole-saturated buffer, the polysomes were isolated in 0.01 M Tris buffer, pH 7.5 containing 0.5 mM $MgCl_2$. These investigators presented evidence for the presence of dimers to pentamers, and for changes in the polysome profile during various phases of the cell cycle. However, the yields of polysomes were low, and the peak heights from dimers to pentamers decreased, an indication of polysome degradation during the isolation procedure (4). In our hands, the difficulties in preparing polysomes from *Tetrahymena* could be overcome by rupturing the cells by intracytoplasmic cavitation of nitrogen in a medium containing calcium.

MATERIALS AND METHODS

Tetrahymena pyriformis, strain GL (obtained from Dr. O. Scherbaum), or *Tetrahymena pyriformis*, strain W (obtained from Dr. I. R. Gibbons), were grown at room temperature in medium containing 1.0% tryptone, 0.5% yeast extract, and 0.5% NaCl, neutralized to pH 7.0. Cells were harvested during the

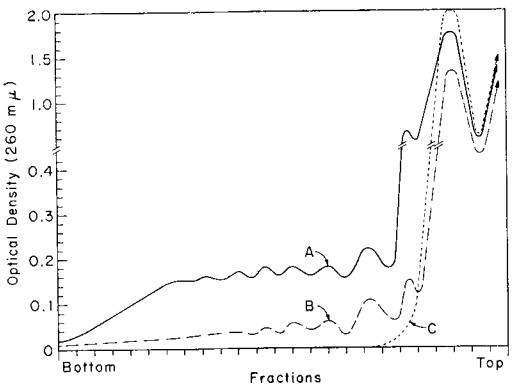

FIGURE 1 Approximately 8 × 10^6 cells of *Tetrahymena pyriformis*, strain W, were centrifuged, washed, and suspended in 6 ml of buffer. The cells were disrupted by intracytoplasmic cavitation of nitrogen, and the intracellular organelles were removed by centrifugation at 11,000 *g* for 10 min. 2 ml of the supernatant fraction were layered on 27 ml of a 15–30% (w/w) sucrose gradient and centrifuged for 2 hr at 25,000 rpm. Absorbance profiles of the gradients at 260 mμ are depicted. *A*, The buffer used contained 0.12 M KCl, 0.0075 M $MgCl_2$, 0.01 M $CaCl_2$, and 0.01 M Tris, pH 7.4. *B*, Same buffer as *A*, except that $CaCl_2$ was omitted. *C*, Same buffer as *A*, but the lysate was pretreated with 10 μg/ml of RNase for 10 min at 20°C. The single ribosome peak is labeled 75S; peaks to the left represent classes of polysomes of increasing size.

exponential growth phase when the density was 0.8–1.0 × 10^5 cells/ml, centrifuged, and resuspended in buffer containing 0.12 M KCl, 0.01 M Tris, 0.0075 M $MgCl_2$, and 0.01 M $CaCl_2$, pH 7.4. The cells were recentrifuged and resuspended in a smaller volume of buffer so that the final cell density was about 1–2 × 10^6.

The cells were disrupted by nitrogen cavitation (1) at 4°C after equilibration with nitrogen gas at 600 lb/in² pressure, using a high pressure homogenizer adapted from the design of Hunter and Commerford (2). The lysate was centrifuged at 11,000 *g* for 10 min to sediment nuclei, mitochondria, and lysosomes; 2 ml of the supernatant fraction were layered onto 27 ml of a 15–30% (w/w) linear sucrose gradient in buffer. After centrifugation for 2 hr at 25,000 rpm in a Spinco No. 25.1 rotor at 0°C, fractions were collected from the bottom of the tube for absorbance measurements at 260 mμ and assays of radioactivity.

RESULTS AND DISCUSSION

Typical absorbance profiles of the sucrose gradients are shown in Fig. 1. The use of buffer without calcium gave a low yield of polysomes; the profile showed decreasing peak height from dimers to pentamers, indicating degradation during preparation. *Tetrahymena* are rich in lysosomes and hydrolytic enzymes (3), which may account for difficulties in preparing polyribosomes and for the susceptibility to degradation.

With calcium present in the buffer, a greater yield of polysomes was obtained, and a slight increase in peak height was observed from dimers to pentamers. In addition, a broad peak was observed which represents hexamers and polysomes of greater size. The yield of polysomes could not be increased further by use of sucrose, dextran sulfate, or an excess of low-molecular-weight RNA during the preparative procedures.

Treatment of the cytoplasmic supernatant with 10 μg/ml bovine pancreatic RNase for 10 min at room temperature prior to layering on the sucrose gradient resulted in loss of absorbance in the polysome region of the profile and a corresponding increase in the region of single ribosomes. The activity of the polysomes was assessed by in vivo amino acid incorporation studies. After pre-incubation in buffer for 30 min, 5 μCi/ml U-amino

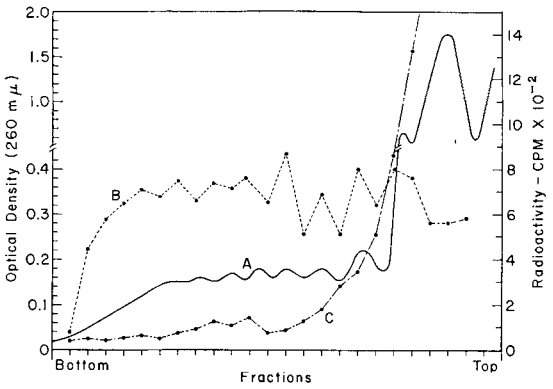

FIGURE 2 Approximately 8 × 10^6 cells of *Tetrahymena pyriformis*, strain GL, were incubated in 5 ml of buffer (0.12 M KCl, 0.0075 M $MgCl_2$, 0.01 M $CaCl_2$, 0.01 M Tris, pH 7.4) containing 25 μCi of U-amino acids-^{14}C for 5 min at 25°C. The suspension was added to 55 ml of ice-cold buffer, and the cells were sedimented, washed, resuspended in buffer, and disrupted by intracytoplasmic cavitation of nitrogen. The lysate was sedimented in a 15–30% (w/w) sucrose gradient. *A*, An absorbance profile of the gradient at 260 mμ. *B*, A radioactivity profile of the gradient. *C*, Same as *B*, but the lysate was pretreated with 10 μg/ml of RNase for 10 min at 20°C.

acid-^{14}C mixture was added, and the cells were harvested after further incubation for 3 min. Most of the radioactivity in the sucrose gradients was found in the polysome region, owing to amino acid incorporation into nascent polypeptides. Treatment of the cytoplasmic supernatant fraction with RNase resulted in a shift of the radioactivity in a subsequent sucrose gradient to the fractions containing single ribosomes (Fig. 2).

Experiments with synchronized cultures are underway to study changes in the polysome profile and amino acid incorporation at various stages in the cell cycle.

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REFERENCES

1. DOWBEN, R. M., T. A. GAFFEY, and P. M. LYNCH. 1968. Isolation of liver and muscle polyribosomes in high yield after cell disruption by nitrogen cavitation. *Fed. Eur. Biochem. Soc. Letters.* **2**:1.
2. HUNTER, M. J., and S. L. COMMERFORD. 1961. Pressure homogenization of mammalian tissue. *Biochim. Biophys. Acta.* **47**:580.
3. MÜLLER, M., P. BAUDHUIN, and C. DE DUVE. 1967. Lysosomes in *Tetrahymena pyriformis*: Some properties and lysosomal localization of acid hydrolases. *J. Cell Physiol.* **68**:165.
4. RICH, A. 1967. *In* Methods in Enzymology. L. Grossman and K. Moldave, editors. Academic Press Inc., New York. **22**:481.
5. WHITSON, G. L., G. M. PADILLA, and W. D. FISHER. 1966. Cyclic changes in polyribosomes of synchronized *Tetrahymena pyriformis*. *Exp. Cell Res.* **42**:438.