POLYRIBOSOME PROFILES FROM HEAT SYNCHRONIZED TETRAHYMENA*

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SUMMARY

Polyribosome profiles were obtained from cultures of Tetrahymena pyriformis synchronized by cyclical temperature changes. After release from heat shock, heavy polyribosomes were prominent in the profile, suggesting that heavy polyribosomes are more resistant to disruption at elevated temperatures. Prior to cell division, light polyribosomes were prominent in the profile. The light polyribosomes may be associated with the specific proteins required for the process of cell division. Very heavy polyribosomes were not found although the cilia contain an ATPase which resembles muscle myosin.

Nuclear division in Tetrahymena, a ciliated protozoan, is delayed by incubation above the maximal growth temperature. Cell viability remains unaffected, and abnormally large cells are produced by the continuation of growth in the absence of cell division. The cells in a culture subjected to a series of "heat shocks" divide synchronously upon continued incubation at 29° C (1). Byfield and Scherbaum (2-4) showed that RNA decays at a rapid rate during heat shock, and suggested that the increased destruction of m-RNA might be responsible for the decreased amino acid incorporation into protein during the cyclical temperature treatment. Another possible factor in causing diminished protein synthesis may be the more rapid denaturation of ribosomes from Tetrahymena at temperatures a few degrees above the maximal growth tem-

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perature (5). Changes in the polyribosome profiles of cells from synchronized cultures of Tetrahymena are reported in this communication.

MATERIALS AND METHODS

Tetrahymena pyriformis, strain GL (obtained from Dr. O. H. Scherbaum), was grown at 29° C in a medium containing 1.0 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, pH 7.0 to a density of 4-6 X 10^4 cells/ml. The cultures were synchronized by five cycles of incubation, first at 29° for 30 minutes and then at 34° for 30 minutes. The cells were harvested at various time intervals after the last heat shock and washed with buffer containing 0.12 M KCl, 0.0075 M $MgCl_2$, 0.01 M $CaCl_2$, 0.01 M tris, pH 7.4. Polyribosome profiles were obtained after cell disruption by nitrogen cavitation as described previously (6) using the cytoplasmic supernatant from approximately 8 X 10^7 cells in each gradient.

RESULTS AND DISCUSSION

Polyribosome profiles of Tetrahymena at several time intervals after release from heat shock are shown in Figure 1. Immediately after release from heat shock, a prominent broad peak of heavy polyribosomes (containing 8 to 20 ribosomes) was found in the profiles. Our data differ from the changes observed in the polyribosome profiles of slime mold subjected to heat shock (7) At 30 and 60 minutes after release, the heavy polyribosome peak became less prominent compared to smaller polyribosomes, which were present in larger amounts. At 85 minutes after release, just prior to cell division, the total amount of polyribosomes was somewhat diminished while the amount of single ribosomes was markedly increased. The total quantity of ribosomes (single ribosomes plus polyribosomes) increased continually during the period from release after heat shock to cell division.

If the instability of m-RNA (3) and the thermal denaturation of ribosomes (5) led to the disruption of polyribosomes in the process of transla-

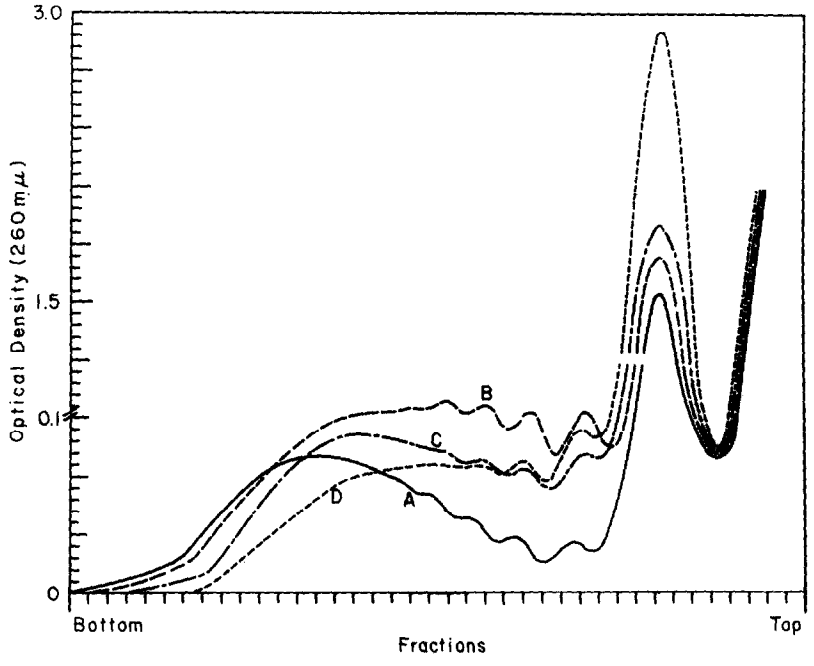

Figure 1. Polyribosome profiles from Tetrahymena after release from heat shock. Approximately 2.5 X 10^8 cells were suspended in 6 ml buffer containing 0.12 M KCl, 0.0075 M $MgCl_2$, 0.01 M $CaCl_2$, 0.01 M tris, pH 7.4 and disrupted by nitrogen cavitation at 4° C after equilibration with nitrogen gas at 600 lb/in^2 for 20 minutes (6). The lysate was centrifuged at 11,000 g for 10 minutes; 2 ml of the supernatant was layered on 27 ml of a linear 15-30 % (w/w) sucrose gradient and centrifuged for 2 hours at 25,000 rpm in a Spinco 25.1 rotor. Absorbance profiles of the gradients are depicted for cells obtained immediately after heat treatment (A), 30 minutes after release (B), 60 minutes after release (C), and 85 minutes after release (D).

tion with release of partially synthesized polypeptide chains, we would expect a greater relative disruption of heavier polyribosomes. The finding of a prominent heavy polyribosome peak immediately after heat shock indicates that the formation of a polyribosome stabilizes m-RNA and ribosomes. The greater stability of the larger polyribosomes may be due to the nature of the forces holding the polyribosomes together. Because polyribosomes are held together principally by the cooperative effects of multiple van der Waals interactions, disruption of larger polyribosomes containing more interacting sites will require higher temperatures than the disruption of smaller polyribosomes. The increased destruction of m-RNA and ribosomes during incubation at elevated temperatures probably occurs to free m-RNA and single ribosomes.

New RNA synthesis and protein synthesis after release from heat shock is required before cell division can take place (8-11). Cells appear to synthesize specific proteins during the last quarter of the cell cycle which are required for the process of cell division. Inhibition of synthesis of these proteins is thought to be the mechanism by which heat shock prevents cell division. The profiles obtained in our experiments show a prominent increase of small polyribosomes before the onset of cell division. If the specific proteins required for cell division are of low molecular weight, the preferential disruption of small polyribosomes during heat shock may prevent their synthesis while permitting sufficient synthesis of other proteins to maintain cell viability.

The cilia of Tetrahymena contain an ATPase, dynein, of molecular weight 600,000 which resembles myosin from skeletal muscle in its solubility properties (12). A class of very heavy polyribosomes containing 55-60 ribosomes is associated with the synthesis of myosin in chick embryo muscle. However, no very heavy polyribosomes were found in the profiles of Tetrahymena.

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