SHORT COMMUNICATION

Changes in Activities of Three RNAases During the Heat-synchronized Cell Cycle of *Tetrahymena pyriformis* ST

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Acid RNAases of *Tetrahymena pyriformis* ST were resolved into one minor and two major peaks by Sulphopropyl Sephadex chromatography. The same three peaks were obtained from exponentially growing cells and from heat-synchronized cells. The two major RNAases (peaks 2 and 3) were also separated by polyacrylamide gel electrophoresis at pH 4.5 and 8.3. The stepwise increase in total RNAase activity during synchronous growth was primarily caused by a large and preferential increase in the activity of the peak 3 RNAase during the first hour after the heat shock.

INTRODUCTION

Synchrony of *Tetrahymena pyriformis* can be induced by giving a series of heat shocks spaced one generation time apart (Zeuthen, 1964, 1971, 1974). Using this system, changes in the activities of nine different enzymes have been assayed during the cell cycle. Activities of nucleoside phosphotransferase (Bols & Zimmerman, 1977; Young *et al.*, 1978) and three mitochondrial enzymes, succinate dehydrogenase, succinate-cytochrome reductase and malate dehydrogenase (Cowan & Young, 1978), increased continuously through the cell cycle. Activities of NADH-cytochrome *c* reductase (Cowan & Young, 1978) and two lysosomal hydrolases, α - and β -glucosidases (Zeuthen, 1974), increased stepwise at about the time of cell division. The activity of thymidine kinase, which was recently found in this organism (Yuyama *et al.*, 1978), showed a peak during the S phase (Young *et al.*, 1978). These changes in enzyme activities were apparently not influenced by the heat-shock regime which was used for synchrony induction. In sharp contrast, the activity of acid RNAase was stimulated by the heat shock and this stimulation determined the step pattern of RNAase activity during the cell cycle (Tarnowka & Yuyama, 1978).

In *T. pyriformis*, RNAases have been resolved by cation exchange chromatography into two species in the W strain (Wu Tching, 1969) and three species in the GL strain (Lazarus & Scherbaum, 1967). The present paper describes fractionation of the RNAases of the ST strain using the same procedure, and the changes of each RNAase during the heat-induced synchronous division cycle.

METHODS

Cell culture. Methods for cell culture, synchronization, determination of cell density and division index, and assay for RNAase were those described previously (Tarnowka & Yuyama, 1978), except that the concentration of yeast extract in the PPY medium was increased to 0.2%, since this led to higher and more reproducible division synchrony. Also the volume of each culture sample was increased from 2 ml to 800 ml.

Fractionation of RNAase. Acid RNAases were fractionated by methods modified from those employed by Lazarus & Scherbaum (1967) and Wu Tching (1969). Cells in 800 ml culture were collected, washed, chilled

to 4 °C and lysed at pH 7.0 as described previously (Tarnowka & Yuyama, 1978). These procedures took about 20 min (i.e. much longer than the 5 to 6 min in the previous method; Tarnowka & Yuyama, 1978). The pH of the lysate was then adjusted to 4.5 by adding sodium acetate buffer to a final concentration of 0.1 M (cells were lysed at pH 7.0, because they would not lyse at pH 4.5). RNAase was assayed at pH 4.5; its activity remained in the soluble fraction at this pH, so after standing for 30 min at pH 4.5, the lysate was centrifuged (10 min, 12000 g) and the supernatant was passed through a Sephadex G-25 column equilibrated with 0.1 M-sodium acetate (pH 4.5). The macromolecular fraction, adjusted to contain 3200 to 4000 enzyme units per separation, was loaded on to a Sulphopropyl (SP) Sephadex C-50 column (1.6 × 10 cm). After washing out the non-specifically bound material (having only 1 % enzyme activity), the protein was eluted (0.8 ml min⁻¹) with a linear gradient of NaCl (0 to 0.2 M) in 0.1 M-sodium acetate (pH 4.5). Fractions (8 to 12 ml) were collected and samples (0.5 ml) of alternate fractions were assayed for RNAase activity.

Gel electrophoresis. The RNAases were concentrated by (NH₄)₂SO₄ precipitation (30 to 70 % saturation, 2 °C), dissolved in 0.05 M-Tris/HCl (pH 7.0) and then desalted (Sephadex G-25; 0.1 M-sodium acetate, pH 4.5) before SP-Sephadex fractionation as above. SP-Sephadex fractions corresponding to each RNAase peak were pooled separately and concentrated by a combination of evaporation, addition of dry Sephadex G-25 and centrifugal desalting (Neal & Florini, 1973). The concentrated samples were analysed on 7 % (w/v) acrylamide gels (0.5 × 6.5 cm) prepared at pH 4.5 (Reisfeld *et al.*, 1962) or at pH 8.3 (Davis, 1964). Following electrophoresis, the gels were cut into serial sections, and the RNAase activity in each slice was determined by incubating it in 1 ml of a solution containing 20 A₂₆₀ units of RNA.

RESULTS AND DISCUSSION

Changes in total cellular RNAase activity during the cell cycle

We have shown previously that during the heat-synchronized cell cycle of *T. pyriformis* ST the RNAase activity changes in a stepwise manner, the step occurring immediately after the heat shock (Tarnowka & Yuyama, 1978).

When the RNAase activity of the crude cell lysate and that of the 12000 g supernatant were assayed at different times during the cell cycle, the profiles of the RNAase activity were essentially the same, although the supernatant fraction contained about 20 % less RNAase activity than the crude cell lysate. Thus, the 20 % loss of RNAase activity was not cell-cycle dependent. The RNAase activities of both the crude cell lysate and the 12000 g supernatant were stable for several days when stored at 0 to 3 °C. All the experiments reported in this paper were conducted within 24 h of lysis.

Changes in activities of individual RNAases during the cell cycle

Samples were taken at four times during the cell cycle and RNAases were fractionated by SP-Sephadex chromatography. Time 1 (Fig. 1*a*) was at the middle of the heat shock, during which time the total RNAase activity did not change; time 2 (Fig. 1*b*) was at the completion of the rapid increase in the total RNAase activity after the heat shock; time 3 (Fig. 1*c*) was the time of synchronous division; time 4 (Fig. 1*d*) was equivalent to time 1 in terms of the cell cycle stage. The RNAases were resolved into one minor peak and two major peaks (peaks 1, 2 and 3, respectively, in Fig. 1). The patterns of the RNAase fractions were qualitatively similar at all three stages during the cell cycle. The results, when quantified as units of RNAase activity per ml culture (Fig. 2), showed that within 60 to 70 min after the heat shock, peak 1 RNAase activity increased by 50 %, peak 2 RNAase by 75 % and peak 3 RNAase by 170 %. Thus peak 3 RNAase was preferentially stimulated by the heat shock, and is primarily responsible for the rapid increase in total RNAase activity that occurs following the heat shock. Subsequently, the activities of peak 1 and peak 2 RNAases continued to increase, while the activity of peak 3 RNAase underwent a pattern of decline and increase. Each RNAase approximately doubled its activity over one complete cell cycle, as expected.

Some of the RNAase activity loaded on to the column was not eluted from it (Fig. 2).

The relative proportions of the three RNAases were 9, 38 and 53 % at time 1 (during the heat shock) and 6, 30 and 64 % at time 2 (60 to 70 min after the heat shock), respectively,

Fig. 1. Fractionation on SP-Sephadex of acid RNAses from heat-synchronized *Tetrahymena pyriformis* ST: —, RNAase activity; ---, estimated NaCl gradient. (a) 15 min before the end of the 6th heat shock (EH); (b) 63 min after EH; (c) 110 min after EH; (d) 185 min after EH, i.e. 15 min before the end of the 7th heat shock. Arrows indicate peaks 1, 2 and 3.

Fig. 2. Changes in the activities of the three RNAses during the heat-synchronized cell cycle of *Tetrahymena pyriformis* ST: —, normalized RNAase activities of peak 1 (▲), peak 2 (■), peak 3 (●) and total RNAase activity (□); ---, RNAase activity not recovered; ···, normalized cell concentration. 6HS and 7HS indicate the 6th and 7th heat shocks; EH designates the end of the 6th heat shock.

in heat-synchronized cells. In exponentially growing cells, their relative proportions were 6, 41 and 52%, respectively. These results indicate that exponentially growing cells and heat-synchronized cells during the time of the heat shock (time 1) have similar proportions of the three RNAses, whereas at time 2, the proportion of peak 3 RNAase is greater. These observations also support the view that peak 3 RNAase is preferentially stimulated by the heat shock.

Polyacrylamide gel electrophoresis at two pH values confirmed that RNAase peaks 2 and 3 were different proteins. At pH 4.5, peak 2 RNAase migrated at about 40% of the rate of the marker dye, toluidine blue, and the peak 3 RNAase at 60% of the rate of the dye. At pH 8.3, peak 2 RNAase migrated at about 50% of the rate of the marker dye, bromophenol blue, while peak 3 RNAase hardly entered the gel.

The mechanisms of the complex pattern of behaviour of RNAases during the cell cycle are not clear. However, the sum of all RNAase activities (including the loss of activity) during the three different phases of the cell cycle agrees well with the profile of total cellular RNAase activity during the cell cycle of this organism (Tarnowka & Yuyama, 1978).

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