REGULATION OF RNA SYNTHESIS IN
TETRAHYMENA PYRIFORMIS: SECRETION
OF REGULATORY FACTORS

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SUMMARY

The rate of ribosomal RNA synthesis varies greatly with the population density in both exponentially and synchronously growing populations of Tetrahymena pyriformis. Shortly after inoculation of the population— at relatively low cell densities—a gene-dose effect dominates the picture, and a doubling in the gene number is immediately followed by a doubling in the rate of RNA synthesis. However, also other mechanisms are controlling the rate of RNA synthesis. Generally one finds high rates of RNA synthesis in the lag phase of newly inoculated cells, decreasing rate of RNA synthesis during most of the exponential growth phase and very low rate of synthesis in stationary phase cells. We now have results which show that the repression of RNA synthesis in densely populated cultures is caused by a dialysable factor, which is secreted by the cells. If cells are inoculated on a medium which contains this factor the high initial rate of RNA synthesis normally observed is prevented, but the cells multiply and grow with normal generation time until normal stationary-phase population densities are reached.

INTRODUCTION

It has previously been described by Keiding & Andersen (1978) that the rate of RNA synthesis is constant during most of the cell cycle in heat-shock-synchronized populations of Tetrahymena pyriformis. Furthermore, the rate of RNA synthesis was found to increase abruptly early in the macronuclear S-period, concomitant with replication of the genes coding for ribosomal RNA (Andersen & Engberg, 1975). It was suggested that the rate of ribosomal RNA synthesis depends on gene dose in these cells. On the other hand, also other mechanisms control the rate of ribosomal RNA synthesis in Tetrahymena, since the synthesis is strongly repressed in very dense populations and derepressed when the cells are inoculated into fresh growth medium (Cameron & Guile, 1965; Conner & Koroly, 1972), and this without any dramatic variation in the cellular content of ribosomal genes (Engberg & Pearlman, 1972; Marcaud, Portier & Milet, 1976). It has been reported (Cameron & Guile, 1965) that cells in lag phase following inoculation derepress ribosomal genes and synthesize RNA in high amounts before the exponential growth phase is initiated. Furthermore, that during exponential growth the doubling in cell number exceeded the rate of doubling in RNA. This would lead to a steady decrease in the cellular RNA content. Our previous results (Keiding & Andersen, 1978) on synchronously growing populations supported this view.
We have now studied these problems in more detail in exponentially growing as well as in synchronized cells. Our results agree with the idea that the rate of RNA synthesis is gene-dose dependent and this is most pronounced in cultures with relatively low population densities. However, at higher cell densities we observe that extracellular factors, secreted by the cells, become of increasing importance in repression of the RNA synthesis. Therefore, not only gene dose, but also the number of cells in the population and the time the cells have spent on the medium is of importance for the rate of RNA synthesis within the single cell in a Tetrahymena population.

**MATERIAL AND METHODS**

*Tetrahymena pyriformis*, amicronucleate strain GL, was grown at 28 °C on a medium containing 0.75 % proteose peptone (Difco), 0.75 % yeast extract (Difco) and salts according to Plesner, Rasmussen & Zeuthen (1964). Synchronized populations were obtained by the procedure described by Zeuthen (1971). Cell counting was performed by an electronic cell counter after fixation of the cells in 4 % formaldehyde. DNA synthesis was inhibited by addition of hydroxyurea (HU) to a final concentration of 10 mM. In the pulse-labelling experiments 1-ml samples were withdrawn at various times and incubated for 20 min with 0.25 μCi/ml of [14C]uridine, [14C]leucine or [14C]thymidine. Then the cells were precipitated by addition of ice-cold trichloroacetic acid (TCA) to a final concentration of 5 %. The samples were filtered on WHATMAN GF/C filters and washed 3 times with 5 % TCA. The filters were dried and counted in a liquid scintillation counter (BECKMAN, LS 200). To obtain dialysed medium a relatively dense population (2–3 x 10⁶ cells/ml) was placed in the dialysis bag and dialysed for 4 h against an equal volume of normal medium containing penicillin G and streptomycin (37 mg/100 ml of each).

**RESULTS**

The results presented in Fig. 1 show the variation of the rate of RNA synthesis per cell (RRSC) during 16 h following inoculation of *Tetrahymena* into fresh medium. A dense population (3.5 x 10⁶ cells/ml) was diluted 20-fold (to about 1.7 x 10⁵ cells/ml) and after a lag phase of about 3 h the cells began to multiply. They maintained a constant generation time for the rest of the experiment (~ 12 h) reaching a cell density of about 3 x 10⁵ cells/ml. Other experiments show that the cells go through another 1–2 doublings, and enter stationary phase between 6 and 9 x 10⁵ cells/ml. Figure 1B shows the RRSC during the experiment. RRSC increases dramatically in the lag phase, then it is nearly constant for about 6 h during which period the cells multiply to about 8 x 10⁴ cells/ml, and thereafter there follows a steady decrease in RRSC during the rest of the experiment. This decrease continues into the stationary phase. Chemical determinations of RNA in parallel experiments show that the RNA contents of the cells indeed fluctuate with the rates of synthesis measured by pulse incorporation with radioactive uridine. This is so for all the experiments presented here. Thus we conclude from the results presented in Fig. 1 that the RRSC decreases when a cell density of approx. 8 x 10⁴ cells/ml is reached, although the cells multiply at the same rate as previously for at least 2 more doublings.

The results of Fig. 1 are not due to depletion of nutrients of the medium. Addition of extra proteose peptone and/or yeast extract (up to a final concentration of 1.5 % of
Fig. 1. Rate of RNA synthesis in an exponentially multiplying population. A, cell number in the population with the time. A dense population (3.5 × 10^6 cell/ml) was diluted 20-fold. After a lag period of about 3 h the cells multiply exponentially. B, the rate of RNA synthesis per cell (RRSC) at various times during the experiment. RRSC is expressed as cpm/cell in TCA-precipitated material after 20-min pulse labelling with ^14^C]uridine. It appears that the RRSC increases more than 6-fold during the first 3-4 h, then it stays more or less constant for about 6 h and thereafter decreases steadily during the rest of the experiment.
each) or bubbling air through the culture at the time when repression commences stimulates neither RNA synthesis, nor cell multiplication. Therefore the RRSC seems to depend on the cell density, and this is the case also in synchronously growing populations, as can be seen from the results presented in Fig. 2. A shows a typical growth curve for a population which has been treated with one heat shock per generation for 6 successive generations. After heat shock no. 6 the growth temperature was kept constant and 3 synchronized cell divisions followed ('free running' synchrony). In the first synchronous division the cell density increases from approx. 4 to $8 \times 10^4$ cells/ml. A similar increase in RRSC followed after the division, as shown in 8. However, after the second synchronous division (from approx. $8$ to $16 \times 10^4$ cells/ml) only a very low increase in RRSC was observed. When gene replication is prevented by addition of hydroxyurea (HU) prior to the second synchronous division, there is progressive repression of RNA synthesis from the second to the third synchronous cell division.

![Fig. 2. Rate of RNA synthesis in a synchronously multiplying population. A, cell multiplication. B, rate of RNA synthesis per cell at various times during the experiment. Addition of hydroxyurea (at arrow) prior to the second synchronous division blocks the gene replication and a decrease in RRSC is observed (x) at the time when the ribosomal genes are replicated. ○, control.](image)
RNA synthesis in Tetrahymena

The increase in RRSC induced in newly inoculated cells is not confined to the lag phase, but may take place also in dividing cells. The increase in RRSC and the duration of the lag phase depends on the conditions of the inoculated cells and the size of the inoculum. In general, a population inoculated with many cells from an actively growing population (< $10^6$ cells/ml) begins multiplication immediately or shortly after inoculation, and the increase in RRSC will be relatively low, whereas the increase in RRSC will be high and the lag period relatively long when few cells from a stationary population are transferred to a new culture flask. Therefore, the variations in RRSC shown in Figs. 1 and 2 are typical only for cells under identical conditions, not only in the actual populations, but also in the previous cultures from which the cells have been inoculated.
How can cell density influence the RRSC when the growth medium is in no respect exhausted? To ensure that the growth medium was in no way exhausted, we have in addition to providing extra proteose peptone and yeast extract, as mentioned above, also examined if the medium from a dense culture could support growth of a new population. The cells were removed from the dense population ($3 \times 10^5$ cells/ml) and new cells were inoculated on the medium. The new population grew at normal rate and reached normal stationary cell density. However, we observed that cells inoculated into such 'used' medium failed to show the high RRSC values which were found when identical cells were inoculated into fresh medium. This observation agrees with the
notion that the first population had formed factors which repress RRSC in the new population inoculated on that medium, and that such factors are without effect on cell multiplication. In other words, the cells seem to secrete factors which inhibit RNA synthesis. The results shown in Fig. 3 demonstrate that these factors are dialysable. A relatively dense population \((2.5 \times 10^6 \text{ cells/ml})\) was placed in a dialysis bag and dialysed for 4 h against an equal volume of normal medium (the recipient medium). Thereafter 2 populations were inoculated with cells from the same culture which contained \(2 \times 10^6 \text{ cells/ml}\). One culture was started on the recipient medium and the other on normal medium. The 2 populations were compared with respect to cell multiplication and RRSC. We found that cells on a medium which had been dialysed against a dense population would start cell divisions before the cells on the normal medium (Figs. 3, 4A). Furthermore, the rate of RNA synthesis per cell increased several-fold in the control (5-fold in Fig. 3B; cf. Fig. 1) whereas this high increase in RRSC failed to appear in cells on dialysed medium. Apart from these differences the 2 populations seemed to grow in parallel and entered stationary phase at the same time. Fig. 4 shows the rates of protein synthesis and the rates of DNA synthesis in 2 parallel populations and they appear to follow identical patterns. An increase in DNA replication is observed prior to the onset of the cell divisions, and the gene replication seems to be accompanied by an increase in RRSC, especially in the repressed population on dialysed medium (cf. Fig. 3B).

DISCUSSION

The results presented in the present paper strongly indicate that *Tetrahymena* cells secrete a dialysable factor which represses RNA synthesis in the population. Characterization of the factor, and analysis of its mechanisms of action are now in progress.

The chemical composition of cells multiplying exponentially is far from constant, and it seems hardly possible to cultivate these cells in batch cultures and obtain balanced growth. Thus the ratio between protein and RNA in the cells here studied varied within the range of 0.4 to 0.15 at the constant multiplication rate of about 0.4 doublings/h. If it is assumed that the efficiency of the ribosomes in protein synthesis is the same in cells of different composition it follows that *Tetrahymena* over a wide growth range has a high amount of inactive ribosomes. Furthermore, it means that the efficiency of the single ribosome in protein synthesis is higher than previously calculated by Leick (1973). He found that 6–7 amino acids were incorporated into protein per 80 s ribosome/s at the growth rate of about 0.4 doublings/h. With the same assumption as made by Leick our maximum rate is, however, about 11–12 amino acids/ribosome/s in cells with the lowest RNA/protein ratio we have observed. The discrepancies are easily explained by differences in growth conditions of the cells studied. As it is unlikely that we have obtained extreme values of the RNA/protein ratio under the growth characteristics used in the present study, the maximum rate of protein synthesis per ribosome may be even higher than here indicated.

If *Tetrahymena* cells are kept in very sparse populations, by continuous dilution of
the cells, the cells tend to accumulate RNA, and we have measured concentrations of RNA/cell which are about twice as great as the highest values obtained with cells growing at the population densities normally studied (10⁴–10⁶ cells/ml). Therefore, in cells where transcription is fully derepressed the amount of RNA/cell more than doubles in each cell generation, and it is probable that other regulatory mechanisms are controlling the chemical composition of the cells in such very diluted continuous-flow populations. Further studies will have to be done to elucidate these problems.

REFERENCES


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