THE DYNAMIC STATE OF *TETRAHYMENA PYRIFORMIS* CYTOSOL PROTEINS DURING CULTURE DEVELOPMENT

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1. Introduction

The dynamic state of cellular proteins has been demonstrated extensively in recent years [1–4]. The renewal of protein populations occurs apparently under strict control and appears to be linked to changes in physiological conditions of the cells [5–7]. Although the knowledge of the mechanisms of this regulation is far from being understood, it has become clear that the turnover of cellular proteins is a highly selective process [8]. In the past the belief has been that microorganisms proteins were stable during growth and became subject to a degradative metabolism only under resting conditions. This argument has been recently questioned. Experimental evidence is compatible with significant protein breakdown during growth [9,10].

The present paper deals with an experimental study of protein stability in cells of *Tetrahymena pyriformis* that initiate a new cultural growth-cycle. It is shown that under these conditions the proteins of higher molecular weight turnover at a relatively high rate, whereas those of small size accumulate.

2. Materials and methods

Tetrahymena pyriformis GL was cultivated in a medium consisting of 0.2% peptone, 0.2% yeast extract and 0.5% glucose as described [11]. Cells were collected from 100 ml cultures by centrifugation at 1000 × *g* for 2 min. In all subsequent steps the material was maintained at 2–4°C. Cells were washed 6–8 times with 10 mM succinate buffer, pH 6.7 and suspended in 20 ml lysing buffer (3 mM sodium succinate, pH 6.7; 3 mM $CaCl_2$; 0.5 mM $MgCl_2$; 0.5 mM KCl; 0.5 mM NaCl). After 10 min the cells were homogenized for 1 min in a Potter homogenizer provided with a Teflon-pestle. The preparation was centrifuged at 10 000 rev./min for 10 min. The supernatant was centrifuged at 100 000 × *g* for 70 min. The supernatant was concentrated to 1/10 vol. by cell filtration in a Diaflo System (Amicon), using a PM 10 membrane, with 3 changes of 100 mM Tris–HCl buffer, pH 7.5. The sample (SF) was treated and analysed by polyacrylamide electrophoresis, according to the procedure of Laemmli [12], under the following conditions: 12 cm separating-gel, 10% acrylamide and 2 cm stacking-gel 3% acrylamide in a glass tube 15 × 0.5 cm, 3 mA/tube, 12 h with cooling and recycling of buffer. The samples contained 100 μg protein/100 μl. The gels were stained with 0.2% Coomassie Brilliant Blue in methanol/acetic acid/water (45:10:45, v/v/v) and destained with 7.5% acetic acid. Densitometry measurements were performed with an ISCO scanning apparatus. A standard curve of molecular weights was obtained with the following proteins (all from Sigma Co.): bovine serum albumin, alpha-amylase, creatine phosphokinase, deoxyribonuclease, lysozyme and cytochrome *c*. For radioactivity measurements the gels were fractionated in a Savant gel crusher. Fractions of 1 mm were collected in vials containing 0.5 ml of water. The flasks were kept at 37°C overnight. Scintillation mixture made of toluene: Triton X-100/PPO (1000:2000: 12, v/v/w) in 10 ml was added to each flask. Radio-

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activity was determined in a Beckman LS/50 liquid scintilator, with efficiency of 98% for ^{14}C and 60% for ^{3}H.

3. Results and discussion

Figure 1 compares the electrophoretic patterns of SF obtained from stationary- and from lag-phase cells. Three broad peaks could be distinguished in the scanning of stationary-phase preparation (fig.1a), corresponding to proteins of higher (B_1), medium (B_2) and lower (B_3) molecular weight. The pattern was conserved in the preparation of lag-phase cells (fig.1b), but the proportion of the areas of the peaks changed. This is better appreciated in fig.1c in which the profiles of fig.1a and 1b were normalized with respect to B_1.

It can be seen that the relative amount of protein is increased in B_2 and more markedly in B_3. The change in B_2 was localized in the right edge of the peak. There was no significant modification in the height of B_1 and B_2. In contrast, the apex of B_3 was raised. There results suggest that either there was accumulation of the B_3 components or the proteins of B_1 and B_2 were degraded, or both events occurred. In order to have a more sensitive test for these findings, an experiment was performed using a procedure of continuous labelling, followed by chase. In this experiment cells were continuously labelled with [^{3}H]leucine (C.E.A France: 1 μCi/ml; 1 Ci/mol) during a growth-cycle (40 h) and sampled. The remaining cells were transferred to a new medium, free from the isotope, and after 3 h another sample was collected. The distribution of radioactivity in the electropherogram of each sample is shown in fig.2.

The general pattern of three broad groups of proteins, observed in the previous experiment, was apparent in each preparation. It can be seen that the chase led to a marked decrease of the radioactivity

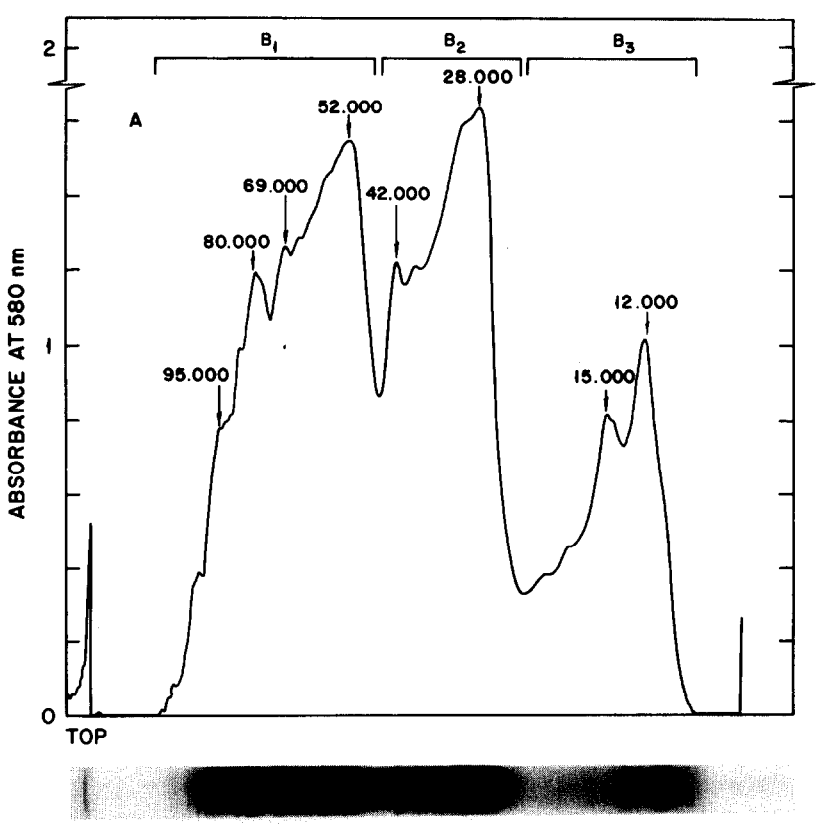

Fig.1a

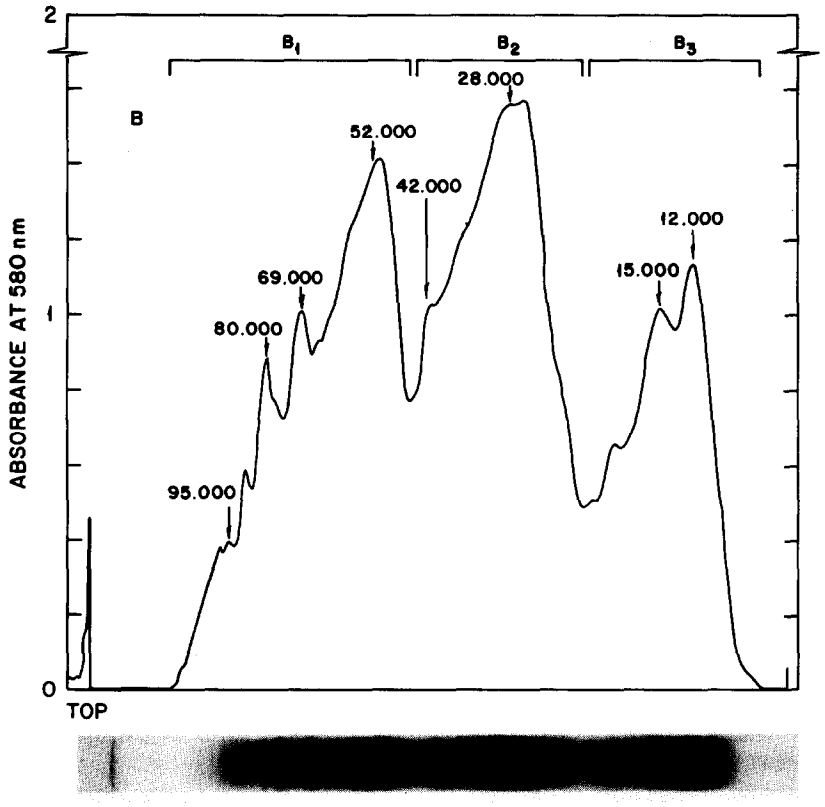

Fig.1b

of the B_1-group and somewhat less of B_2. In this latter peak the decrease is more conspicuous along the left edge. On the other hand the radioactivity of B_3 can be observed to increase discretely in some regions of the peak (fractions 82–85 and 89–94). This result shows that during the lag of 3 h a significant proportion of the proteins of B_1 and some of B_2 were degraded. The small increase in B_3 can indicate the utilization of radioactive amino acids coming from the degraded proteins.

A complementary information on the state of the proteins, under the experimental conditions adopted, can be obtained with the use of a double-label procedure. For an experiment of this kind the cells were labelled during a complete growth-cycle with [^{14}C] leucine (New England Nuclear: 0.5 μCi/ml; 305 mCi/mmol) and a sample of the culture was taken for the preparation of the soluble fraction. The cells of the remaining culture were washed in isotope-free medium and suspended in new medium containing ^{3}H leucine (C.E.A., France: 2.5 μCi/ml; 1 Ci/mmol). After 3 h the cells were collected for the preparation of the soluble fraction. Both preparations were analysed by electrophoresis.

In fig.3 the radioactive profiles corresponding to each isotope are compared. It is seen that, during the lag-phase, the proteins in each of the three main peaks were labelled to about the same extent. This result indicates that the rate of synthesis is of nearly equal magnitude for the different populations of protein molecules.

The data described support the conclusion that in cells that initiate a new growth-cycle there is a relatively high turnover of proteins of higher molecular weight and, to a lesser extent, of those of medium molecular weight. Proteins of small molecular weight are relatively stable. During the lag-phase the synthesis of protein is equivalent in each of the 3 groups. This condition leads to an accumulation of the proteins of low molecular weight and to a smaller scale, of those of medium weight. The fact that the proportions of B_1- and B_2-groups do not

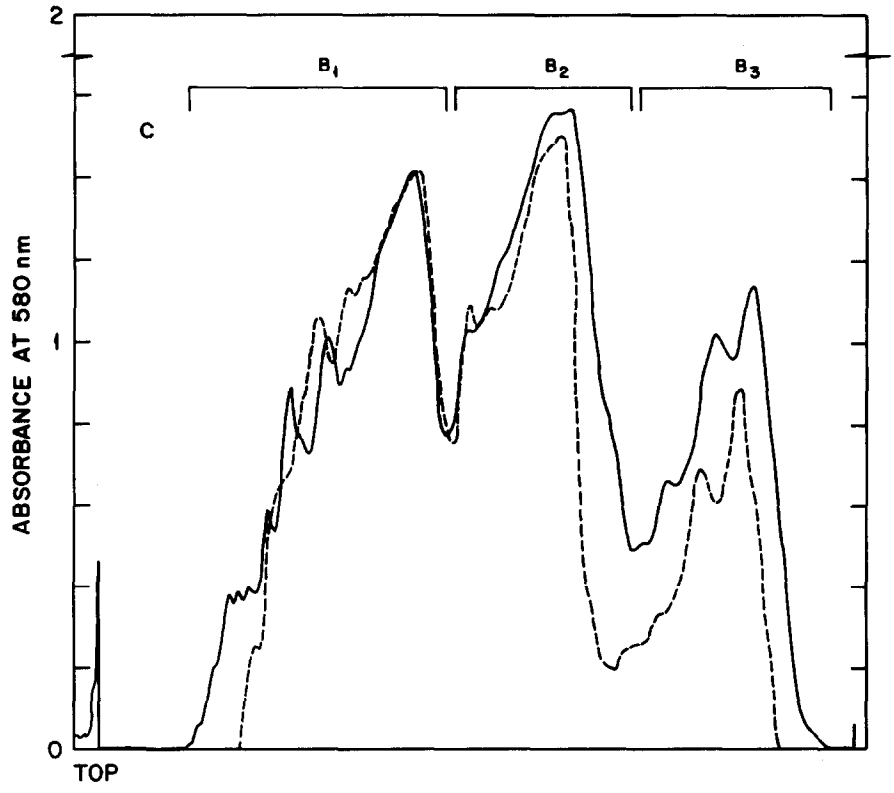

Fig.1c

Fig.1. Separation of the proteins of the cytosol of *Tetrahymena pyriformis* by electrophoresis on polyacrylamide gel. The figures above the arrows indicate the range of molecular weight of the protein population. For purposes of comparison a picture of the gel is included. (a) Stationary-phase, (b) lag-phase, (c) A and B profiles normalized with respect to peak B_1. (——) Stationary-phase, (– –) lag-phase.

change appreciably from stationary to lag conditions suggests that during this time most of the proteins degraded in B_1 are resynthesized.

The rate of degradation of different cellular proteins has been shown to vary widely [9]. Dehlinger and Shimke [13] were the first to call the attention to the fact that proteins of higher molecular weight are more rapidly degraded than smaller ones. This condition has been shown to prevail in a variety of tissues [14–17]. So far, however, no evidence has been presented for a correlation between molecular size and the increase in prótein degradation known to

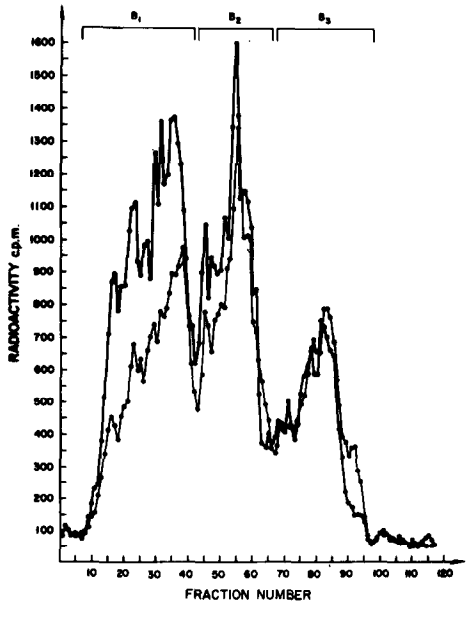

Fig.2. Radioactivity profiles of the cytosol proteins of *Tetrahymena pyriformis* cells labelled continuously with [^{3}H] leucine (1 μCi/ml; 1 Ci/mmol). (•) Stationary-phase, (○) after 3 h chase.

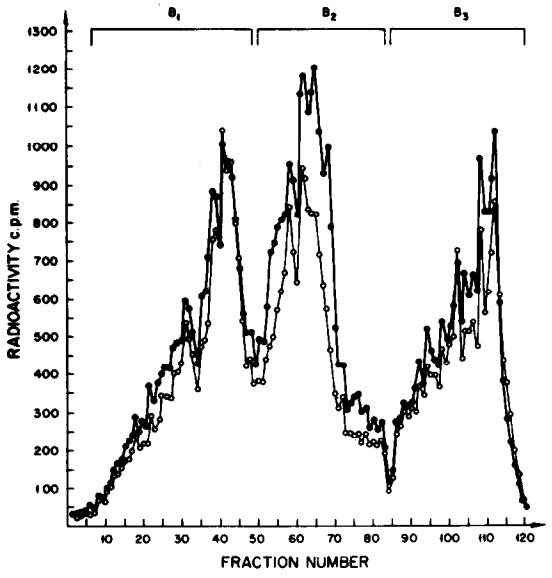

Fig.3. Radioactivity profile of the cytosol proteins of *Tetrahymena pyriformis*. (•) [^{14}C]Leucine, continuous labelling (stationary-phase). (○) [^{3}H]Leucine, pulse labelling (lag-phase).

occur under certain physiological conditions of the cell [1].

The results here presented show that the change in the physiological state of *Tetrahymena* cells is accompanied by a measurable degradation of the proteins of the cytosol, predominantly of the heavier ones. The concomitant synthesis seems to attain the generality of the soluble protein populations. The net result is a partial renewal of the heavier proteins and accumulation of the lighter ones.

The meaning of the present results, as of protein turnover in general, remains open to speculation. The degradation and accumulation of proteins reported in the present paper involve whole heterogeneous populations of molecules. It seems thus unlikely that a regulatory mechanism, concerned with adjustment of enzyme levels, would account for the changes observed. Although the occurrence of single regulatory changes, in any of the groups of proteins present in the electropherograms is clearly admissible, the whole regulatory pattern observed suggests the existence of a more general regulation operating in the control of protein turnover.

Acknowledgements

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References

[1] Goldberg, A. L. and Dice, J. F. (1974) Ann. Rev. Biochem. 43, 835–869.
[2] Schimke, R. T. (1973) Adv. Enzymol. 37, 135–148.
[3] Pine, M. J. (1972) Ann. Rev. Microbiol. 26, 103–126.
[4] Pine, M. J. (1965) Biochem. Biophys. Acta 104, 439–456.
[5] Mandelstam, J. (1969) J. Symp. Soc. Gen. Microbiol. 19, 377–402.
[6] Sussman, M. and Sussman, R. R. (1969) Symp. Soc. Gen. Microbiol. 19, 403–435.
[7] Millward, S. J. (1970) Clin. Sci. 39, 591–603.
[8] Goldberg, A. L., Olden, K. and Prout, W. F. (1973) in: Intracellular Protein Turnover (Schimke, R. T. and Katunuma, N. eds) pp. 17–55, Academic Press, New York.
[9] Pine, M. J. (1966) J. Bacteriol. 92, 847–850.
[10] Nath, K. and Koch, A. L. (1971) J. Biol. Chem. 246, 6956–6957.
[11] De Castro., Jacy, F., Carvalho, J. F. O., Moussatché, N. and De Castro, F. T. (1975) Antimicrob. Agents Chemother. 7, 487–493.
[12] Laemmli, U. K. (1970) Nature 227, 680–685.
[13] Dehlinger, P. J. and Schimke, R. T. (1971) Biochem. Biophys. Res. Comm. 40, 1473–1479.
[14] Dice, J. F. and Schimke, R. T. (1972) J. Biol. Chem. 247, 98–111.
[15] Tweto, J., Dehlinger, P. J. and Larrabee, A. R. (1972) Biochem. Biophys. Res. Comm. 48, 1371–1374.
[16] Dehlinger, P. J. and Schimke, R. T. (1971) J. Biol. Chem. 246, 2574–2583.
[17] Dice, J. F. and Schimke, R. T. (1973) Arch. Biochem. Biophys. 158, 97–105.