Putrescine and Spermidine Changes in Heat-synchronized Tetrahymena Populations

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Putrescine and spermidine were the dominant polyamines in Tetrahymena cells. Spermine was present only in very small amounts. Changes in the amount of putrescine during and after the synchronizing heat treatment followed a definite quantitative pattern. Changes in the amount of spermidine were similar, though not so pronounced.

The changes may be due to a varied influx of polyamines into the cells. Moreover, the exchange of polyamines between the cells and the culture medium must be specifically regulated since there seems to be a relatively greater accumulation of spermidine than of putrescine in the cells, and since spermine was almost excluded from the cells.

Putrescine, spermidine and spermine have been found in all cells so far examined. Spermidine and spermine are dominant in animal cells, while putrescine and spermidine are the most frequent of these amines in bacteria. In most cases, these amines are referred to as polyamines, although putrescine is a diamine. Their postulated role as stabilizers of nucleic acids has attracted much attention. In vitro experiments have yielded evidence that polyamines compete with metal ions at the organization of ribosomes (for references see STEVENS) as well as with histones at nucleoprotein formation.

The presence of putrescine, spermidine and spermine in Tetrahymena cells has earlier been established by WELLER, RAINA and JOHNSTONE, and ARLOCK, HEBY and HOLM. These observations are confirmed in the present paper. The high amounts of polyamines in the cell suggest that they may participate in ionic regulation. The polyamine content during and after heat treatment changes inversely with the amount of bound Mg and in parallel with the stability of DNA previously registered.

Material and Methods

Experimental cultivation and sampling: The ciliate Tetrahymena pyriformis, amicronucleate strain GL, was cultured at 28 °C in Fernbach flasks containing 500 ml medium, which consisted of 2 per cent proteose peptone (Difco), 0.3 per cent yeast extract (Difco) and inorganic salts in the following amounts per 1: 10 g MgSO₄ x 7H₂O, 0.5 g CaCl₂ x 2H₂O, 7.5 g CaCl₂ x 6H₂O, 0.125 g FeCl₃ x 6H₂O, 0.05 g MnCl₂ x 4H₂O, 6 mg ZnAc x 2H₂O, 2.5 g Fe(NH₄)₂(SO₄)₆ x 6H₂O.

After inoculation of each flask with 5 ml of 2-days old stock cultures, and subsequent exponential growth for 14 hours, the cultures were synchronized by 7 heat shocks at 34 °C, lasting 30 min each, during a total period of 7 hours, according to SCHEERBAUM and ZEUTHEN. The cultures were mixed and distributed again during the interval at 28 °C between the fourth and fifth shocks. 200 ml samples were withdrawn, two at a time, at appropriate intervals during the heat treatment, and the subsequent period up to and including the second synchronous division. The samples were centrifuged and the cells were rapidly washed in 0.4 per cent NaCl, frozen and stored at —20 °C. Small samples (2 x 1 ml) were removed from each 200 ml sample before centrifugation and the number of cells per ml was counted in a Celloscope. The times and the degree of synchronous divisions were established by calculating the maximum values of the ratio between the number of cells in division and the total number of cells.

Preparation and analysis of polyamines: Frozen cell samples of 2.0—2.5 ml volume were homogenized in ice-cold 0.9 per cent NaCl with a Teflon homogenizer. The proteins in the homogenates were precipitated by addition of ice-cold trichloroacetic acid and removed by filtration. The supernatants were extracted three times with diethyl ether to remove the acid, and the amines were then extracted into n-butanol according
to RAINA. The n-butanol layer was acidified and lyophilized. The residues were dissolved in 0.1 M HCl and stored in small test-tubes until analysis.

The polyamines were separated by thin-layer chromatography according to HAMMOND and HERBST. 5 μl samples were applied on thin-layer plates coated with Whatman Cellulose CC 41. The solvent system used was ethylene glycol monomethyl ether : propionic acid : water (70:15:15) saturated with NaCl. The solvent was removed by heating and the plates immersed in a 1 per cent solution of ninhydrin in absolute ethanol containing 1 per cent 2,4,6-trimethylpyridine. The plates were developed at 60 °C for 15 minutes and the coloured spots were scraped off and aspirated quantitatively into 70 per cent ethanol. When the colour had been eluted, the cellulose powder was sedimented by centrifugation. The absorbance of polyamines at 575 nm was used for the quantitative determinations.

Standard solutions of spermine tetrahydrochloride, spermidine trihydrochloride and putrescine dihydrochloride (Calbiochem) were included in duplicate on each thin-layer plate.

Results

Of the polyamines, putrescine and spermidine appeared in the highest amounts in the Tetrahymena cells. Spermine was present, but only in very small amounts. Cadaverine, which had earlier been obtained, could not be detected by the present method.

The changes in the amounts of intracellular putrescine between the fourth shock and the second synchronous division are shown in Fig. 1. The peak during the last shock is statistically significant (f = 10, t = 3.38 and P<0.01) as is the increase from EH (EH = end of heat treatment) to 60 minutes after EH (f = 10, t = 3.49 and P<0.01). The peak between the synchronous divisions is also significant (f = 5, t = 3.05 and P<0.05). The changes in spermidine content are not so pronounced (Fig. 1). The peak at 60 minutes after EH is, however, significant (f = 10, t = 2.13 and P<0.05). The intracellular amount of putrescine exceeded that of spermidine by more than a factor of 2.

The amounts of putrescine and spermidine in the heat-synchronized cell cultures at EH were about three times higher than in exponentially growing cultures. The latter contained 21.7±1.8 nmoles putrescine and 6.8±0.4 nmoles spermidine per million cells. The pure medium was found to contain 180 nmoles putrescine, 47 nmoles spermidine and 40 nmoles spermine per ml respectively. These values were compared with the polyamine concentration in the Tetrahymena cells. By estimating the cell volume at EH to be 70 μl per million cells, according to ZEUTHEN and SCHERBAUM, it was calculated that the approximate concentration of putrescine and spermidine in the cells was 5 and 8 times higher, respectively, than in the medium.

Discussion

The variation in the amount of putrescine in heat-synchronized Tetrahymena cultures closely parallels the change in the uptake of 3H-putrescine from the medium. As there are polyamines in the sur-

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rounding medium, it seems reasonable to assume that part of the increase may be due to an increased influx into the cells. Results earlier obtained suggest a very low or non-existent spermidine synthesis in *Tetrahymena*; no $^3$H-putrescine was found to be transformed to $^3$H-spermidine \(^13\). An endogenous production of putrescine in *Tetrahymena* cannot be confirmed until it has been shown that possible precursors such as arginine and ornithine are incorporated into putrescine.

The exchange of polyamines between the cells and the surrounding medium must be specifically regulated since there seems to be a relatively greater accumulation of spermidine than of putrescine in the cells. Spermine was almost excluded from the cells. The uptake and extrusion of polyamines by *Tetrahymena* is probably involved in those mechanisms, which have been proposed to actively regulate the intracellular content of different ions, amino acids and other organic compounds \(^14\).

The intracellular amounts of polyamines increase during those phases which are characterized by an increase in the stability of DNA \(^7\). This is in line with the generally accepted role of polyamines as physiological stabilizers of nucleic acids. It has been shown in vitro that the efficiency with which some relevant ions stabilize calf thymus DNA, determined as the increase in the melting point ($T_m$), is dependent on their respective concentrations. 5 times more Na is required to equal a certain concentration of putrescine, 3 times more putrescine to equal Mg and 7 times more Mg to equal spermidine with regard to the binding efficiency to DNA in vitro \(^15\). These simple relationships between some cations may not be relevant under the more complicated conditions in vivo. However, the magnitude of the changes in the ion content of heat-synchronized *Tetrahymena* suggests that the variations in polyamine content must be taken into account. During the seventh shock the amount of bound Mg increased by about 25 nmoles per million cells while the putrescine content decreased by about 40 nmoles per million cells. During the same period the influx of Na reached a maximum, which, however, was compensated by a simultaneous outflux of K. There was also an increase in Ca amounting to about 100 nmoles per million cells \(^8\).

The extensive changes in the amounts of intracellular ions, including putrescine and spermidine, must greatly affect the synthesis of RNA and protein. There is a parallelism between ribosomal RNA and polyamine changes in heat-treated cells. This is obvious when the present curves (Fig. 1) are combined with those of Christensson \(^16\). This is in line with results obtained in other cells \(^1\). Raina and Jänne \(^17\) have shown that putrescine and spermidine stimulate the activity of nucleolar RNA polymerase in rat liver nuclei. The polyamines may also prevent the degradation of newly synthesized RNA \(^17\). Petersen and Kröger found that both spermidine and monovalent salts were necessary for maximal RNA synthesis \(^18\).

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