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The Relationship between the Synthesis of Protein and the Synthesis of Ribonucleic Acid in the Connective-Tissue Cell

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In a previous paper (Harris, 1959) the use of quantitative radioautographic techniques for the study of ribonucleic acid turnover in the nucleus and cytoplasm of animal cells was described. These techniques were used in the present experiments to examine some aspects of the relationship between the synthesis of ribonucleic acid and the synthesis of protein in the connective-tissue cell of the rat. The synthesis of protein was measured by the incorporation of tritium-labelled valine and methionine, the synthesis of ribonucleic acid by the incorporation of tritium-labelled adenosine and cytidine. The cells were cultivated in media containing β -2-thienylalanine and 8-azaguanine and the effect of these compounds on the rates of synthesis of both nuclear and cytoplasmic ribonucleic acid and protein were measured.

EXPERIMENTAL

The connective-tissue cells were obtained from the hearts of 5-day-old rats and were cultivated as dissociated cells in small coverslip chambers (Harris, 1955a, b, 1956). The

culture medium consisted of 80% (v/v) of Eagle's soln. (Eagle, Oyama, Levy, Horton & Fleischman, 1956) and 20% (v/v) of bovine serum which had been dialysed for 18 hr. against an excess of 0.85% NaCl soln. When β -2-thienylalanine was added to the medium, phenylalanine was omitted from the Eagle's soln. The β -2-thienylalanine was added to make a final concentration of 5 mM, the 8-azaguanine to make a final concentration of 2 mM.

The tritium-labelled adenosine, cytidine, thymidine and valine were used at a concentration of 5 μ Ci/ml. of medium; the tritium-labelled methionine was used at a concentration of 1 μ Ci/ml. The tritium-labelled adenosine, cytidine and thymidine were obtained from Schwarz Laboratories Inc., Mt. Vernon, N.Y., U.S.A., and had specific activities of 1.0, 0.36 and 1.9 Ci/m-mole respectively. These compounds were labelled in the purine or pyrimidine ring. Tritium-labelled methionine was obtained from The Radiochemical Centre, Amersham, Bucks.; this compound was generally labelled and had a specific activity of 28 mCi/m-mole. Tritium-labelled valine was prepared by an exchange reaction between tritium gas and the vapour of the ethyl ester of valine on a platinized platinum electrode at 50°. The tritium gas was obtained from the Atomic Energy Research Establishment, Harwell, and contained a maximum of 95% of tritium in hydrogen. The

(a)

(b)

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method used was essentially that described by Farkas & Farkas (1937). The tritium-labelled ester was hydrolysed and the valine was isolated and purified by paper chromatography. The specific activity of the purified valine was 1.5 c/m-mole. All the tritium-labelled compounds used were free of readily exchangeable tritium.

The technique of preparing radioautographs of the fixed cells and of measuring by grain counts the amount of radioactive precursor incorporated into the nucleus and the cytoplasm has been described (Harris, 1959). Cells not in the phase of deoxyribonucleic acid (DNA) synthesis were selected for study; these could be distinguished from those in the phase of DNA synthesis by their failure to incorporate tritium-labelled thymidine (Harris, 1959). In cells not in the phase of DNA synthesis labelled adenosine or cytidine which was incorporated into material insoluble in 0.3N-trichloroacetic acid at 0° was considered to have been incorporated into ribonucleic acid (RNA). Labelled valine or methionine which was incorporated into material insoluble in 0.3N-trichloroacetic acid at 90° was considered to have been incorporated into protein (Harris & Watts, 1958).

Fourteen chambers containing comparable populations of cells were used for each experiment. Pre-warmed medium containing tritium-labelled adenosine and thymidine was introduced into one chamber, and similar medium containing tritium-labelled valine and thymidine into another. The cells were incubated in the radioactive media for 20 min. and then fixed in methanol. Medium containing β -2-thienylalanine was then introduced into the other 12 chambers. At intervals of 2 hr. medium containing thienylalanine together with tritium-labelled adenosine and thymidine was introduced into one chamber and similar medium containing tritium-labelled valine and thymidine into another; the cells were again fixed after incubation for 20 min. The fixed cells were extracted with 0.3N-trichloroacetic acid at 0°, washed under running water and then dried. Radioautographs of the preparations were made by the technique previously described, and the cells were stained through the emulsion with nuclear fast red (Harris, 1959). Similar experiments were carried out with tritium-labelled cytidine and methionine, and the whole series was repeated with 8-azaguanine instead of β -2-thienylalanine.

The radioautographs were exposed for periods from 4 to 16 days, depending on the nature and specific activity of the radioactive precursor. The exposure time was adjusted

to give grain densities suitable for counting. Forty cells not in the phase of DNA synthesis were selected in each preparation and the number of grains over the nucleus and the cytoplasm counted. It had previously been shown that with a sample of 40 cells, the standard error of the mean number of grains over the nucleus or the cytoplasm was less than 10% of the mean (Harris, 1959).

The number of grains over each cell was a measure of the amount of radioactive precursor incorporated during the 20 min. period of incubation. Differences in the number of grains over the nucleus or the cytoplasm from one 20 min. period to another thus reflected changes in the rate of incorporation of the precursor into the nucleus or cytoplasm. Since the specific activities of the precursors and the length of exposure of the radioautographs varied, no comparison could be made between the absolute rates of incorporation of one precursor and another; but changes in the rate of incorporation of one precursor could be compared with changes in the rate of incorporation of another. It was therefore possible to measure the effects of β -2-thienylalanine and 8-azaguanine on the rate of incorporation of precursors into RNA and compare these with similar measurements on the rate of incorporation of precursors into protein. Since only changes in rate were compared, the results were plotted on an arbitrary logarithmic scale with all the controls, that is, the rates at which the precursors were incorporated in the absence of thienylalanine or azaguanine, set at the same level.

RESULTS

Incorporation of precursors into protein and ribonucleic acid

Plate 3 (a) shows a radioautograph of a cell, not in the phase of DNA synthesis, which had been incubated for 20 min. in medium containing tritium-labelled adenosine. Plate 3 (b) shows a radioautograph of a similar cell which had been incubated for 20 min. in medium containing tritium-labelled valine. In the cell exposed to tritium-labelled adenosine the nucleus is more heavily labelled than the cytoplasm, indicating that more adenosine was incorporated into nuclear RNA during the 20 min. period than into cytoplasmic RNA (Harris, 1959). In the cell exposed to tritium-labelled valine, the nucleus as a whole is not more heavily labelled than the cytoplasm, although there is rather heavy labelling over the nucleoli. Grain counts indicate that the ratio of the amount of adenosine incorporated into nuclear RNA to the amount incorporated into cytoplasmic RNA is about 6:4; the ratio of the amount of valine incorporated into nuclear protein to the amount incorporated into cytoplasmic protein is about 1:4. With tritium-labelled cytidine the distribution of radioactivity between nucleus and cytoplasm was essentially similar to that obtained with labelled adenosine, although, as mentioned in the previous paper (Harris, 1959), the nucleoli were more heavily labelled with cytidine than with adenosine. The

EXPLANATION OF PLATE 3

- (a) Radioautograph of a connective-tissue cell, not in the phase of DNA synthesis, which was incubated for 20 min. in medium containing tritium-labelled adenosine. The nucleus is more heavily labelled than the cytoplasm.
- (b) Radioautograph of a connective-tissue cell, not in the phase of DNA synthesis, which was incubated for 20 min. in medium containing tritium-labelled valine. The nucleus as a whole is not more heavily labelled than the cytoplasm, although there is rather heavy labelling over the nucleoli.

For purposes of illustration these radioautographs were exposed for a longer period than was suitable for grain counting. Magnification $\times 1400$.

results with labelled methionine were similar to those with labelled valine. It is therefore clear that there is no simple relationship between the rate of incorporation of nucleosides into RNA and the rate of incorporation of amino acids into protein, which holds good for both nucleus and cytoplasm: the ratio of the amount of nucleoside incorporated to the amount of amino acid incorporated is about six times as high in the nucleus as in the cytoplasm.

Effect of β -2-thienylalanine

Over a period of 12 hr., which is less than half the mean generation time of the connective-tissue cells grown under the present conditions, 5 mM- β -2-thienylalanine produced no change in the rate of incorporation of labelled valine or labelled methionine into nuclear or cytoplasmic protein. But the incorporation of labelled adenosine and cytidine into cytoplasmic RNA was rapidly suppressed, and there was a gradual fall in the rate of incorporation of these compounds into nuclear RNA. At the end of 12 hr. the rate of cytoplasmic RNA synthesis, as measured by incorporation of nucleosides, had fallen to about 10% of the control value and the rate of nuclear RNA synthesis to about 50% of the control value. These results are shown in Fig. 1.

Fig. 1. Effect of 5 mM- β -2-thienylalanine on the rate of incorporation of tritium-labelled adenosine into RNA and tritium-labelled valine into protein. ▲, Incorporation of adenosine into nuclear RNA; ●, incorporation of adenosine into cytoplasmic RNA; △, incorporation of valine into nuclear protein; ○, incorporation of valine into cytoplasmic protein.

Effect of 8-azaguanine

This is shown in Fig. 2. The results were essentially similar to those obtained with thienylalanine, although with azaguanine there was some inhibition of protein synthesis. The fall in the rate of protein synthesis was not large, and, within the limits of experimental error, there was no difference between nucleus and cytoplasm. The rate of cytoplasmic RNA synthesis fell much more rapidly than the rate of nuclear RNA synthesis: at the end of 12 hr. the former was about 10% of the control value, the latter about 50%. The results obtained with cytidine and methionine were again similar to those obtained with adenosine and valine.

DISCUSSION

The present findings show that there is no mandatory coupling between the synthesis of protein and the synthesis of ribonucleic acid, at least in so far as mandatory coupling implies a constant relationship between the overall rate of the one process and the overall rate of the other. It is possible to reduce the rate of RNA synthesis in the cytoplasm to about 10% of the normal value, and in the nucleus to about 50% of the normal value, without affecting the rate of protein synthesis. These

Fig. 2. Effect of 2 mM-8-azaguanine on the rate of incorporation of tritium-labelled adenosine into RNA and tritium-labelled valine into protein. ▲, Incorporation of adenosine into nuclear RNA; ●, incorporation of adenosine into cytoplasmic RNA; △, incorporation of valine into nuclear protein; ○, incorporation of valine into cytoplasmic protein.

results do not, however, exclude the possibility that there might be coupling between the synthesis of certain proteins and the synthesis of some particular fraction of the ribonucleic acid.

Whether protein synthesis can be suppressed without affecting RNA synthesis remains undecided, since the amino acid analogue, somewhat paradoxically, inhibited RNA synthesis much more readily than protein synthesis. Both thienylalanine and azaguanine suppressed cytoplasmic RNA synthesis more rapidly than nuclear RNA synthesis, thus indicating that there is no rigid coupling between these two processes either. No dissociation of nuclear from cytoplasmic protein synthesis was observed: thienylalanine produced no change in the rate of either nuclear or cytoplasmic synthesis over 12 hr., and azaguanine reduced the rate of synthesis in nucleus and cytoplasm to about the same extent.

SUMMARY

1. Radioautographic methods were used to study the effect of β -2-thienylalanine and 8-azaguanine on the synthesis of protein and ribonucleic acid in the nucleus and cytoplasm of the rat connective-tissue cell.

2. With β -2-thienylalanine it was possible to

reduce the rate of ribonucleic acid synthesis in the cytoplasm to about 10% of the normal value, and in the nucleus to about 50% of the normal value, without affecting either nuclear or cytoplasmic protein synthesis.

3. With both β -2-thienylalanine and 8-azaguanine, cytoplasmic ribonucleic acid synthesis was much more rapidly suppressed than nuclear ribonucleic acid synthesis.

4. The results indicate that there is no mandatory coupling between the synthesis of ribonucleic acid and the synthesis of protein, as measured by the overall rates of the two processes, either in the nucleus or in the cytoplasm.

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The Purification of Human Caeruloplasmin

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Almost all of the copper in plasma is bound to a single protein. Holmberg & Laurell (1948) isolated a cuproprotein from porcine and human plasma, and because of its blue colour they called it caeruloplasmin. It had a molecular weight of about 151 000 and contained 0.34% of copper, which corresponded to eight atoms of copper per molecule. Like a number of other copper-proteins, it is an enzyme, having oxidase activity, though its physiological substrates, if any, are unknown. In Holmberg & Laurell's method of preparation (1948) caeruloplasmin was precipitated from serum with the globulin fraction by ammonium sulphate, dissolved in water and dialysed. Some impurities were precipitated at pH 6.2 and 5.5 and other impurities by two treatments with 50% alcohol-chloroform at room temperature; caeruloplasmin

was extracted with sodium chloride solution from the resulting precipitate, which was mostly denatured protein. Details of percentage yields were not given. Scheinberg & Morell (1957) used caeruloplasmin prepared from Cohn's fraction IV by methanol-chloroform fractionation, but details of the method were not given. Most published work on caeruloplasmin has been related to various disease processes. There is little published on the structure and properties of caeruloplasmin, mainly because of the labour and difficulties in preparing purified material in sufficient quantity. Recent interest in caeruloplasmin has been concerned with its deficiency in hepatolenticular degeneration (reviewed by Cumings, 1959) and its possible significance in mental disease (e.g. Melander, 1960).