DYNAMICS OF THE RIBOSOMAL RNA CONTENT AND GROWTH OF SYNCHRONIZED CHINESE HAMSTER CELLS

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Changes in the ribosomal RNA (rRNA) content, the dimensions of the cells and the nucleo-cytoplasmic ratio were studied throughout the various periods of interphase in a synchronized culture of Chinese hamster cells. Cytophotometric measurements showed a gradual increase in the rRNA content during interphase, reaching a maximum in the G_2-period. The dimensions of the cells and their nuclei showed parallel increases. The nucleo-cytoplasmic ratio decreased in the G_1-period, it increased at the end of the S-period, and decreased again in the G_2-period.

Although equal attention has been paid in recent years to interphase as to mitosis, most workers have concentrated their efforts on the study of the duration and variation in its component periods [2, 13]. The cytochemical features of interphase (except for the S-period) have received much less study [8]. Information is even more limited on the interphase growth of cells and most of it has been obtained by the study of protozoa [6, 9].

With these facts in mind it was decided to study changes in the content of ribosomal RNA (rRNA) and in the dimensions of the nucleus and cytoplasm between two cell divisions.

EXPERIMENTAL METHOD

A synchronized culture of Chinese hamster cells (strain B11düFAF-28, line 237) was used. Synchronization of the cells was carried out in the exponential stage of growth by selective sampling of cells blocked with colcemid (0.04 μg/ml) in metaphase [4]. Preliminary tests with thymidine-H^3 and analysis of the mitotic index enabled the duration of interphase and of its various periods to be determined (G_1 4-5 h, S 6-7 h, G_2 1-1.5 h). Material was fixed in Carnoy's fluid (10 min at 4°C) every h throughout interphase. Three or four slides were fixed simultaneously at each time. From 50 to 60 cells were studied in each preparation. To determine the RNA content the slides were stained with gallocyanin and chrome alum by Einarson's method. After staining, the cells were tagged and the RNA concentration in the cytoplasm was determined with a probe cytophotometer by the plug method ($\lambda = 550$ nm). The cells themselves were then photographed. The area of the nuclei and of the cells was determined on the outlines obtained from the negatives by means of a planimeter. The content of the complex of RNA with gallocyanin and chrome alum in the cytoplasm was calculated as the product of optical density and area of cytoplasm. At the end of interphase partial desynchronization of the population usually takes place, and at that time besides cells in the G_2-period there are other cells exhibiting late replication of DNA. To exclude these, thymidine-H^3 (4 μCi/ml, specific activity, 3.7 Ci/mmole) or thymidine-C^{14} (1 μCi/ml, specific activity 0.2 Ci/mmole) was added at the end of interphase. After cytometric measurements, the preparations were coated with type M emulsion and exposed for 5 (thymidine-H^3) or 20 (thymidine-C^{14}) days. By analysis of the tagged cells on these slides, cells in the S or G_2 stage could be differentiated. The experiments were repeated three times. Student's criterion was used for the statistical analysis of the data.

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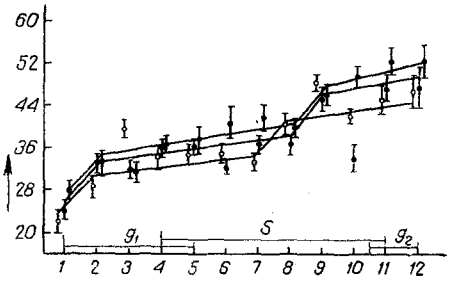

Fig. 1

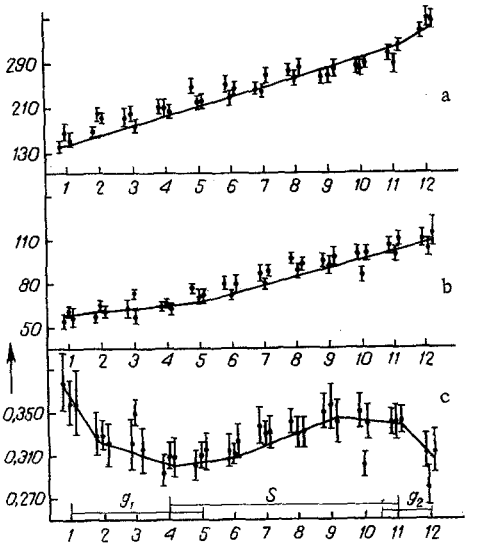

Fig. 2

Fig. 1. Changes in the RNA content in the cytoplasm of synchronized Chinese hamster cells in interphase. Abscissa, time from beginning of interphase (in h); ordinate, RNA content (in conventional units).

Fig. 2. Changes in area of cells (a) and their nuclei (b) and in the nucleocytoplasmic ratio (c) during interphase of synchronized Chinese hamster cells. Abscissa, time after beginning of interphase (in h); ordinate: in a) and b) area of cells (S_c) and nuclei (S_n) (in conventional units), in c) ratio S_n/S_c.

EXPERIMENTAL RESULTS

The method used to determine RNA enabled the dynamics of rRNA accumulation in the cytoplasm of the Chinese hamster cells to be studied. In all three experiments similar changes were found in rRNA in interphase. It will be clear from Fig. 1 that after the cells had escaped from the colcemid block a gradual increase in the RNA content took place, to reach a maximum at the end of the G_2-period. Experiments have shown [14] that 10-15 min is required for newly synthesized RNA to leave the nucleus into the cytoplasm, and for that reason the small increase in the rRNA content in the cytoplasm during the first few hours after escape from the colcemid block points to intensification of the synthesis and transport of rRNA after mitosis. The second considerable increase in the rRNA content begins at the middle of the S-period 7-8 h after mitosis. A general tendency was observed for the rRNA content to be approximately doubled (2, 1.95, and 2.1 times) in the cytoplasm of the cells at the G_2 stage compared with G_1. The cytophotometric data also showed that the RNA concentration is maintained at a constant level at the beginning and end of interphase. In all three experiments there was only a small decrease in optical density toward the middle of the S-period. Doubling of the rRNA content in the cytoplasm took place simultaneously with an increase in the size of the cell (Fig. 2a). The results are in good agreement with those obtained by other workers [10, 11]. Intensification of the incorporation of uridine-H^3 was observed in the last few hours before mitosis in autoradiographic investigations [12]. The increase in the RNA content observed in the present experiments in the second half of interphase was probably connected with replication of the DNA cistrons responsible for rRNA synthesis at that moment [12, 15]. Correlation between DNA replication and the intensification of rRNA synthesis has also been demonstrated by the nucleic acid hybridization method [7]. Autoradiographic observations in the writer's laboratory have shown that the period of accumulation of rRNA in the cytoplasm is preceded by an increase in the incorporation of uridine-H^3 into the nuclei of Chinese hamster cells, where the synthesis of ribosomal precursors is known to take place. The increase in the RNA content at the end of interphase corresponds to the observed increase in the intensity of protein synthesis in that period of the cell cycle [3], for the number of ribosomes is known to be one of the factors limiting protein synthesis.

In a normally growing cell population a balance is maintained between growth and reproduction. In this connection it is interesting to study the changes in the size of the cell as an index of growth during interphase. During the period of the cell cycle studied the area of the cells was approximately doubled

(Fig. 2a); during the G_1- and S-periods the increase in area followed a linear course, while in the G_2-period there was a sharp increase in the size of the cell. It must be accepted, in view of the many observations showing that the cell volume doubles in interphase [6], that the height increases only very slightly in the cells studied from one division to the next. Whatever the case, the marked increase in size of the cell observed in the G_2-period can evidently be explained by an intensive increase in the protein content in the cytoplasm at this time. The karyometric data showed (Fig. 2b) only very slight changes in the size of the nucleus in the G_2-period. During the S- and G_2-periods the area of the nucleus increased by about 1.5 times. This increase was probably due not so much to doubling of the DNA content as to an increase in the content of nonchromosomal protein in the nucleus, for after the beginning of the S-period protein transport from the cytoplasm into the nucleus is accelerated [17].

According to Hertwig's hypothesis, the difference in growth of the nucleus and cytoplasm during interphase gives rise to "critical stress" which stimulates cell division. However, subsequent investigations have shown that growth of the cell has only an indirect effect on mitosis, by inducing DNA synthesis, for example [8, 10, 16]. The particular features of the increase in size of the cytoplasm and nucleus vary in different cells [1, 6], evidently because of changes in the direction and intensity of protein and RNA migrations between the nucleus and cytoplasm in the course of the mitotic cycle. Analysis of the ratio between the area of the nucleus and area of the cell in culture showed (Fig. 2c) that during the G_1-period cytoplasmic growth and accumulation of protein in the cytoplasm to nucleus is intensified (the ratio S_n/S_c becomes stabilized), and at the end of the period of DNA synthesis growth of the nucleus is rather faster than growth of the cell (the ratio S_n/S_c rises). At the end of interphase, however, cytoplasmic growth is intensified, with the result that S_n/S_c falls.

During interphase regular changes thus take place in the ratio between the areas of nucleus and cytoplasm, reflecting the growth patterns of the principal parts of the cell. The rRNA accumulating intensively in the cell cytoplasm may possibly participate in the building of the mitotic apparatus [1, 5] and also in synthetic processes connected with cell growth.

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