DYNAMICS OF URIDINE-H^3 INCORPORATION INTO RNA DURING INTERPHASE AND ROLE OF NEWLY SYNTHESIZED RNA IN FORMATION OF THE MITOTIC APPARATUS IN SYNCHRONIZED CHINESE HAMSTER CELLS

L. S. Strochkova and V. V. Kazan'ev

UDC 612.398.145.1

The dynamics of RNA synthesis during interphase in synchronized Chinese hamster cells was studied with the air of uridine-H^3. Incorporation of the isotope increased gradually as the cells passed through the mitotic cycle and reached a maximum in the G_2-period. Investigation of the distribution of grains of silver above the dividing cells showed that label incorporated into RNA synthesized at the beginning of the S-period is localized above the zone of the first division spindle, whereas label incorporated into RNA at the end of the S- and during the G_2-period is localized above the zone of the second division spindle.

During interphase substantial changes in the synthesis of RNA, DNA, and protein macromolecules take place in the cells [3, 8]. Data on the character of RNA synthesis are contradictory. Some workers have demonstrated a linear pattern of uridine-H^3 incorporation into RNA at the beginning of interphase [5] whereas others have demonstrated a relatively constant rate of RNA synthesis during the G_1-period, with marked acceleration during DNA synthesis [8, 12]. The stable form of RNA is also known to participate in the formation of the mitotic apparatus [4]. However, it is not known at which period this type of RNA is synthesized.

The object of this investigation was to study the dynamics of RNA synthesis during interphase and to attempt to determine the time of synthesis of the RNA participating in the formation of the mitotic apparatus. RNA synthesis was estimated from the intensity of incorporation of uridine-H^3, the most specific precursor of RNA.

EXPERIMENTAL METHOD

In the experiments of series I uridine-H^3 was added (5 μCi/ml; 16.1 Ci/mmole) every hour throughout interphase to three or four flasks containing Chinese hamster cells synchronized with colcemid [3]. After incubation for 1 h with the isotope the cells were fixed in Carnoy's fluid for 10 min at 4°C. To remove acid-soluble precursors the preparations were treated for 5 min with TCA at 4°C and washed with running water for 1.5 h. They were then stained with type M (Research Institute of Photographic Chemistry) emulsion and exposed for 3-5 days. After exposure, the cells were stained with Carazzi's hematoxylin. The number of grains of silver in 50-70 cells was counted on each slide – in the nucleolus, the nucleus (the total number and the number of grains per unit area in the extranucleolar region), and in the cytoplasm. The results were subjected to statistical analysis. To determine the specificity of uridine-H^3 incorporation into RNA, control specimens were treated with ribonuclease (0.5 mg/ml, 1 h, pH 6.5),

Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 1, pp. 70-73, January, 1974. Original article submitted June 20, 1973.

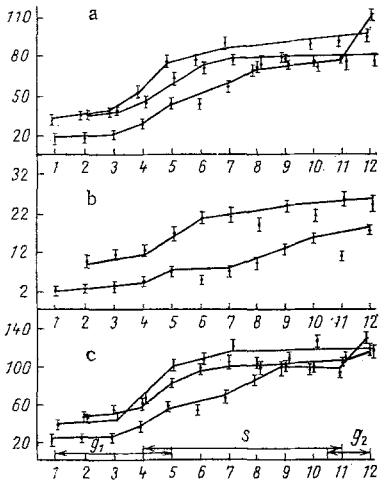

Fig. 1

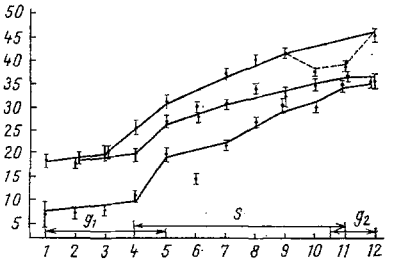

Fig. 2

Fig. 1. Dynamics of incorporation of uridine-H^3 into RNA of Chinese hamster cells during interphase (experiments repeated three times): a) dynamics of incorporation of uridine-H^3 into nuclei of Chinese hamster cells during interphase; b) dynamics of appearance of labeled RNA in cytoplasm of Chinese hamster cells during interphase; c) change in total labeling by uridine-H^3 in Chinese hamster cells. Abscissa, periods of mitotic cycle and time (in h); ordinate, number of silver grains.

Fig. 2. Changes in number of silver grains per unit area in extranucleolar zone of nucleus of synchronized Chinese hamster cells in interphase (experiments repeated three times). Abscissa, periods of mitotic cycle and time (in h); ordinate, number of grains of silver.

deoxyribonuclease (0.5 mg/ml, 1 h, pH 6.0), and also with ribonuclease and deoxyribonuclease together. Data are available for changes in the absorption of β-particles of H^3 depending on the concentration of the substance [6, 9, 11]. To introduce corrections for decay of the tritium emission depending on the density of the absorber the concentration of dry substance was measured in the individual components of the cell with the aid of a shearing interference microscope. For this purpose synchronized colorless cells suspended in distilled water were used to determine the path difference of the beam for the first layer, including the nucleolus and the layers of the nucleus and cytoplasm above and below it, for the second layer, including the nucleus and adjacent layers of the cytoplasm, and for the third (cytoplasmatic) layer. Measurements of the dry weight of these parts of 30-40 cells were made at various times of the G_1-, S-, or G_2-periods. Allowing for the concentrations of dry substance of the individual components of the cell, a correction could be introduced for the change in absorption, using curves of self-absorption of H^3 β-particles.

In the experiments of series II synchronized cells were incubated with uridine-H^3 (5 μCi/ml; 16.1 Ci/mmole) for 1 h in the G_1-, S-, or G_2-periods, and after contact with the isotope the cells were transferred to medium containing an excess of unlabeled uridine (0.03 mg/ml), in which incubation continued at 37°C. The material was fixed during either the first or the second wave of mitoses after addition of the isotope. The distribution of grains of silver above the dividing cells was investigated.

EXPERIMENTAL RESULTS

RNA synthesis in the cell falls off sharply during metaphase-anaphase of mitosis and recovers rapidly in late telophase during decondensation of the chromosomes. In the Chinese hamster cells used as the experimental model incorporation of uridine-H^3 varied during interphase. The total quantity of label in the cells increased gradually as the cells passed through the cycle and reached a maximum in the G_2-period (Fig. 1c). RNA synthesis was at a low level in the G_1-period. As the cells passed into the S-stage the incorporation of uridine-H^3 into the RNA of the nucleus increased sharply (by 1.8-2.7 times compared with the beginning of interphase) (Fig. 1a). This increase was repeated from one experiment to another. It is perhaps connected both with an increase in the number of templates for RNA synthesis in the

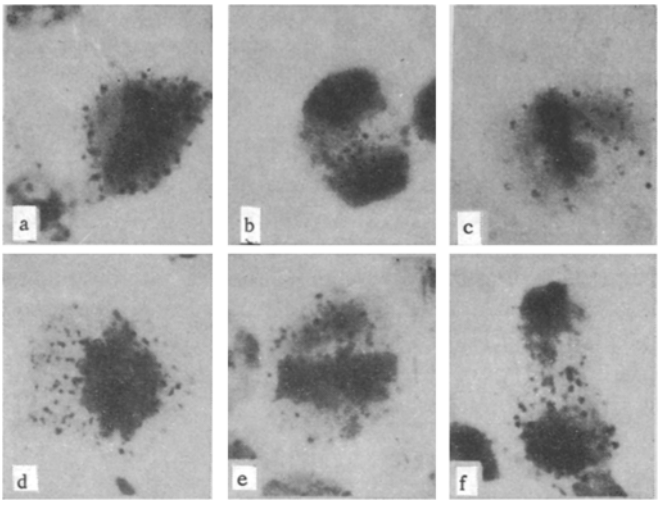

Fig. 3. Distribution of grains of silver and uridine-H^3 over dividing cells in first and second mitoses: a) incorporation of uridine-H^3 into RNA in G_1-period of interphase: metaphase of first mitosis, label localized around periphery of cell; b) incorporation of uridine-H^3 into RNA at beginning of S-period of interphase: label localized above zone of mitotic apparatus of first mitosis; c) incorporation of uridine-H^3 into RNA in G_1-period of interphase: diffuse distribution of silver grains above dividing cell in second mitosis; d, e) incorporation of uridine-H^3 into RNA at the end of S-period and in G_2-period of interphase: metaphases of second mitosis, label localized above zone of mitotic apparatus; f) incorporation of uridine-H^3 into RNA at end of S-period and in G_2-period of interphase: intensive labeling of interzonal region of anaphase of second mitosis.

course of DNA synthesis and also with activation of hitherto inactive genes [8, 12]. There is evidence that early-replicating DNA is more active in RNA synthesis than the late-replicating variety [14]. Determination of the number of silver grains per unit area in the extranucleolar zone of the nucleus (Fig. 2) showed that the intensity of RNA synthesis increased gradually during interphase and reached a maximum in the G_2-period. At the end of interphase the incorporation of uridine-H^3 into the cells was 2.5-4.5 times higher than in the G_1-period. RNA synthesis in the nucleus was balanced with its liberation into the cytoplasm. The dynamics of appearance of the label in the cytoplasm of the Chinese hamster cells (Fig. 1b) is evidence that migration of the newly synthesized RNA from nucleus into cytoplasm is negligible in the G_1-period. The maximal quantity of label in the cytoplasm was found at the end of the S-period and in the G_2-period. This could be connected both with an increase in the number of RNA molecules leaving the nucleus and entering the cytoplasm and with an increase in the rate of transfer of the molecules. The character of incorporation of the label into the cytoplasm at the end of interphase and also into the nucleolus (at the end of interphase incorporation into the nucleolus was 1.9-6.7 times greater than at the beginning of interphase) correlated with the cytophotometric data for the increase in the content of ribosomal RNA in the cell cytoplasm during this period of the cell cycle. Variations in the intensity of incorporation of the isotope observed from one experiment to another could be explained by the very slight differences in density of the cell population, variations in the composition of the culture medium, and so [13].

The dynamics of RNA synthesis correlated with the changes in protein synthesis [1] and in the dry weight of the cell [3] during interphase. Determination of the concentration of dry substance of the cells at individual times of the G_1- and S-periods and in the G_2-period showed only slight changes in the course of the cell cycle. Concentration of dry substance of the cell nucleus with the adjacent layers of cytoplasm be-

gan to increase from the middle of the S-period, correlating with the increase in mass of the nucleus during DNA synthesis. The concentration of dry substance of the nucleolus with the adjacent layers of the nucleus and cytoplasm increased considerably at the end of interphase. This is confirmed by data in the literature on the synthesis of nucleolar material at the end of the S-period and in the G_2-period.

At the same time an attempt was made to determine the period of interphase when the RNA participating in the formation of the mitotic apparatus is synthesized. Investigation of the distribution of silver grains above the dividing cells showed that the RNA synthesized in the G_1-period cannot be found in the mitotic spindle of either the first or the second mitosis (Fig. 3a, c). RNA synthesized at the beginning of the S-period is incorporated in the first mitosis throughout the cytoplasm and to some extent into the mitotic spindle of the first division (Fig. 3b). In metaphase the label was localized above the zone of the mitotic apparatus, while in anaphase-telophase it was located in the interzonal region, where the microtubules of the spindle participate in the formation of the intermediate bodies. Label incorporated into RNA at the end of the S-period and in the G_2-period was localized mainly above the zone of the mitotic spindle of the second mitosis (Fig. 3d, e, f). Although the utilization of newly synthesized RNA chiefly through one mitosis agrees with data in the literature [7, 10], the possibility that certain subsidiary factors (redistribution of the label, incorporation of the isotope into the pool of RNA precursors, and so on) may have affected the results cannot be completely ruled out at this stage. The results of these experiments thus show that the long-living RNA synthesized at the beginning of interphase is partly used to build the mitotic spindle in the first mitosis, while that synthesized at the end of interphase participates in the building of the mitotic spindle of the second mitosis.

LITERATURE CITED

1. V. V. Kazan'ev, Dokl. Akad. Nauk SSSR, 201, 711 (1971).
2. V. V. Kazan'ev, L. S. Strochkova, and V. V. Akopov, Byull. Éksperim. Biol. i Med., No. 12, 71 (1973).
3. R. Baserga, Cell Tissue Kinet., 1, 167 (1968).
4. L. K. Chu, J. R. Stetson, M. Hardividjaja, et al., J. Cell Biol., 55, 43 (1972).
5. M. D. Enger and R. A. Tobey, J. Cell Biol., 42, 308 (1969).
6. P. J. Fitzgerald, M. J. Eidinoff, J. E. Knoll, et al., Science, 114, 494 (1951).
7. E. V. Gaffney and R. M. Nardone, Exp. Cell Res., 53, 410 (1968).
8. R. R. Klevecz and E. Stubblefield, J. Exp. Zool., 165, 259 (1967).
9. W. Maurer and E. Primbsch, Exp. Cell Res., 33, 8 (1964).
10. C. Mittermayer, R. Braun, and H. P. Rusch, Biochim. Biophys. Acta, 91, 399 (1964).
11. S. R. Pelk and M. G. E. Welton, Nature, 216, 925 (1967).
12. S. Pfeiffer, J. Cell Physiol., 71, 95 (1968).
13. P. J. Stambrook and J. E. Sisken, J. Cell Biol., 52, 514 (1972).
14. A. Zellweger and R. Braun, Exp. Cell Res., 65, 424 (1971).