CYTOCHEMICAL INVESTIGATION OF DYNAMICS OF RNA SYNTHESIS DEMONSTRATED IN METAPHASE CHROMOSOMES OF HeLa CELLS

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The dynamics of synthesis of DNA and chromosomal RNA, demonstrated in metaphase chromosomes of HeLa cells, was investigated by methods of selective extraction of cells in mitosis from the monolayer and pulse labeling. The results showed that chromosomal RNA is itself synthesized chiefly at the end of the G_1- and beginning of the S-period. The synthesis of chromosomal RNA was found in the G_2-period or during mitosis.

Key words: chromosomes; RNA synthesis; mitosis; colchicine.

The study of the dynamics of RNA synthesis during the cell cycle is now a rapidly developing aspect of the general problem of the investigation of the mechanisms lying at the basis of cell proliferation. Research that has demonstrated the presence of RNA in metaphase chromosomes is of special interest [3, 4, 11, 14]. The writer showed previously [1] that metaphase chromosomes of PHA-blasts contain their own chromosomal RNA, characterized by high resistance to RNAase in solutions of high ionic strength. Its synthesis is relatively resistant to actinomycin D. It is regularly arranged in metaphase chromosomes. However, as yet hardly anything is known of the nature and function of this RNA and the dynamics of its synthesis has not been revealed.

The object of the present investigation was to discover whether metaphase chromosomes of HeLa cells contain intrinsic chromosomal RNA and to study the dynamics of its synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on HeLa cells obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology. Cells of this strain were mycoplasma-free. The HeLa cells were grown in medium No. 199 with the addition of 10% bovine serum. After a monolayer had been obtained the medium was replaced by Hanks's solution with the addition of 10% bovine serum and 20 μCi ml uridine-H^3 (specific activity 14 Ci/mmole).

To prevent the incorporation of uridine into DNA, 5 μg/ml of cold thymidine was added to the medium. The cells were labeled for 1 h, after which the monolayer was washed 3 times with Hanks's solution to remove the labeled precursor. Fresh medium No. 199 with 10% bovine serum at 37°C was then added. After each 2 h cells in mitosis were removed from this monolayer by a selective method [5]. Preparations of metaphase plates were obtained from these cells. Some of the preparations were treated with RNAase (3 μg/ml, 20 min, 37°C) in a solution of high ionic strength to remove RNA not bound with the metaphase chromosomes. All films were treated with cold 5% TCA for 20 min and then coated with type M (NIIKhimfoto) emulsion. The number of silver grains above 100 metaphase plates was counted in the autoradiographs and the mean number of grains per plate was calculated.

As a control and to determine the duration of the cell cycle, the dynamics of DNA synthesis of HeLa cells was studied by a modified method of selective isolation of cells in mitosis, preparation of metaphase plates, and autoradiography. The silver grains were counted in the same way as to determine the dynamics

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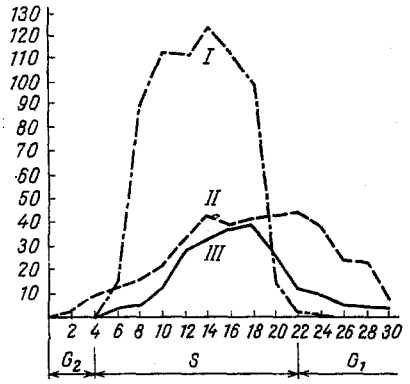

Fig. 1. Dynamics of synthesis of DNA and chromosomal RNA demonstrable in metaphase chromosomes of HeLa cells: I) DNA synthesis; II) synthesis of total RNA demonstrable in metaphase chromosomes; III) synthesis of purely chromosomal RNA. Abscissa, time (in h) counted from mitosis, i.e., in the reverse direction; ordinate, number of grains.

of synthesis of RNA demonstrated in the metaphase chromosomes.

EXPERIMENTAL RESULTS AND DISCUSSION

The dynamics of DNA synthesis in the HeLa cells is illustrated in Fig. 1. The first preparations with labeled metaphase chromosomes were observed only 6 h after pulse labeling. The end of the synthetic period and the duration of the G_2-period, in this case 4-6 h, could thus be determined. The plateau of thymidine-H^3-labeling in the metaphase chromosomes was observed between 8 and 18 h, counting from the time of pulse labeling. The duration of the S-period was thus 14-16 h. The most intensively labeled metaphase chromosomes were found 14 h after pulse labeling. The method of selective isolation of cells in mitosis and the method of autoradiography as used thus enabled the dynamics of synthesis of total RNA and of purely chromosomal RNA, demonstrable in the metaphase chromosomes of HeLa cells, to be determined. The distribution of total labeled RNA detected in metaphase chromosomes over the cell cycle can also be seen in Fig. 1. During the first 2 h the amount of label in the metaphase chromosomes was negligible, but later the curve rose. The most intensively labeled metaphase chromosomes were found between 14 and 22 h. Less intensive incorporation took place 30 h after pulse labeling.

To determine whether RNA synthesis can take place in the metaphase chromosomes of HeLa cells themselves, three experiments were carried out in which an initial colchicine block was applied to accumulate cells in metaphase, after which the cells were pulse-labeled with uridine-H^3 (colchicine was still present all the time in the culture medium) and autoradiographs were prepared. No silver grains could be found above the metaphse chromosomes, whereas the interphase nuclei were labeled. It thus follows that the metaphase chromosomes of HeLa cells do not synthesize RNA, but RNA synthesis takes place in interphase nuclei even in the presence of colchicine in the medium.

To study the dynamics of synthesis of purely chromosomal RNA detectable in metaphase chromosomes the preparations were treated with RNAase. This treatment led to complete removal of the label from the spaces between the chromosomes and of some of the label located above the metaphase chromosomes (Fig. 2).

In preparations treated with a solution of RNAase, labeled metaphase chromosomes were visible only after the 6-h period. A rise of the curve was then observed and the most intensively labeled metaphase chromosomes were found 18 h after pulse labeling. A sharp decrease in the quantity of labeled RNA in the metaphase chromosomes of the HeLa cells began after 22 h. The dynamics of synthesis of purely chromosomal RNA thus differed from the dynamics of synthesis of total RNA.

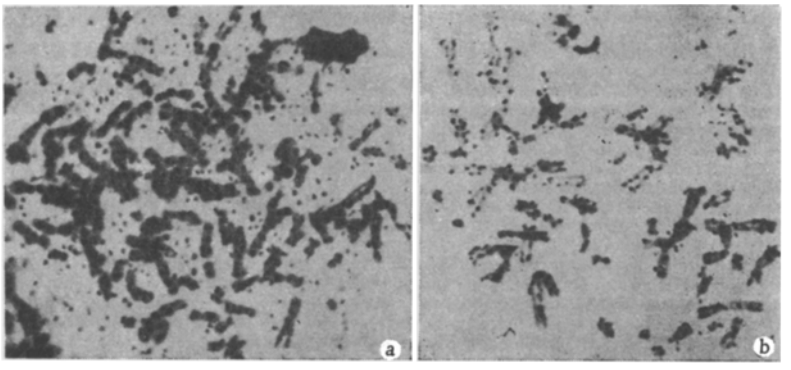

Fig. 2. Labeled RNA in metaphase chromosomes of HeLa cells before (a) and after (b) treatment with RNAase.

It is also clear from Fig. 1 that the synthesis of purely chromosomal RNA takes place chiefly at the end of the G_1- and beginning of the S-period of the cycle, i.e., before the beginning of and during DNA synthesis, and ends at the end of the S-period, in good agreement with the dynamics of DNA synthesis. No synthesis of purely chromosomal RNA was found in the G_2-period or during mitosis.

Analysis of the autoradiographs shows that purely chromosomal RNA was associated with the chromosomes from the moment of its synthesis until the beginning of anaphase, i.e., that it is long-living. This is in good agreement with biochemical investigations showing that a low-molecular-weight RNA (3.5S) exists in constant association with the nucleus [10, 12]. A series of communications [6-8] describing the presence of a distinctive low-molecular-weight RNA (3.4S) with a high content of dihydrouridylic acid, covalently bonded with proteins, and also reports [9, 13] of investigations showing that for DNA synthesis to take place the synthesis of an RNA with the function of an initiator is necessary are very interesting for the interpretation of the present results. The coincidence established in the present experiments between the times of synthesis of DNA and of purely chromosomal RNA provides a basis for a detailed analysis of the problem of whether the RNA now described can perform that particular function.

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