ELECTRON-AUTORADIOGRAPHIC STUDY OF RNA TRANSFER FROM NUCLEUS TO CYTOPLASM

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Electron-autoradiographic investigation of RNA synthesis in mouse liver cells revealed considerable differences in the content of labeled RNA in the nucleoli of the same nucleus. In areas of the cytoplasm located near the nuclear membrane, with an adjacent nucleolus, the concentration of silver grains was frequently much higher than elsewhere in the cytoplasm. On the basis of these findings it is suggested that the nucleoli of one nucleus synthesize RNA at different times, transfer it to the nuclear membrane, and also discharge RNA into the cytoplasm at different times. This would explain the rhythm of RNA liberation into the cytoplasm. Under pathological conditions the number of nucleoli may be increased, with a consequent increase in the frequency of liberation of RNA portions into the cytoplasm.

KEY WORDS: electron-microscopic autoradiography; RNA synthesis; nucleolus; biorhythm.

RNA is known to be synthesized in the nucleolus and chromatin, and most of it is then subsequently transferred into the cytoplasm. Strocker et al. [4] and Harris [3] have found that the nucleolus plays an important role in this transfer of RNA. Clearly [3], the appearance not only of ribosomal RNA (rRNA), synthesized in the nucleolus itself, but also of messenger RNA (mRNA), synthesized in the chromatin, is connected with the function of the nucleolus. However, the mechanism by which the nucleolus performs its transfer function has not yet been explained. To study this problem the method of electron-microscopic autoradiography was used.

EXPERIMENTAL METHOD

A subcutaneous injection of 0.2 ml of a 40% solution of carbon tetrachloride (CCl_4) in peach oil was given to noninbred albino mice. The electron-autoradiographic investigation of the liver tissue was carried out 24 h after the injection of CCl_4, when the animals had developed necrosis of the central zones of the hepatic lobules, and a compensatory increase in the rate of RNA synthesis had developed in the hepatocytes located at the periphery of the lobules. At intervals of 30 min and 2 and 4 h before fixation of the material the experimental and control (not receiving CCl_4) animals were given injections of RNA precursors: orotic acid-5H^3 or uridine-5H^3 in doses of 50-100 μCi/g (specific activity 25 Ci/mmole). Pieces of liver were fixed with glutaraldehyde and OsO_4 and embedded in Epon. Thin (500-1000 A) and semithin (0.5-1 μ) sections were cut from the blocks for investigation in the electron and light microscopes. The sections were coated with M emulsion, exposed for 15-40 days, and developed with D-19 developer. The density of the silver grains above the nucleolus, nucleoplasm, chromatin, granular endoplasmic reticulum, and mitochondria was determined on the electron-autoradiographs. The number of grains of silver above the nucleolus and above the remainder of the nucleus was counted in light-optical autoradiographs.

EXPERIMENTAL RESULTS

Usually one or several nucleoli, most frequently from 2 to 4, were found in the nuclei of the hepatocytes of the experimental and control animals. On the average the nucleoli had the highest density of

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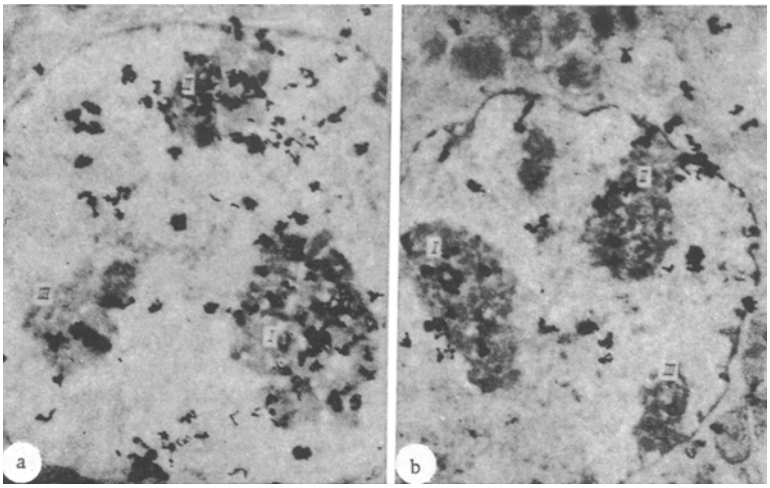

Fig. 1. Differences in content of newly synthesized RNA in hepatocyte nucleoli: a) many grains of silver in two nucleoli (I and II) and fewer in the third (III). 15,000×; b) high content of labeled RNA in one nucleolus (I), less in another (II) with an increased concentration of silver grains in the area of cytoplasm closest to the nucleolus, and absence of grains in a third nucleolus (III). 15,000×.

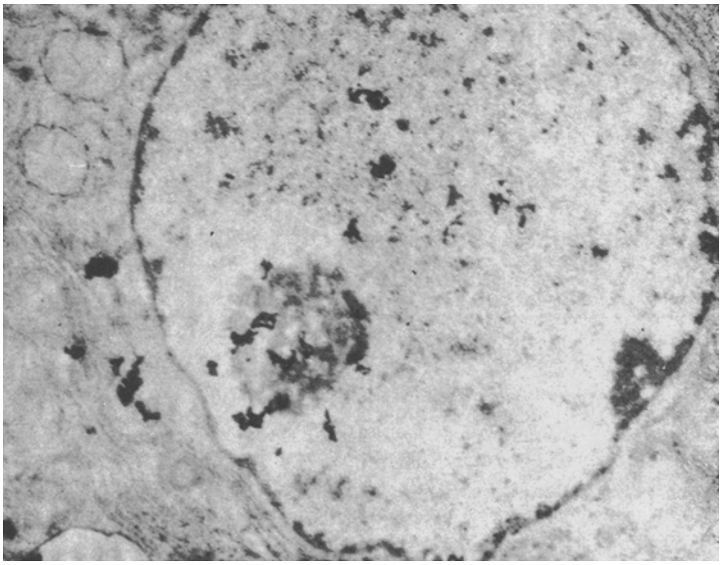

Fig. 2. Transfer of newly synthesized RNA from nucleolus to cytoplasm. 15,000×.

silver grains compared with other structures of the cells. The concentration of silver grains above the zone nearest to it in density was 2.5-3 times smaller. Meanwhile the nucleoli of one nucleus often differed considerably in the density of the silver grains above them. Heterogeneity in the content of label was particularly characteristic of nucleoli near the nuclear membrane or close to it. Some of them, judged from the number of grains of silver, contained much of the labeled product (Fig. 1a); above the others there were fewer grains, but the concentration of label in the cytoplasm opposite such a nucleolus was higher than in the rest of the cytoplasm (Figs. 1b and 2); finally, no silver grains whatever were found above individual nucleoli located near the nuclear membrane (Fig. 1b).

The difference in the content of labeled RNA in the nucleoli indicates that its synthesis in the different nucleoli of the same nucleus is evidently not synchronized but takes place at different times. There is a corresponding lack of synchronization of the "discharge" of newly synthesized RNA into the cytoplasm

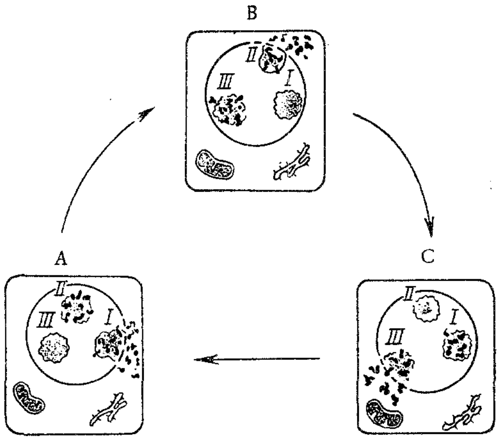

Fig. 3. Diagram showing variation in times of RNA synthesis in each of the nucleoli and its transfer into the cytoplasm.

at the nuclear membrane. Allowing for both these phenomena the following picture can be drawn of the dynamics of the process as a whole: the nucleoli synthesize rRNA in turn and transfer it, also in turn, to the nuclear membrane, picking up a certain quantity of messenger RNA during this movement [3]. They then "discharge" a portion of newly synthesized RNA into the cytoplasm and again start to synthesize rRNA and to pick up mRNA (Fig. 3).

If these arguments are correct, valuable material is now available for solving the problem of the rhythm of nucleo-cytoplasmic relations. Since a transverse movement of the nucleoli toward the nuclear membrane followed by liberation of RNA into the cytoplasm can be seen, there are good grounds for supposing that RNA enters the cytoplasm most probably not continuously but periodically, in separate portions. This, in turn, is evidence in support of the rhythmic character, rather than the smoothness and continuity, of nucleo-cytoplasmic relations, determining the correspondingly rhythmic work of the cell as a whole. The dynamics of RNA synthesis in the nucleolus and its liberation into the cytoplasm thus provide a very clear illustration of the alternating activity of functioning structures as an important principle of the function of biological systems [1, 2]. In this case this principle is expressed at the subcellular (intranuclear) level. Each successive portion of RNA liberated by the nucleolus into the cytoplasm can be regarded as a unique biological "quantum" [2], or an elementary component particle of the continuous process of vital activity. The results confirm the view that the quantum character of elementary processes, combined with the alternating activity of functioning systems, provide for the continuous activity of the system as a whole at the output [1, 2].

Analysis of the light-microscopic autoradiographs revealed an increase in the number of grains of silver in the nuclecli of the experimental animal compared with the control. This fact evidently reflects a compensatory increase in both the synthetic and the transport functions of the nucleolus. In such a situation, in each "working cycle" it liberates an increased quantity of rRNA and template RNA into the cytoplasm, and thus contributes to maintenance of protein synthesis in the cell when damaged by CCl_4 at a level sufficient to preserve life. During long periods of functional stress on a cell the number of nucleoli in it may increase. Under these circumstances the frequency of the supply of newly synthesized RNA into the cytoplasm, i.e., the rhythm of this process, must inevitably be increased.

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