A STUDY OF THE INITIAL INTERACTION OF THE SENDAI VIRUS WITH CELLS, USING THE METHOD OF AUTORADIOGRAPHY

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 7, pp. 67-73, July, 1963
Original article submitted November 23, 1962

In studying the initial stage of interaction between the Sendai virus and tissue culture cells, it was shown that after adsorption on the cells, and after the virus had surmounted the cell barriers [2, 3], it could not be demonstrated inside the cell by virological or serological methods. It may be postulated that, close to the surface of the cell, the virus particle breaks up into subunits – nucleoprotein and proteins of the capsule, as was shown earlier for the grippe virus [7]. If the nucleic and protein components of the virus particle are tagged with various radioactive precursors, the method of autoradiography should show which components of the virus penetrate into the cell, and in which cell structures they are localized.

There are reports on the successful use of radioactive phosphorous in studying the intracellular localization of the nucleic component by the method of autoradiography, and demonstration of the latter in such cell structures as the chromosomes and nucleoli [6, 9, 10]. In connection with this, in connection with this, we inferred the possibility of using P^{32} to tag the nucleic component of the virus. In certain experiments, we used viral preparations tagged with C^{14}-uracil. For tagging the protein component of the virus, we used methionine and cysteine, tagged with radioactive sulfur.

EXPERIMENTAL METHOD

We used strains No. 960 and LM-1 of the Sendai virus, possessing comparatively weak hemolytic activity, which considerably facilitated the process of purification associated with the use of chicken erythrocytes.

The work was performed with transplanted tissue cultures of COTs cells and of human amniotic cells, and also with a primary culture of fibroblasts from chicken embryos.

Radioactive preparations of the Sendai virus were obtained according to the method described earlier [2]. As the radioactive precursors, we used $Na_2HP^{32}O_4$, S^{35}-methionine, S^{35}-cysteine, and C^{14}-uracil.

The radioactivity was determined in a volume of 1 ml of preparation, which was evenly distributed in the center of the targets, dried, and counted with the aid of a surface Geiger-Muller counter. The radioactivity was expressed in impulses per minute per 1 ml of preparation.

The autographs were obtained according to the method described by L. N. Zhinkin and coworkers [5]. After fixation in Carnu's solution and three-fold washing with Henk's solution, the preparations were coated with a gelatin under layer, and then with a light-sensitive emulsion of type R. After exposure for 1 or 3 weeks, the preparations were treated with amidol developer and stained with orange-yellow eosin-methylene blue, according to the modified method of Unna.

TABLE 1. Radioactivity of the Viral Subunits (in Percents of the Radioactivity of the Original Preparation)

Isotopes	Compounds	Nucleoprotein (S-antigen)	Protein (including the V-antigen)
P^{32}	Phosphate	44-56	11-13
S^{35}	Methionine	10.4	47.4

TABLE 2. Distribution of the Cells with Localization of the Granules in Different Cell Structures, Using Virus Tagged with C^{14}-Uracil

Duration of contact (in minutes)	Percent of cells with granules localized		
	in the cytoplasm	in the nucleus	in the nucleolus
10	6	34	60
30	11	38	51
60	14	45	41
120	18	50	32

We started from the fact that P^{32} is included in the nucleic acids and phospholipids of the myxoviruses [8], while the sulfur of methionine and cysteine enters into the protein capsules of the virus [7]. The virus was cleansed of radioactive impurities by twofold adsorption and elutriation on chicken erythrocytes dialysis opposite distilled water.

We purity of the tagged virus preparations was controlled by adsorption of the virus on erythrocytes or by sedimenting it through ultracentrifugation at 30,000 g for one hour. The characteristics of the viral preparations used were presented in a previous report [2].

The specific radioactivity of the different preparations ranged from 3.4 imp/min/HU* to 40.0 imp/min/HU*, depending on the original concentration of isotope.

EXPERIMENTAL RESULTS

For a correct interpretation of the results obtained with the use of autoradiography, it was necessary to carry out a preliminary analysis of the viral preparations for radioactivity of the different viral components, obtained after treating the virus with ether. Table 1 presents the combined results of these investigations.

Thus, when the virus was tagged with P^{32}, the radioactivity accumulated predominantly in the nucleoprotein, while with S^{35} tagging, it was primarily in the proteins of the capsule.

In the experiments with P^{32}, only 10 minutes after contact of the virus with the cells, radioactive granules were distributed over the nuclei and nucleoli, as well as on the cell membrane and the border between the nucleus and the cytoplasm (Fig. 1). After 30 minutes, the majority of granules were over the nucleoli, and after 60 minutes, many nuclei solidly filled with granules. After 2 hours, the number of cells containing granules increased, and the latter were localized in the nuclei, often filling the internucleolar space (Fig. 2a). After 2 and 3 hours, there was an increase in the number of cells with localization of the granules in the cytoplasm, primarily in the perinuclear zone (Fig. 2b). On the basis of mass tallies of the cells inoculated with virus, the dynamics of these changes are presented in Fig. 3.

From these data, it may be concluded that the viral components containing the P^{32} rapidly pentrate into the nucleoli, being somewhat retarded along the way at the cell and nuclear membranes, and that they accumulate here during the 1st hour after contact of the virus with the cell. In the course of the 2nd hour, in a number of cells, the P^{32} passes from the nucleoli to the nucleus, and after 2 hours – into the cytoplasm, being localized in the perinuclear zone. A similar picture was observed in the work with all the indicated types of tissue cultures (human amniotic cells, SOTs, chicken fibroblasts). The portion of cells with radioactive granules in these preparations ranged from 0.5 to 10%.

In control experiments, using contact of the cells with allantoic fluid not containing virus, but obtained under the same conditions and possessing the same radioactivity as the viral preparations, after the 1st hour the granules were still not concentrated in the cellular territories. After 2 hours, we observed individual cells with granules localized in the nuclei. We did not observe accumulation of P^{32} in the nucleoli of the control preparations.

*HU – Hemagglutinizing Unit.

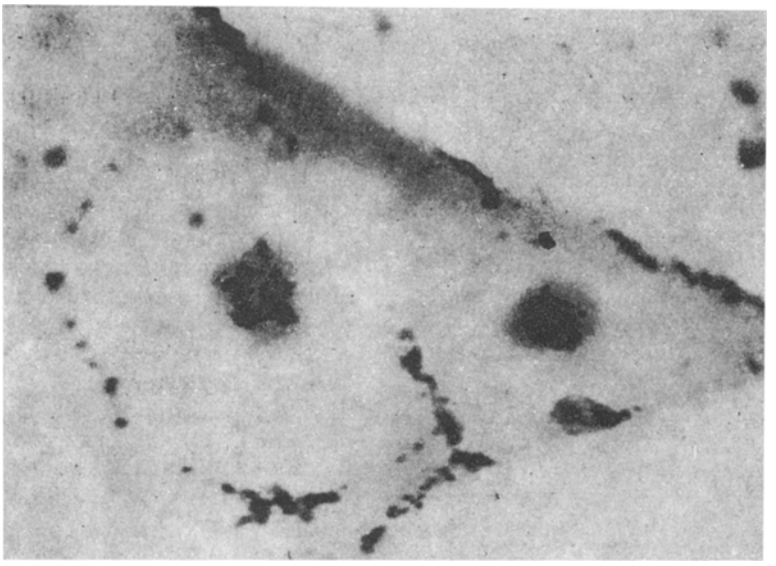

Fig. 1. Autograph of human amniotic cells after 10 minute contact with Sendai virus tagged with P^{32}. The granules are localized over the nucleoli and on the cell and nuclear membranes. Stained by the method of Unna. × 900.

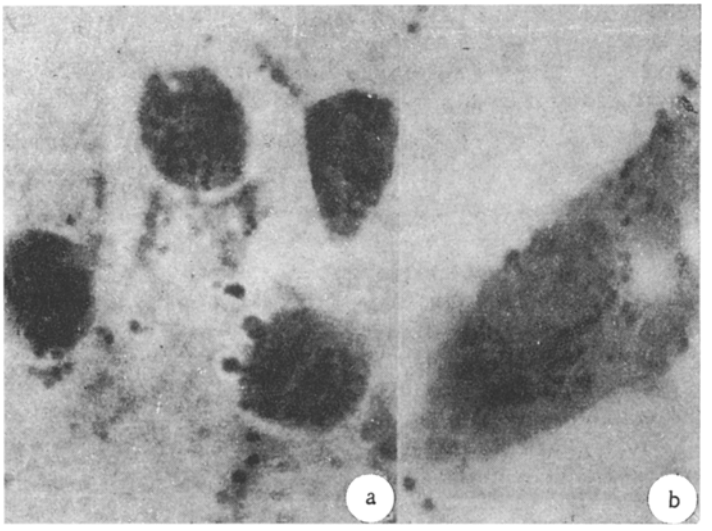

Fig. 2. Autograph of human amniotic cells after 2 hour contact with Sendai virus tagged with P^{32}. a) In the upper left cell, the granules are localized in the internucleolar zone of the nucleus; b) the granules are localized in the perinuclear zone of the cytoplasm. Stained by the method of Unna. × 600.

In the experiments using the virus tagged with C^{14}-uracil, the same principles were noted. The highest percentage of cells with granules localized in the nucleoli was observed after 10 minute contact between the cells and the virus. With contact for 2 hours, the percent of cells with nucleolar localization of the granules decreased, the percent with cytoplasmic localization increased (Table 2). As in the case of the preparations tagged with P^{32}, in the cells with cytoplasmic localization, where the preparations were in contact with the virus for 2 hours, the granules were also observed in the form of conglomerates within the perinuclear zone.

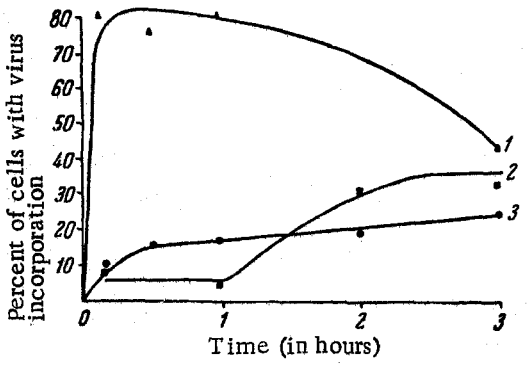

Fig. 3. The dynamics of incorporation of Sendai virus tagged with P^{32} into the cell structures. 1) Nucleoli; 2) cytoplasm; 3) nuclei.

In the control preparations of this series of experiments, not containing virus, after 2 hours of contact with the cells we observed incorporation of the isotope into the nuclei of isolated cells. After 3 hours of contact, a significant number of cells contained radioactive granules; in this case, intranuclear localization of the granules was observed in 90% of the cells, and only in 10% did we see intranucleolar localization.

In the experiments using virus tagged with methionine containing S^{35}, 10 minutes after contact with the cells, granules appeared over the territories of the majority of cells (up to 97%), distributed evenly over them. After 30 minutes, the number of granules increased, and after 60 minutes it attained its maximum. The uniform distribution of the granules over the cell territories was maintained throughout the entire period of observation.

Clear differences were noted in comparing the autographs obtained from contact of the cells with viruses tagged with C^{14}-uracil, P^{32}, S^{35}-methionine and S^{35}-cysteine. While S^{35} was distributed evenly over the territory of the cells during the entire period of observation (3 hours), P^{32} and C^{14}-uracil initially accumulated primarily in the nucleoli and then partially shifted to the nuclei and the perinuclear zone of the cytoplasm.

These differences can be explained by starting with the hypothesis that the components of the virus particle tagged with P^{32} and C^{14}-uracil penetrate into the nucleus and nucleolus of the cell, while those tagged with S^{35} are basically adsorbed onto the cell membranes or are distributed evenly in the cytoplasm. In this case, the number of cells with radioactive granules in the experiments with S^{35} was significantly greater than in the experiments with P^{32}. This circumstance is apparently reflected by the fact that not all the viral particles adsorbed onto the surface of the cells penetrate into the cells.

The data presented justify postulating that after adsorption of the virus onto the surface of the cells, and surmounting of the cell barriers by the entering viral enzymes (RDE and CWDE) [1], the viral particle splits into its components: nucleic acid (or, perhaps, nucleoprotein), and proteins of the capsule; in this case, the viral nucleic acid (nucleoprotein) reaches the nuclei and nucleoli in the course of a short period of time, measured in a few minutes. The fate of the protein component still remains unclear.

As was indicated, localization of the granules to nuclear territory was observed in the early intervals after inoculation of the cells with virus, and increased regularly over the course of the 1st hour of cultivation, not accompanied by a decrease in the radioactivity of the nucleoli. This is evidence that part of the RNA of the virus also settles in the internucleolar area of the nucleus. At 1 hour or more after the inoculation, the granules observed in the internucleolar area were apparently caused by both the RNA of virus that had penetrated into the nucleus and the RNA of virus that had left the nucleolus.

An increase in the number of granules within the cytoplasm arose only after 2 hours of contact between the cells and the virus, when we noted a decrease in the radioactivity of the nucleoli. Apparently, the RNA of the virus or its component begins, at this time, to shift from the nucleolus and nucleus to the cytoplasm. This is confirmed by certain peculiarities in the localization of the granules within the cytoplasm which we noted. In the preparations with 2 and 3 hour contact intervals, we observed concentration of the granules in the region of the cytoplasm near the nuclear membrane, which was not observed in the preparations taken at earlier intervals. The number of these cells, and the number of granules in the foci of concentration, increased with subsequent cultivation of the cells, which also caused a jump in the total number of cytoplasmically localized granules after 2 and 3 hour contacts between the cells and the virus.

Several hours later, intense synthesis of viral antigen begins in the perinuclear zone of the cytoplasm [4].

Thus, the RNA of virus entering the nucleolus and other portions of the nucleus apparently causes the synthesis of RNA of new virus particles, which seems to carry out the functions of information RNA which are necessary for the synthesis of the protein components of the virus. We have not excluded the possibility that in addition to the nucleoli there are other nuclear structures which participate in this process. This question presents considerable interest, since

the mechanism and cell localization of the synthesis of specific types of RNA, associated with inoculation of the cell with RNA-containing viruses, remain unclear. Our results correspond to the latest data on the mechanism of reproduction of other RNA-containing viruses [11].

SUMMARY

A study was made of localization in the cellular structures of the Sendai virus with labeled nucleic (with the aid of uracil-C^{14} and radiophosphorus) or protein (with the aid of methionine and S^{35}-cystine) components. Autoradiography was employed for this purpose. As revealed, during the action of virus preparations labeled with C^{14}-uracil and P^{35} on the chick embryo cells and human amniotic cells, radioactive granules localized in the nucleoli and nuclei during the first hour then partially passed into the perinuclear zone of the cytoplasm. With the use of S^{-35} labeled preparations radioactive granules were evenly distributed in the cells. Basing upon the data obtained it may be concluded that the nucleic component of the virus penetrates into the nuclei and nucleoli whereas the protein component or its considerable part remains on the cell membranes or in the ectoplasm.

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