A new device for electron microscope autoradiography of whole cultured cells

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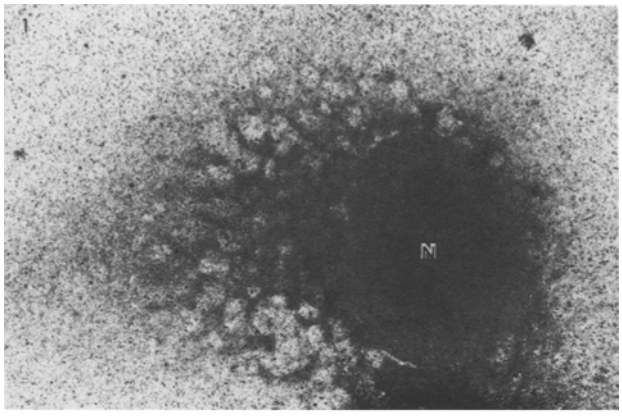
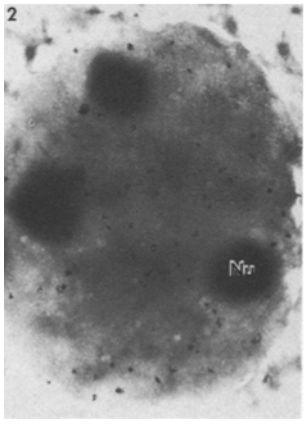
Summary. A new procedure which enables us to observe whole cultured cells autoradiographically by electron microscopy is described. By this method, only a relatively small amount of radioisotope and a relatively short exposure period are needed to get a large enough number of silver grains over the cell. The location of radioactive substances can be examined in relation to internal cell structures, especially the nucleus.

Thin sections have commonly been used for autoradiographical studies by electron microscopy. Since the amount of radioisotope retained in a thin section is small, it takes quite a long period of exposure to get a sufficient number of silver grains over the section for analysis by electron microscope autoradiography (EMARG)[1]. Hama and Hirosawa reported that the period of exposure could be considerably reduced by the observation of thick sections, employing a high voltage electron microscope[2]. Buckley and Porter and Buckley and Raju have developed a procedure for studying cellular structures in whole cells grown on grids coated with formvar membrane employing the critical point drying method[3,4]. Also, we could observe the internal structures of cultured whole cells by negative staining[5,6]. In this paper EMARG of whole cells grown on formvar- and carbon-coated grids and labelled by [^{3}H]-thymidine is described.

Materials and methods. A cell line of African green monkey kidney (CV-1) was inoculated into Falcon plastic petri dishes containing nickel grids which were coated with formvar and carbon. For the tissue culture medium, a 1:1 mixture of YLE medium and Eagle Minimum Essential Medium (Nissui, Tokyo) supplemented with 10% donor calf serum (GIBCO, USA) was used. The cultures were incubated at 36 °C in a humidified CO_2 incubator (5% CO_2: 95% air) until the cells became subconfluent. CV-1 cells at this stage were labelled by [^{3}H]-thymidine (1 μCi/ml and 19 Ci/mmole) for 1 h. After labelling, cells were washed carefully with 0.01 M phosphate-buffered saline (pH 7.2) 5 times, and fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The fixed cells on the grids were washed with distilled water and dried. The reverse surface of the grid was thinly coated with carbon and hydrophilic processing was carried out by UV-irradiation. Nuclear track emulsion (Sakura NR-H_2 diluted 1:5 with water) was mounted onto the reverse surface of the grid by the touching method. Exposure time was 4 or 8 days and during this time the grids were kept in a dry light-tight box at room temperature. Development was with Copinal (Fuji, Tokyo) at 20 °C for 2 min and was followed by fixing and washing with water. For electron microscopical observations, a Hitachi HU 12A electron microscope was used at 100 kV.





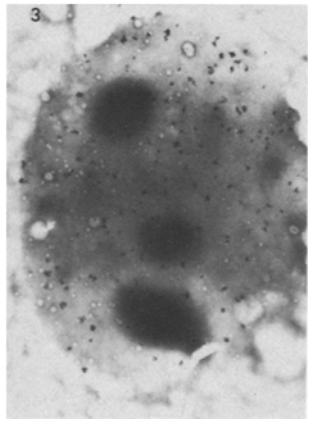


Fig. 1. CV-1 cell mounted by nuclear track emulsion without further processing. N: nucleus, × 1700.

Fig. 2. Autoradiogram of CV-1 exposed for 4 days. Nu: nucleolus, × 2350. Fig. 3. Autoradiogram of CV-1 exposed for 8 days, × 2350.

Results and discussion. CV-1 cells could grow and spread on formvar- and carbon-coated grids as well as on Falcon plastic petri dishes. Figure 1 shows a cell which was mounted with the nuclear track emulsion without further processing. The cell was covered with the emulsion fairly uniformly, while the emulsion was repulsed when it was mounted directly on the cell surface coated with carbon. Figures 2 and 3 are autoradiograms which were exposed for 4 and 8 days respectively. Silver grains are located uniformly and exclusively over the nuclei. In this experiment the cells were not synchronized, so the density of the grains varied from cell to cell. When the grids were exposed for 4 days, the mean number of grains over 1 nucleus was 64 ± 33 ($n = 23$), while it was 121 ± 46 ($n = 25$) for 8 days' exposure. Electron microscope autoradiography of whole cultured cells needs a relatively small amount of radioisotope, and it shortens the exposure period. With this technique, the resolution is not so good as with the thin section technique because of the thickness of the cells, but, as long as tritium is used as a tracer, there will be no problem of resolution since beta-particles do not travel long distances[2,7]. Moreover, it appears that we can examine the relationship between the location of radioactive substance and cellular structure from the standpoint of whole cells. Thus experiments employing this technique are now in progress for the analysis of the movement of progeny herpes simplex virus particles in cells after infection of CV-1 by the virus.

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