Scanning Electron Microscopy of the Nuclei Isolated from Chicken Liver

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Recently, isolated nuclei have been employed as materials for biochemical studies on the structure and function of the chromatin as well as nucleoli by a considerable number of investigators [1, 3, 4, 6]. In order to assess the validity of isolated nuclei as a reliable system for studies of chromatin, it is necessary to verify the fine structural integrity of chromatin by electron microscopy. However, there have been few electron microscopic studies on the chromatin in isolated nuclei. The present paper describes the scanning electron microscopic observations on the fine structure of the isolated nuclei of hepatic cells.

Chicken liver used in the present study was one of the most suitable materials for morphological and biochemical studies of the chromatin. The chicken was easily bred with commercial foods in the laboratory, and the desirable age of individuals could be obtained.

Nuclei were prepared from the liver of a 5-week old chicken by the method of Chauveau *et al.* [2] with some modifications. The liver was homogenized in 20 vol of 2.2 M sucrose-TKM buffer (10 mM Tris-HCl, pH 7.5, 25 mM KCl and 5 mM MgCl₂). The homogenate was centrifuged at 35,000×g for 60 min, and the resulting pellet was homogenized in 0.3 M sucrose-TKM buffer. After centrifugation at 800×g for 5 min, the pellet of nuclei was suspended in 0.3 M sucrose-TKM buffer. Then, the isolated nuclei were fixed for 1 hr with 1.8% glutaraldehyde in 0.3 M sucrose-TKM buffer at 4°C. The isolated nuclei were embedded in gelatin in order to minimize the loss of the nuclei during dehydration, freeze-cracking and critical point-drying. For embedding, a small amount of the fixed nuclei was mixed with 15% gelatin solution warmed at about 40°C, and kept for 30 min at room temperature. The gelatin containing the nuclei was cut into small blocks about 1×1×3 mm, and fixed with 2% glutaraldehyde for 1 hr. The gelatin blocks were conductive-stained with 2% tannic acid and 2% osmium tetroxide, dehy-

drated in a graded series of ethanol, and freeze-cracked with a razor blade in liquid nitrogen. Finally, they were critical point-dried with liquid carbon dioxide, and sputter-coated lightly with gold. The specimens, thus prepared, were examined under a JEOL JSM-T300 scanning electron microscope operated at an accelerating voltage of 25 kV.

A representative electron micrograph of the freeze-cracked surface of a nucleus isolated from chicken liver is shown in Fig. 1. Chromatin in the isolated nucleus appeared as a fibrous structure composed of randomly distributed thin fibers with a diameter of 20–40 nm and thick fibers with a diameter of 60–100 nm. The thick fibers were observed to be formed by coiling or twisting of the thin fibers. Furthermore, in some thin fibers, cross striations with a pitch of 10–15 nm were discernible, suggesting that the thin fibers were themselves made up of thinner subfibers. The nucleolus appeared as a roundish mass surrounded with chromatin components. Its cracked surface showed a compact structure which appeared to consist of thin fibrils with a diameter less than 10 nm. Isolation and observation of nuclei were repeated 4 times, and the results were always essentially the same. The present study demonstrated that the 60–100 nm fibers were formed by coiling or twisting of the 20–40 nm thin fibers composed of the 10–15 nm subfibers.

In order to examine whether the nuclei isolated by the present method maintain a chromatin structure similar to that *in situ*, comparative scanning electron microscopic observation was performed on the chromatin of *in situ* nuclei of hepatic cells. The interior of the *in situ* nucleus is shown in Fig. 2. The chromatin was revealed as a three-dimensional meshwork composed of thin (20–40 nm) and thick (60–100 nm) fibers. The thick fibers were formed by coiling or twisting of the thin fibers. A similar structural organization of chromatin was previously demonstrated by Tanaka and Iino [5] who examined the cracked surface of rabbit hepatic cells with a high resolution scanning electron microscope.

Fig. 1. Freeze-cracked surface of a nucleus isolated from chicken liver. N: nucleolus, a: thick fiber, b: thin fiber. Bar indicates $0.5\ \mu\text{m}$. $\times 40,000$.

Fig. 2. Freeze-cracked surface of an *in situ* nucleus in a chicken hepatic cell. Arrowheads denote thick fibers formed by thin fibers. N: nucleolus. Bar indicates $1\ \mu\text{m}$. $\times 25,000$.

The present study revealed that the chromatin observed in the isolated nuclei was very similar in appearance to that examined *in situ* in the hepatic cells, indicating that the *in situ* chromatin structure was well preserved in the isolated nuclei by the present method. In morphological as well as biochemical studies of chromatin, the isolated nuclei can be employed as useful materials. They have an advantage over the *in situ* nuclei that the chromatin can be investigated under a wide variety of experimental conditions. For example, effects of irradiation with ultraviolet or X-rays on the chromatin are studied without disturbance of cytoplasmic components. Furthermore, the chromatin in the isolated nuclei seems to be more identical in structure and function with that in *in*

situ than the extracted chromatin from the nuclei.

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要 約

ニワトリ肝臓の単離核の走査電顕による観察(短報): 荒井惣一郎・中西 宥(酪農学園大学放射線獣医学教室)
——ニワトリ肝臓から単離した核を固定後凍結切断し、走査電顕により観察した。クロマチンは直径 20~40nm の細い線維、およびその回旋またはよじれ合いにより形成された直径 60~100nm の太い線維から構成され、クロマチンの構築は、細胞核内でのそれとほとんど同様であった。