QUANTITATIVE ELECTRON MICROSCOPE RADIO-AUTOGRAPHY OF INTRAMITOCHONDRIAL NUCLEIC ACID SYNTHESIS

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The present author has been studying the nucleic acid synthesis in the mitochondria of various vertebrate cells *in vitro* by means of electron microscope radioautography. Among the problems concerning these studies, this paper deals with the stochastic analyses on the problems of quantitative techniques.

When cells were incubated in media containing either ^3H -thymidine or ^3H -uridine as the precursors and fixed in glutaraldehyde and osmic acid, embedded in Epon, sectioned, radioautographed with Sakura NR-H2 emulsion, exposed and developed in elon-ascorbic acid developer after gold-latensification, the sites of DNA synthesis (Fig. 1) and RNA synthesis (Fig. 2) were indicated by the appearances of comma-shaped (Figs. 1 & 2) or dot-shaped (Fig. 3) silver grains with the diameters of 300–800 Å according to the developing conditions. The resolution of the electron microscope radioautograms has been discussed by many authors. However, there have been discrepancies between them. Caro (1962), Salpeter *et al.* (1969) and Nadler (1971) maintain the resolution to be 1000–1600 Å, while Uchida and Mizuhira (1971) insisted a smaller range as 100 Å. According to the present author's experience, the appropriate range seemed to be 250 Å, a radius of the silver grains developed by elon-ascorbic acid developer (Nagata, 1972).

As for the localization of nucleic acid synthesis in the mitochondria, there exist two problems, first the localization of silver grains in the mitochondrial compartments and second the classification of labeled and unlabeled mitochondria. Deciding the intramitochondrial localization of incorporated silver grains, the localization can be classified into four compartments, i.e. matrix (A), crista (B), mitochondrial membrane (C) and outside of the mitochondria (D). By deciding the localization of grains in previous studies, a silver grains touching any membrane was supposed to be due to the membrane (Figs. 3 & 4). When HeLa cells were labeled with ^3H -uridine, a model of an averaged mitochondrial section can be depicted as in Fig. 4, having an ellipsoidal contour with a long axis of 8124.72 ± 865.31 Å and a short axis of 6231.29 ± 573.93 Å and 2.14 ± 1.35 cristae. By counting the number of silver grains appeared in 300 mitochondria from 30 photomicrographs taken at random, the number of grains appeared in A, B, and C (designated as x_i) resulted to be 10.8; 4.7; 5.5. However, when the theoretical probabilities were computed from the model on the assumption that the grains could appear with the same probabilities in the three unit square compartments, the probabilities of the three compartments (designated as p_i and $\sum p_i = 1$) were 0.4779; 0.1775; 0.3446. On the other hand, when expected values of these three were calculated as $y_i = N p_i$, where N was expressed as $N = \sum x_i$, we can test the null hypothesis $H_0 : p_1 : p_2 : p_3$ with χ^2 -goodness-of-fit test, where $\chi_0^2 = \sum \frac{(x_i - y_i)^2}{y_i}$ had

the distribution of chi-square with $f=n-1$ degree of freedom. As the result, $\chi_0^2=102.505 > \chi^2(0.001)=13.815$ was obtained. Therefore, the observed value of A (x_1) appeared significantly less than the anticipated value (y_1) and the hypothesis was rejected, which means that the observed value of B and C contained A. Likewise, the stochastic analysis revealed that the silver grains observed on the crista and the membrane were mostly due to the mitochondrial matrix. From these results, it was concluded that the silver grains localized in the mitochondria were due to the incorporation of nucleic acids in the mitochondrial matrix.

The second problem on the classification of labeled and unlabeled mitochondria will also be discussed and some examples of stochastic analysis on cultivated mouse kidney cells and HeLa cells labeled with either ^3H -thymidine or ^3H -uridine will be shown.

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FIG. 1. Electron microscope radioautogram of HeLa cell incubated in a medium containing 20 $\mu\text{C}/\text{ml}$ ^3H -thymidine for 3 hr. $\times 6,720$. N: nucleus, M: mitochondria.

FIG. 2. Electron microscope radioautogram of a HeLa cell incubated in a medium containing 20 $\mu\text{C}/\text{ml}$ ^3H -uridine for 2 hr. $\times 5,950$. Nc: nucleolus.

FIG. 3. Electron microscope radioautogram of part of a HeLa cell cytoplasm incubated in a medium containing 20 $\mu\text{C}/\text{ml}$ ^3H -uridine for 3 hr. Three mitochondria with silver grains can be seen. $\times 32,000$.

FIG. 4. Diagram showing a model of an averaged mitochondria in HeLa cells labeled with ^3H , indicating four types of silver grain localization in the mitochondria. Dark zones sandwiching the membranes and cristae indicate the resolution boundary of the radioautogram.