

FINE STRUCTURES OF INTERPHASE NUCLEI

IV. The Behavior of Late Replicating Chromatin during a Late Portion of the S Period as Revealed by Electron Microscope Radioautography

TSUNEYOSHI KUROIWA and NOBUNORI TANAKA. From the Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan

INTRODUCTION

Radioautographic studies at the electron microscope level have suggested that DNA synthesis is active in regions of dispersed chromatin but not in regions of condensed chromatin (1-5). However, it is not yet clear how the replicated DNA in such regions becomes organized into mitotic chromosomes. In *Crepis capillaris*, the S phase nuclei were classified into three periods: early S, mid S, and late S periods, on the basis of morphological distinctions and differences in incorporation patterns of thymidine-³H.¹ A comparative

analysis of the grain counts among these types has revealed that the DNA replicated in a late portion of the S period was condensed more rapidly into chromonema-like dense chromatin than that replicated either in mid S or in early S period.¹

In the present experiments, aimed at learning by what morphological steps the chromonema-like dense chromatin was organized, the behavior of the DNA which was replicated during a late portion of the S period was studied by means of electron microscope radioautography.

¹ Kuroiwa, T., and N. Tanaka. 1971. Fine structures of interphase nuclei. III. Asynchronous condensation

of DNA replicated during various portions of the S-period as revealed by electron microscopic autoradiography. Manuscript in preparation.

MATERIALS AND METHODS

Seeds of *Crepis capillaris* were germinated on wet filter paper in Petri dishes according to the method described previously (6). When the primary roots were approximately 4 mm in length, the seedlings were transferred to dishes which contained a thymidine-³H aqueous solution. After immersion in a 50 μ Ci/ml (SA 25.3 Ci/mole) thymidine-³H aqueous solution for 10 min, the roots were removed from the isotopic solution and, after washing in distilled water, they were placed in a nonisotopic solution which contained an excess amount of unlabeled thymidine (100 times the concentration of the labeled thymidine) and were harvested 0, 10, and 20 min later. They were fixed in a 6% glutaraldehyde solution buffered with phosphate at pH 6.8 for 2 hr. After washing in buffer solution at pH 6.8, they were postfixed in a 1% OsO₄ solution buffered with phosphate at pH 6.8 for 12 hr. They were dehydrated in a graded series of ethanol and propylene oxide, and finally embedded in Epon (7). Ultrathin sections were cut longitudinally on a Porter-Blum microtome with a glass knife and were mounted on grids which were coated with a Formvar membrane (Chicago, Ill.). Then, radioautographic preparations were made according to the procedures employed previously (8, 9).

RESULTS

Fig. 1 shows an electron micrograph of a portion of the nucleus fixed immediately after thymidine-³H treatment for 10 min. The chromonema-like dense chromatin masses form a network, and are distributed randomly throughout the nucleoplasm. On the other hand, the distribution of silver grains is not random; i.e. silver grains occur in groups in certain areas of the nucleoplasm. As seen in the left half of Fig. 1, a number of silver grains are located over the electron-transparent regions (long arrows), while others are clustered over the peripheral regions of the homogeneous, dense chromatin masses. It will be noticed that over some parts of the peripheral region of the dense chromatin masses many grains form condensed flocks, as indicated by short arrows in the

lower right portion of the figure. Such dense flocks of grains appear localized in regions of the nucleoplasm which have small numbers of silver grains over the electron-transparent areas.

Similar distribution patterns of silver grains are observed in the late S period nuclei fixed 10 min after thymidine-³H treatment, except that condensed flocks of grains appear more frequently. These results may indicate that almost all of the DNA synthesis occurs in the dispersed chromatin, as described previously (1-5), and in the adjacent peripheral region of the dense chromatin areas, and also that some of the DNA replicated in the latter areas is reorganized rapidly into the peripheral regions of the dense chromatin masses.

Fig. 2 *a* shows an electron micrograph of a nucleus fixed 20 min after thymidine-³H treatment. The distribution pattern of the chromonema-like chromatin and of several groups of silver grains in the left half area of the nucleoplasm is similar to that of the nucleus illustrated in Fig. 1. However, the density of the grains is heavier in Fig. 2 *a* than in Fig. 1.

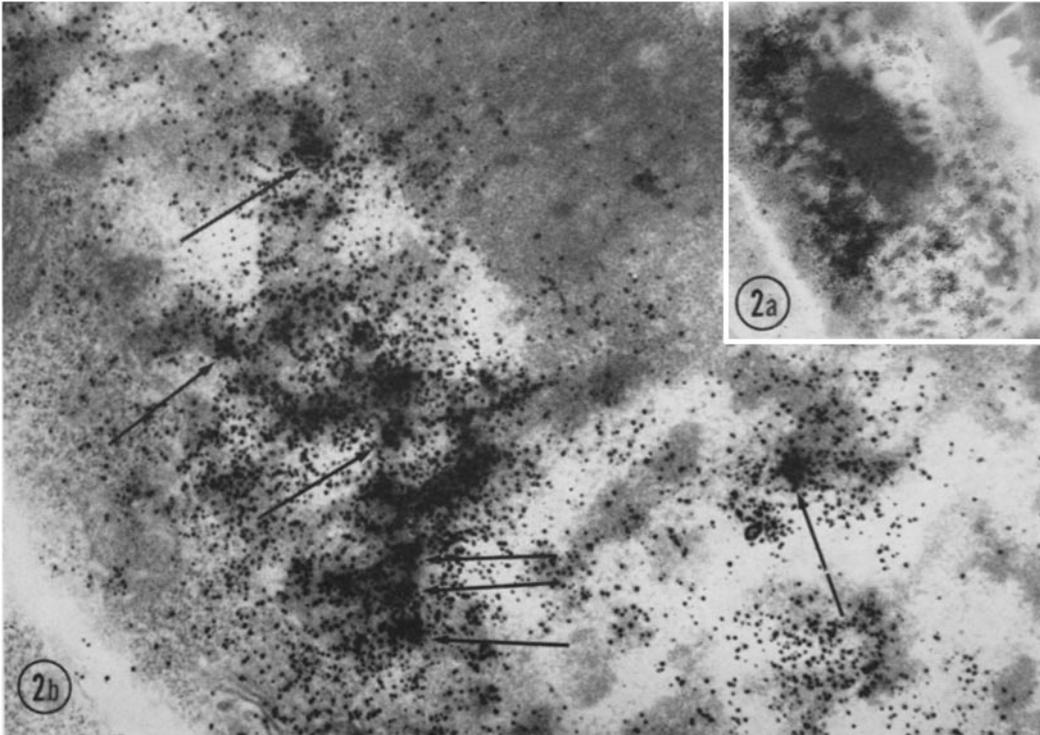
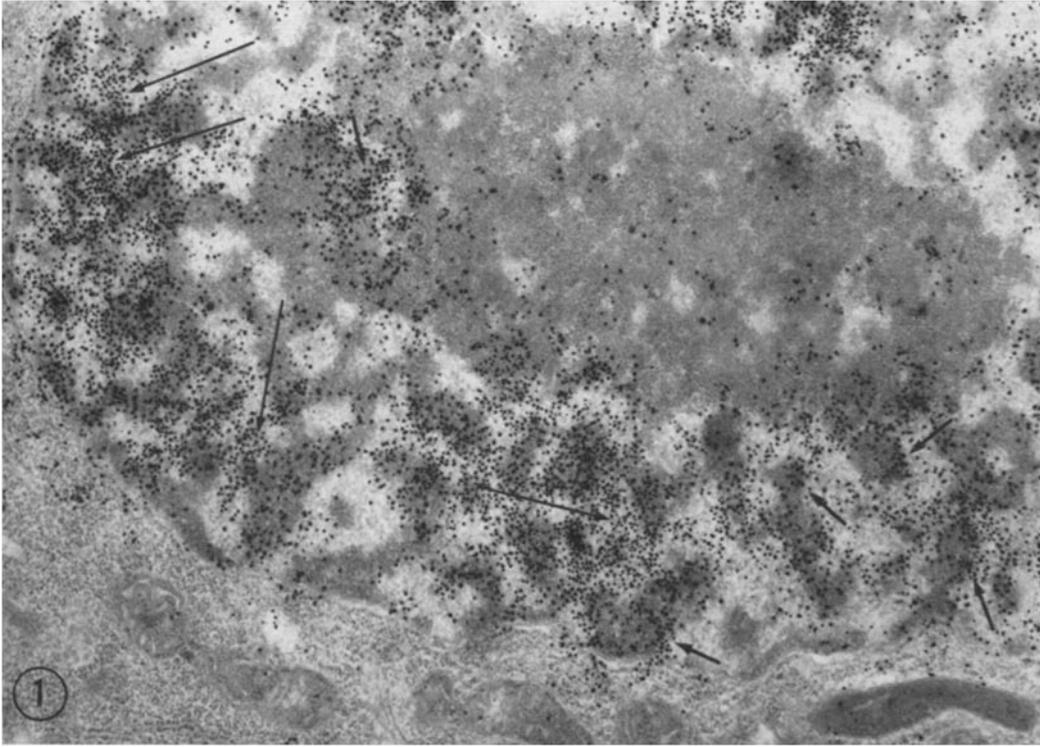
Fig. 2 *b* is a higher magnification micrograph of a portion of Fig. 2 *a*. It is quite clear that a number of silver grains are clustered over the dense chromatin masses, and in several places the dense chromatin masses are covered with the dense flocks of grains (long arrows). This observation suggests strongly that the DNA replicated during the late portion of the S period is reorganized into chromonema-like chromatin in about 20 min. And further, the differential appearance of heavily labeled regions in the dense chromatin may reflect the presence of independently replicating units in the chromonema.

DISCUSSION

It has been observed in synchronized KB cells that during a late portion of the S period the nuclear periphery is more active in DNA synthesis than are other parts of the nucleus (4). However,

FIGURE 1 Electron micrograph of the late S period nucleus fixed immediately after thymidine-³H treatment for 10 min. A number of silver grains are distributed over some of the electron-transparent regions (long arrows) and the peripheral areas of dense chromatin masses (short arrows). $\times 16,500$.

FIGURES 2 *a* and 2 *b* Electron micrographs of late S period nuclei fixed 20 min after thymidine-³H treatment. A few groups of silver grains are observed over the nucleus (Fig. 2 *a*). At higher magnification (Fig. 2 *b*), the condensed flocks of grains can be seen over the dense chromatin masses (long arrows). Fig. 2 *a*, $\times 5100$, Fig. 2 *b*, $\times 25,400$.



this work has dealt with the irregular-shaped nuclei of animal cells with irregular and marginated chromatin masses. In the present experiments, however, in which typical plant "reticulate nuclei" were used, such peripherally localized patterns of incorporation were not found.

By two techniques, i.e. acetic orcein staining and radioautography, it has been shown that heterochromatic segments in mitotic chromosomes of *Crepis capillaris* corresponded to the late replicating region (6). Accordingly, the appearance of the silver grains over the electron-transparent region of nuclei fixed immediately after thymidine-³H treatment suggests that the late replicating heterochromatic segments of mitotic chromosomes are decondensed to dispersed chromatin and are replicated during a late portion of the S period. Some of the replicated DNA in the dispersed chromatin may have reorganized to form peripheral regions of the dense chromatin mass in several minutes, and may have condensed further to the chromonema-like chromatin, since in the nucleus fixed immediately after thymidine-³H treatment for 10 min a great majority of silver grains had shifted to the peripheral region of the dense mass. In fact, in preparations fixed 10 min after thymidine-³H treatment the grains increased in number, and after 20 min of incubation many flocks of silver grains, in fact almost all grains, appeared over the dense chromatin masses.

Furthermore, the presence of patchlike, condensed flocks of silver grains over the chromonema-like chromatin in nuclei fixed 20 min after thymidine-³H treatment indicates that there are many units of replication along the chromonema-like chromatin.

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