DNA SYNTHETIC PATTERN IN THE NUCLEOLUS

II. Chinese Hamster Cells

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In the preceding note (2), we showed an asynchronous pattern of DNA synthesis in the nucleoli of chicken fibroblast cells, in which the rRNA cistrons (about 100 per genome) (10) are accumulated on one chromosome (12). We reported that in these cells nucleolar DNA synthesis takes place twice in the S phase, once at about the 4th hr and again at about the 10th hr, the total length of the S phase being 11 hr. However, we could not decide whether DNA in one of two nucleoli duplicates at the early S phase and in the other at the late S phase, or whether DNA in both nucleoli duplicates at both phases but asynchronously. That means: part of both polycistronic organizing regions may replicate at the early S phase, and the rest during the late S phase; in addition, it must be assumed that in both phases the respective segments of both chromosomes replicate one after the other.

As was already mentioned in the previous paper, Kasten and Strasser (6) reported labeling of nucleoli, asynchronous with the majority of autosomes, twice during the S phase: once at the early, and again at the late, S phase. For their investigations, those authors used a human tumor cell line (CMP). They did not mention that any asynchrony of labeling between nucleoli within one nucleus was observed. The photographs presented in their paper and in the other papers (3, 8) show, so far as we were able to see, labeling over both discernible nucleoli. In the human genome, the number of chromosomes which carry the information for the synthesis of rRNA is, so far, not precisely known. According to different sources (see 5, 11), it is supposed that in the diploid cell up to 10–12 nucleolus-producing sites are present. When cells that possess so many rDNA-regions have only two distinct nucleoli, nothing can be said about asynchrony in DNA synthesis between different organizing regions.

To get more information as to whether the phenomenon of asynchrony in nucleolar labeling with thymidine-^{3}H may also be found in other species, we decided to work with cells of the Chinese hamster, in which, in general, the cells show two to six nucleoli.

Phillips and Phillips (7) have reported that in this species nucleolus-producing sites are located

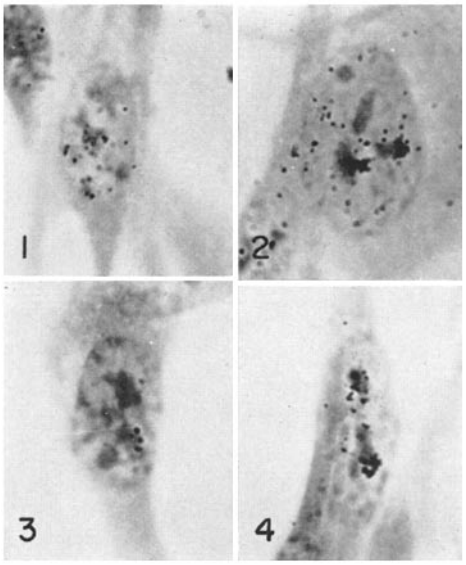

FIGURE 1 Nucleus with five nucleoli. Only three of them are labeled.

FIGURE 2 Tetraploid cell with four nucleoli. Only two of them are labeled.

FIGURE 3 Nucleus with three nucleoli, only one of which is labeled.

FIGURE 4 Nucleus with two nucleoli. Both are labeled. The lower one seems to be a fusion-product of smaller nucleoli.

Magnification of Figs. 1–4, 1,200.

on almost all of the 11 chromosomes of the genome. They were able to produce multimicronucleated cells by the action of colchicine, and they observed that, in most—if not all—of the micronuclei, nucleoli were visible which could not be distinguished from normal nucleoli either by light microscopy or by electron microscopy. In a normal diploid nucleus, however, not more than six to ten nucleoli are usually found. This phenomenon may be explained in different ways, as mentioned in the preceding paper (2): either a fusion of nucleoli may take place (1, 4), or some of the regions that are able to code for rRNA may be in an inactive state, probably heterochromatized (7, 9).

The cells were grown as monolayer cultures on tube slips. A 5-min pulse with thymidine-^{3}H (final concentration 1 μc/ml of the medium) was used to label the nucleoli. It was followed by a chase with a 40-fold stronger, concentrated solution of nonradioactive thymidine. The cells were fixed after 1 hr. (For further procedures, see the preceding paper (2)).

50 cells in which labeling was mainly confined to the nucleoli were used for the following observations: in no case, in cells exhibiting more than two nucleoli, were all the nucleoli found to be labeled. In most cells, some of the nucleoli were labeled, whereas the other nucleoli belonging to the same nucleus were without any label. However, in the cells showing two nucleoli, either both or none of the nucleoli were found to be labeled.

Fig. 1 exhibits a nucleus with five nucleoli (the upper nucleolus probably represents two smaller fused nucleoli). Only three nucleoli are labeled. Fig. 2 shows a cell, probably tetraploid, with four nucleoli. Here labeling is confined to two nucleoli. Fig. 3 represents a nucleus with three distinct nucleoli, of which only one is labeled. In Fig. 4, both nucleoli of the nucleus are seen to be labeled. At least one of these two nucleoli has a morphological appearance which indicates that it has arisen by fusion of two, if not more, smaller nucleoli, one of which is definitely devoid of any labeling.

The results are thus in clear agreement with our previous findings in chicken cells, that DNA synthesis in the nucleoli takes place asynchronously. This speaks in favor of the assumption that some of the nucleolus-organizing regions are involved in replication but that others continue to synthesize rRNA.

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