COMPARISON OF INCORPORATION OF DNA PRECURSOR AT- AND GC-BASE PAIRS INTO CHINESE HAMSTER CHROMOSOMES

L. P. Tupitsyna

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The chronology of DNA synthesis at the end of the S-period of the mitotic cycle was studied autoradiographically in chromosomes of a culture of Chinese hamster fibroblast-like cells with the use of thymidine-H^3 and deoxycytidine-H^3. Qualitative analysis of the autoradiographs showed that no segments distinguished by predominant incorporation of either precursor could be found among the late-replicating regions of the chromosomes by this method.

KEY WORDS: thymidine-H^3; deoxycytidine-H^3; autoradiography; chromosomes.

The overwhelming majority of autoradiographic studies of the chronology of DNA synthesis in chromosomes have been carried out with thymidine-H^3, a precursor of the AT-base pairs of DNA, and the time of replication of the AT-containing regions has been regarded as the replication time of the DNA as a whole. However, the use of only one labeled precursor cannot reflect the true chronology of replication if the chromosomes contain sufficiently large segments rich in GC-pairs. Data in the literature on the distribution of the different pairs of nitrogenous bases in late-replicating chromosomal DNA are contradictory [1-4, 6, 7, 9, 10].

It was decided to investigate this problem in Chinese hamster chromosomes using tritiated thymidine and deoxycytidine as labeled precursors.

EXPERIMENTAL METHOD

An aneuploid culture of Chinese hamster fibroblast-like cells (original line B 11d-ii-FAF-28, clone 400 B) was used. The cells of this clone have a hypodiploid chromosome set with modal number 20, with 4 chromosomes containing large late-replicating regions. The karyotype studied differed from that described previously [11] by structural changes in some chromosomes.

Thymidine-H^3 (specific activity 11 Ci/mmole) or deoxycytidine-H^3 (specific activity 10 Ci/mmole) was added to parallel cell cultures in a dose of 0.3 μCi/ml (both compounds from "CEA," France) 4 h before fixation, i.e., at the end of the period of synthesis. Colchicine was added in a dose of 0.5 μg/ml 1.5 h before treatment. The technique of autoradiography corresponded to that described previously [11].

EXPERIMENTAL RESULTS

The character of incorporation of thymidine-H^3 corresponded to that described previously [11] for chromosomes not undergoing structural changes. The distribution of the late deoxycytidine-H^3 label did not differ appreciably from that observed after the addition of thymidine-H^3 (Fig. 1). In addition to the 4

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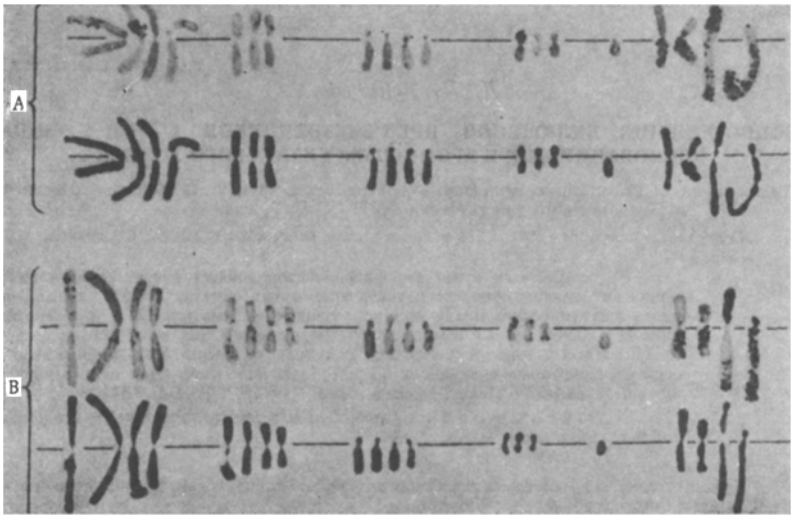

Fig. 1. Distribution of late label in Chinese hamster chromosomes after the use of thymidine-H^3 (A) and deoxycytidine-H^3 (B). In each example (here and in Fig. 2) top line shows arrangement of metaphase plates with characteristic pattern of distribution of late label; Bottom line shows the same plate after removal of the label.

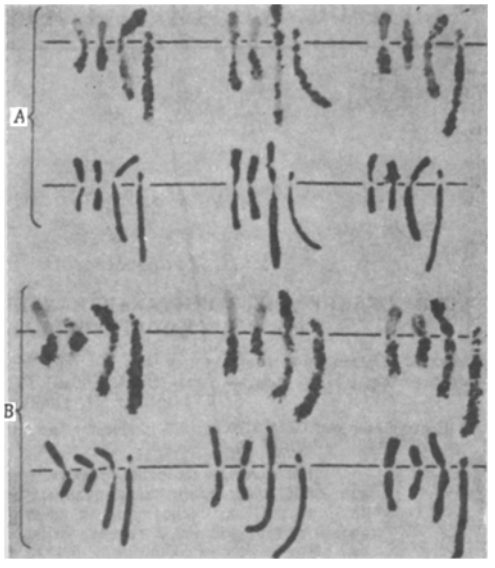

Fig. 2. Marker and sex Chinese hamster chromosomes with characteristic pattern of late labeling after addition of deoxycytidine-H^3 (A) and thymidine-H^3 (B). Sample of chromosomes from 3 cells. Marker and sex chromosomes arranged in the same order as in Fig. 1.

chromosomes containing large, late-replicating regions, late labeling also occurred in the terminal region of one arm of the first chromosome and in one of the small metacentric chromosomes. The character of labeling along the length of the chromosomes remained the same from one cell to another, and differed only in its intensity (Fig. 2). The label was distributed strictly locally, revealing regions of late-replicating chromosomal DNA.

On the basis of qualitative assessment of the distribution of the label it was concluded that the late-replicating regions of the Chinese hamster chromosomes, at least in the cell line used, have no sharp differences in the pattern and intensity of labeling with the precursor of the thymine nucleotide compared with the precursor of cytosine. It is considered that this observation reflects the absence of significant predominance of AT- or GC-base pairs in the regions of the chromosomes large enough to allow evaluation by optical autoradiographic methods and passing through the stage of reproduction in the second half of the S-period.

These results agree with information in the literature showing that satellite DNA cannot be found in the DNA of Chinese hamster cells [5] and that there is a very slight predominance of AT-pairs in the late-replicating fraction of DNA in this species of mammal [4]. It is relevant to note that no appreciable differences in the incorporation of thymidine-H^3 and of deoxycytidine-H^3 likewise have been found in human chromosomes by the autoradiographic method in the final stages of the S-period [8-9].

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