# REPLICATION TIME OF HUMAN CHROMOSOMES STUDIED WITH THE AID OF 5-BROMODEOXYURIDINE

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Cultures of peripheral blood lymphocytes from normal persons of both sexes were treated with 5-bromodeoxyuridine (BDU; 20 μg/ml), 5-fluorodeoxyuridine (FDU; 0.1 μg/ml), and uridine (2 μg/ml). Chromosome preparations were obtained 5 h later, incubated in a solution of the fluorochrome Hoechst 33258, and then stained with a 2% solution of Giemsa's stain in phosphate buffer, pH 5.0. Segments of the chromosomes that incorporated BDU stained weakly with Giemsa's stain, as a result of which the late-replicating regions showed up in each chromosome as weakly stained segments. The late replication pattern of DNA in all human chromosomes was described on the basis of this method. The method is much superior in resolving power to autoradiography.

KEY WORDS: 5-bromodeoxyuridine; chromosomes; differential staining; DNA replication.

All that is now known about the order of DNA replication in the chromosomes of higher organisms has been learned through the use of a single method, autoradiographic determination of the incorporation of labeled DNA precursors. Because of its low resolving power when used with metaphase chromosomes, this method has not been able to provide accurate information on the localization of segments differing in their replication time in chromosomes.

The writers previously demonstrated delay in the condensation of segments of chromosomes incorporating 5-bromodeoxyuridine (BDU) in high concentrations at the end of the period of DNA synthesis. Because of the irregular condensation of the chromosome along its length, the chronology of reproduction of its segments can be assessed in this way [1, 3, 4]. However, the localization only of the largest regions with the greatest delay in replication has proved to be determinable on the basis of irregularity of condensation. Recently the inability of segments of chromosomes incorporating BDU to fluoresce after staining with the fluorochrome Hoechst 33258 has been demonstrated and in this way a difference in the replication time of X chromosomes in human female cells has been found [7, 8]. The technique became simplified when Perry and Wolff [9] showed that chromosome segments incorporating BDU and treated with Hoechst 33258 take up Giemsa stain only weakly.

The authors have made a first attempt to use the phenomenon of weakened staining of chromosomal material incorporating BDU in order to study the order of DNA replication along the length of all human chromosomes. The results of such an investigation for the final stages of the S period are given in this paper.

## EXPERIMENTAL METHOD

Cultures of human blood from two phenotypically normal donors of both sexes were used. Lymphocyte cultures were prepared in Eagle's medium with the addition of 10% group AB human blood serum and phytohemagglutinin (Difco P). After cultivation for 67 h the cultures were treated, 5 h before fixation, with BDU, 5-fluorodeoxyuridine (FDU), and uridine in concentrations of 20, 0.1, and 2 μg/ml, respectively. The last

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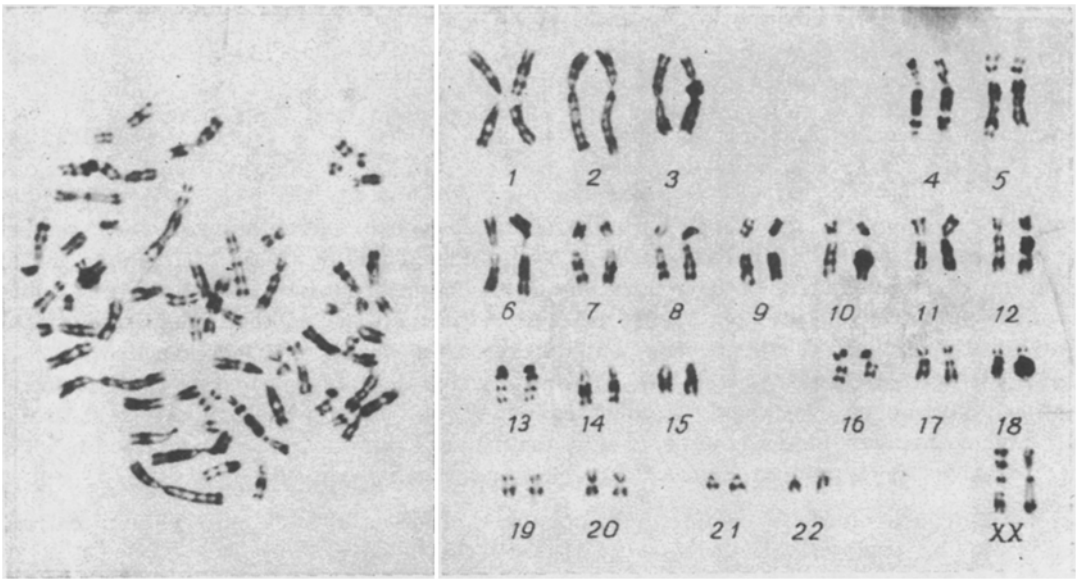


Fig. 1. Differential staining of human chromosomes (normal female karyotype) incorporating 5-bromodeoxyuridine in the final stages of the synthetic period. Unstained or weakly stained segments correspond to areas of late DNA replication.

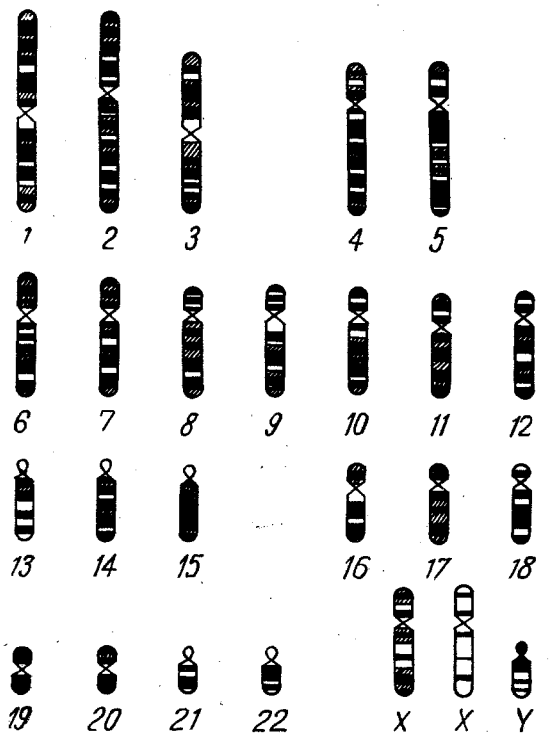


Fig. 2. Scheme of replication structure of human chromosomes in final stages of the synthetic period. Black and white segments correspond to areas of early and late replication; obliquely shaded areas correspond to intermediate times of replication.

two compounds were used to block incorporation of endogenous thymidine into DNA. Colcemid was added to accumulate metaphases 1 h before fixation, in a dose of 0.06 μg/ml. Chromosome preparations were made by the drying method. One half of each preparation was treated for 15 min with a solution of Hoechst 33258 (final concentration 0.5 μg/ml deionized water or physiological saline), and the other half acted as the control. The preparations were then washed in tapwater, mounted in deionized water or physiological saline under a coverslip, kept for a few hours in light, again rinsed with tapwater, and stained for 10 min with a 2% solution of Giemsa's stain made up in phosphate buffer (pH 5.0).

## EXPERIMENTAL RESULTS

Metaphase plates from the preparations treated as described above were stained differentially along their length (Fig. 1). In the control halves of the preparations the chromosomes of all metaphases were uniformly stained. Metaphases with differential staining often differed in the staining pattern of the same chromosomes, and metaphases with homogeneously stained chromosomes were seen. This picture is in good agreement with the nature of the staining; it is due to incorporation of BDU into the chromosomes, the degree of which varies from cell to cell because of their unsynchronized passage through the cell cycle. A careful study of several dozen metaphases under the microscope and in photographs showed that, within a certain range of variation, a most frequent pattern of arrangement of the stained (i.e., replicating before addition of BDU) and unstained (replicating in the presence of BDU) segments could be distinguished in each chromosome (Fig. 2). The following preliminary conclusion can be drawn from an analysis of this picture.

All human chromosomes are structures with linear differentiation into many segments with different DNA replication times. As a rule the segments are small. Each chromosome is characterized by its own specific pattern of arrangement of early- and late-replicating regions, and all human chromosomes can accordingly be easily individualized with reference to this feature. The largest unstained segments correspond to those shown to be late-replicating by autoradiography [5, 6] and described previously as taking part late in condensation under the influence of high concentrations of BDU [1, 2]. Chromosomes carrying such regions include autosomes 1, 4, 5, 6, 9, 13, and 16 and the X and Y sex chromosomes. Differentiation of human chromosomes in comparatively small segments measuring, as a rule, less than 1 μ on the basis of DNA replication time, detectable by this new method, explains why it has not been possible to describe the replication structure of human chromosomes sufficiently definitely by autoradiography.

Comparison of the replication structure of human chromosomes thus revealed with the pattern of P staining shows a large measure of agreement. This confirms the writers' earlier hypothesis of the important role of structural and functional differentiation of chromosomes in the formation of the differential staining pattern [2].

The results described above mark the first step in the study of the replication structure of eukaryote chromosomes by means of a new technique. The comparison of patterns of BDU incorporation at different stages of the S period can result in an accurate and dynamic description of the replication structure of chromosomes.

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