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Differential Repair Activity of Human Chromosomes

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The frequency of radioactive label (^{3}H-thymidine) incorporation in human lymphocyte chromosomes in the course of repair (extra) synthesis of DNA was assessed. Chromosomes 7, 12, and 21 were found to have a lower, and chromosome 22 a higher repair activity than was expected when a uniform incorporation of the label along the genome was hypothesized. Segments were detected that incorporated an increased number of labels in the course of extra DNA synthesis.

Key Words: *repair; human chromosomes; chromosome segments*

Differences in chromosome behavior in the course of replication, spiralization, and disjunction during cell division have been thoroughly studied [1,2]. There are reports of the differentiated activity of chromosomes in genetic processes related to the functioning of individual genes or to their interaction, which manifests itself in an increased frequency of sister chromatid exchanges and breaks at certain sites of chromosomes under the effect of mutagenic factors and substances "provoking" such phenomena [3,5,7]. As such data are accumulated, a picture of the complex behavior of chromosomes in the life cycle of the cell unfolds, observable by light microscopy using various methods. This picture is, however, incomplete because insufficient information is available about chromosome behavior in the course of extra DNA synthesis associated with repair of genome injuries. Such information would help assess the status of repair processes in the cell and its changes under the influence of both endogenous and exogenous factors.

This research was aimed at detecting differences in the activity of chromosomes or their individual sites in the course of repair DNA synthesis in the absence of mutagenic factors.

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MATERIALS AND METHODS

Whole blood of a healthy 30-year-old woman was used in the experiments. Newly isolated blood cells were incubated in a thermostat at 37°C in nutrient medium with ^{3}H-thymidine (final activity 20 μCi/ml) for 3 h. After incubation the cells were washed free of residual label and cultured in nutrient medium with phytohemagglutinin (PHA, PanEko, Moscow). After cell fixation and preparation of metaphase chromosomes, these preparations were coated with photosensitive emulsion and exposed in the dark at 4 C. After development of radioautographs the chromosomes were stained to detect G+ and G- segments. Chromosome identification and label scintillation and localization were carried out using microphotography and a map of the haploid set of chromosomes with 330 segments [4].

RESULTS

Microphotographs of 11 metaphase plates with a complete set of chromosomes were analyzed. A total of 890 labels were localized in chromosome

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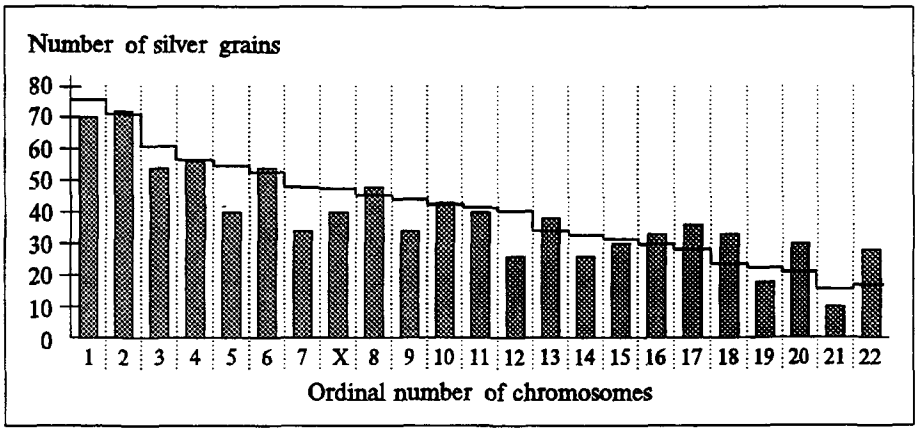

Fig. 1. Histogram of data on the number of silver grains in chromosomes. The line shows the expected number of labels estimated on the basis of data on the relative length of chromosomes.

segments, label incorporation being observed in all chromosome pairs. Figure 1 presents a histogram of label distribution by chromosomes. Of special interest is the label distribution observed in our experiment in comparison with the distribution expected proceeding from the hypothesis of a uniform distribution of labels depending only on the length of chromosomes. The χ^2 test demonstrated ($p<0.05$) that the number of labels was lower than expected in chromosomes 7, 12, and 21 and higher than expected in chromosome 22. Label was detected in 259 segments, the mean number of labels per segment (without zero values) being 2.90. The total number of labels per segment in 11 cells varied from 1 to 12. A complete picture of segment distribution by the number of labels in them is presented in Fig. 2. Statistical comparisons demonstrated that the value "6 labels" reliably ($p<0.05$) differs from the mean number of labels per segment (2.62 with consideration for the zero class), the χ^2 test being equal to 4.3 at freedom number 1. Hence, the 26 segments in which 6 or more labels are localized can be referred to as segments showing an increased activity in extra DNA synthesis (Table 1).

But since the length of chromosome segments varies, the number of labels per segment was reestimated with consideration for the length of the segment in order to draw correct conclusions about segment activity during label incorporation. The following formula was used: $W=(B/C)\times 5.34$, where W is the number of labels per segment of C (arbitrary U) length, B is the observed number of labels in a segment, 5.34 is the mean length of

TABLE 1. Segments with the Highest Number of Labels

Segment	Number of labels		Segment	Number of labels	
	observed	corrected		observed	corrected
1q31	6	2.7	13q13	6	10.7*
1q12	7	3.4	13q33	4	6.1*
1q31	9	3.1	15q26	6	4.6
2p16	6	4.0	15q21	10	6.7*
2q36	6	6.4*	16q12	7	12.5*
3q13	7	3.1	17q24	8	8.5*
3q26	9	4.4	18p11	6	2.9
5p14	7	4.4	18q22	6	4.0
6q22	9	4.6	18q23	6	7.1*
8q23	6	4.3	19q13	7	2.0
8q21	12	7.5*	20c	5	8.9*
9q21	7	3.7	20q13	7	3.6
10q25	6	4.6	22p13	4	7.1*
10q21	8	3.6	22q12	10	13.4*
11q14	6	4.3			

Note. An asterisk shows segments with a reliably higher number of labels in comparison with the mean value.

segments (arbitrary U) [6]. After correction the mean number of labels per segment came to 2.79.

Table 1 shows that some segments which had a reliably higher number of labels *vs.* the mean number but which were relatively longer were not included among the segments actively incorporating the label after correction. It is noteworthy that in 3 cases the number of labels after correction was appreciably higher than the number observed (22q12, 16q12, 13q13).

According to published data, out of the 330 segments analyzed, 109 segments contained 116 fragility sites (FS). Label incorporation was found in 80 sites with FS, or in 36% of segments incorporating the label, with 29% of labels being localized in segments with FS.

The mean number of labels in segments containing FS was 3.25, while that in segments without FS was 2.74. Distribution of the number of segments by the number of labels incorporated conformed to the negative binomial law, and therefore the nonparametrical Mann-Whitney's test was used to compare the mean values. The mean values of these two samplings reliably differed (p=0.03).

Use of a chromosome map with 330 segments helped minimize the effect of variations in segment length (no more than 6% in the present study) in different cells and chromosome homologs on the accuracy of label localization [8], an effect which becomes evident during more precise differentiation of segments (variability can be 10% or higher when there are 500 or more segments). However, insufficient differentiation of segments does not permit a comparative analysis of label distribution by G+ and G- segments, because the majority of segments on the 330-segment map are composite G+/-.

Since no data similar to our findings have previously been analyzed in the literature, investigations of the repair activity of chromosomes and segments in relation to sex and age and of the effects of specific factors of different nature promise to bear fruit.

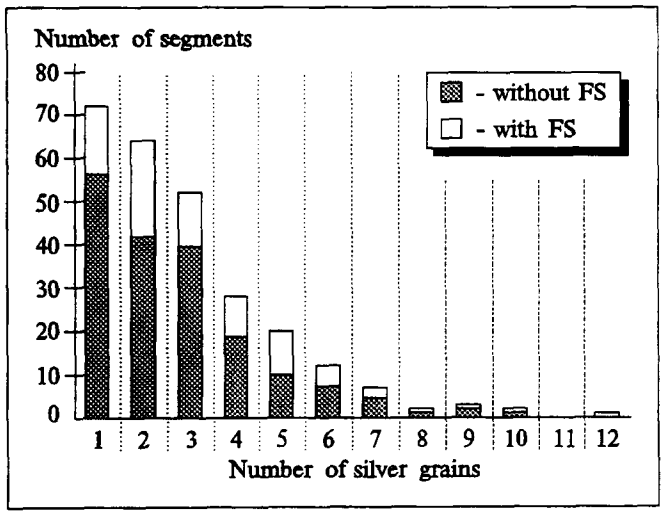

Fig. 2. Distribution of segments by the number of silver grains in them.

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