PREPARATION AND MOLECULAR CYTOGENETIC CHARACTERIZATION OF α-SATELLITE DNA OF HUMAN CHROMOSOME 6

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The structural heterochromatin of chromosomes of eukaryotes consists of "classical" satellite and α-satellite DNA. The study of genomes of primates, including man, has shown that α-satellite DNA consists of frequently repeated tandem arrays 340 base pairs (bp) long, including divergent monomeric sequences [8, 10]. Recent studies of α-satellite DNA have demonstrated its considerable heterogeneity and have isolated groups of alphoid DNA located on separate chromosomes [1, 11]. Determination of the primary nucleotide sequence of chromosome-specific subgroups of the α-satellite revealed the hierarchical organization of these DNA sequences. The repeating units of high order, determining specific hybridization, differ both in their divergent tandem monomers of α-satellite DNA and in the mutual arrangement of the monomers.

Attempts are currently proceeding on a wide scale to discover chromosome-specific alphoid DNA sequences which would not only allow the structural organization of heterochromatic regions of human chromosomes to be studied in more detail, but would also provide a new type of molecular markers, which could be used in medial genetics to determine linkage groups and in the prenatal diagnosis of several hereditary diseases [12].

The aim of this investigation was to obtain and study the localization, structural organization, and polymorphism of the α-satellite DNA of human chromosome 6.

EXPERIMENTAL METHOD

Human DNA was isolated from whole blood leukocytes by the standard method [2]. Deproteinization was carried out by successive extraction with water-saturated phenol, a phenol–chloroform mixture, and chloroform. Restriction hydrolysis of DNA was carried out with a threefold excess of enzyme in the corresponding buffer [3]. Electrophoresis was carried out in horizontal 0.8% agarose gels. DNA was labeled by the "nick translation" method [9], using the phosphorus-labeled precursor P-dCTP ($8.1 \cdot 10^7$ MBq/mmole). Probes for in situ hybridization were obtained by the use of three tritium-labeled nucleotides with specific radioactivity of $1.85 \cdot 10^6$ MBq/mmole. Labeled DNA was purified from nucleotides by gel-filtration on a column with Sephadex G-50 (fine). The specific radioactivity of the probes was $(1\text{-}5) \cdot 10^8$ cpm/μg of phosphorus-labeled DNA, and $(1\text{-}5) \cdot 10^7$ cpm/μg of ^{3}H-DNA. In situ hybridization was done by the method in [6], including denaturation of the preparations in 0.07 N NaOH for 2 min, hybridization for 18 h, washing under standard conditions, and exposure under Ilford emulsion for 10 days. Differential staining of the chromosomes was carried out with Wright's stain for 10 min.

EXPERIMENTAL RESULTS

Fragments of human DNA obtained after hydrolysis with restriction endonuclease Sau 3A were used to isolate chromosome-specific α-satellite DNA. Insertion of these DNA fragments into plasmid pIS 19 was carried out at the BamHI restriction site. The bacterial colonies were screened in two stages. In stage 1 colonies most of which had recombinant plasmids with alphoid DNA were selected. This selection was carried out by using the "nick-translated major" DNA fragment, 340 bp long, obtained after hydrolysis of total human DNA

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TABLE 1. Results of Hybridization of Clone HRS 180 with Chromosomes of Three Individuals

Individual	Mean number of grains of silver (in %)		
	chromosome 6	chromosome 1	remaining chromosomes
64-7*	53,8±3,2	10,8±2,7	35,5±2,8
66-1	61,2±3,2	18,1±2,5	20,7±2,7
66-5	59,2±3,3	23,2±2,8	17,5±2,5

Legend. *) Preparations washed off after hybridization under mild conditions.

TABLE 2. Frequency of Polymorphism of Restriction Fragments of α-Satellite of Human Chromosome 6 according to Results of Investigation of DNA from 35 Individuals

Serial No.	Polymorphic restriction fragments, kbp	Frequency, %
1	2,8	8,6
2	3,0	17,1
3	3,5	5,7
4	26,3	2,8
5	27,3	5,7
6	28,3	2,8
7	2,6, 2,8, 3,0	20,0
8	2,7, 3, 3,5	5,7
9	2,6, 2,7, 3,0	17,1
10	2,6, 2,7, 2,8, 3,0	11,4
11	—	2,8

by restriction endonuclease EcoRI, as the probe. In the second stage of the search clones containing α-satellite DNA were selected in accordance with the principle proposed by the writers previously, whereby tandemly organized repeats can be detected. As a result of this two-stage screening a collection of α-satellite chromosome-specific clones was obtained, of which one (clone HRS 180) was described in another paper.

The results of in situ hybridization with the cloned DNA sequence are given in Table 1. Analysis of the hybridization shows that the clone of α-satellite DNA thus obtained forms clusters in the centromeric region of chromosome 6. The principles of distribution of the grains of silver on metaphase chromosomes of several individuals are summarized in Table 1. As Table 1 shows, we obtained a subgroup of α-satellite DNA localized mainly in the centromeric regions of chromosome 6.

To obtain some idea of the hierarchical organization of the cloned DNA sequence in the genome, the blot hybridization method was used. By this method it is possible not only to estimate the size of the DNA sequence studied, but also to determine the classes of restriction enzymes represented by the largest number of copies. Sequences with the highest copy number and identical length during hydrolysis by several restriction endonucleases, will evidently be close to high-order repeated units, determining the specific chromosomal organization. DNA from one individual, hydrolyzed by different restriction endonucleases, were used in blot hydbridization. Clearly the largest number of copies was possessed by two fragments, one of which was found after hydrolysis by restriction endonuclease BamHI, and measuring 3.4 kbp, the other after hydrolysis by restriction endonucleases Eco RI and EcoRII, and 1.6 kbp long. The results of blot hybridization thus suggested that the length of the high order repeated unit was close to 1.6 or 3.4 kbp. Similar data were obtained during cloning of α-satellite fragments relative to restriction endonuclease BamHI from a human gene bank [7]. The high-order unit for the clone which they obtained for chromosome 6 was found to measure about 3 kbp.

To assess the structural heterogeneity of this subgroup of the α-satellite, blot hybridization with samples of DNA from eight individuals was used. Total human DNA was hydrolyzed with restriction endonucleases EcoRI, EcoRII, EcoRV, HindIII, and PstI, and this was

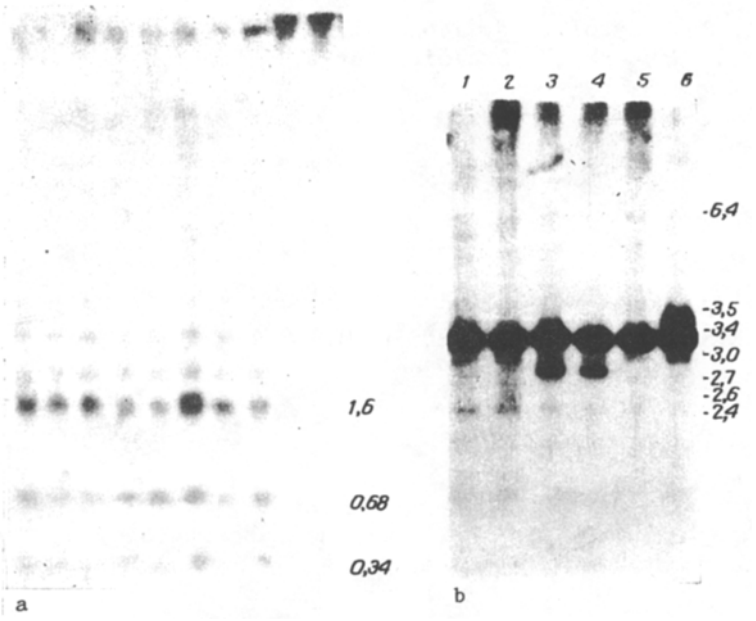

Fig. 1. Results of blot hybridization of clone HRS 180 with samples of DNA from different individuals. a) Eco RI; b) BamHI. Arrows indicate polymorphic restriction fragments (their size is given in kbp).

followed by blot hybridization with clone HRS 180. The results of one such hybridization of the clone thus obtained with DNA hydrolyzed by restriction endonuclease EcoRI is shown by way of example in Fig. 1. The results are evidence of conservatism both of the set of restriction variants and of the relative number of copies of each restriction fragment. Similar results also were obtained for all the restriction endonucleases studied except BamHI. When restriction endonuclease BamHI was used the individual restriction fragments in DNA samples from different individuals were found to differ both in length and in copy number (Fig. 1).

The results of investigation of the distribution of polymorphic fragments in samples of DNA from 35 individuals, not linked by birth, are given in Table 2. Either separate restriction fragments or combinations of them, which could serve as DNA markers, were observed in the DNA samples studied. Thus, when restriction endonuclease BamHI and probe HRS 180 were used, a considerable spectrum of polymorphic DNA fragments was found in DNA from different individuals, with frequencies that are usually employed in the analysis of linkage groups [4].

Results indicating that connection exists between chromosomal aberrations involving chromosome 6 and melanoma development have been published [5]. However, these results have not yet been confirmed by linkage studies using polymorphism for length of restriction fragments of DNA. The clone of the subgroup of α-satellite DNA of chromosome 6 which we obtained reveals considerable polymorphism during blot hybridization and it can evidently be used to determine linkage groups with the melanoma gene.

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INDUCTION AND ELIMINATION OF CYTOGENETIC DISTURBANCES IN LYMPHOCYTES OF MONKEYS EXPOSED TO THIOPHOSPHAMIDE

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To evaluate correctly the results of testing for mutagenicity and to predict the mutagenic affects of external environmental factors it is necessary to describe the dose–time–effect relationships, i.e., the dynamics of the mutation process, which has two components: induction of mutations and elimination of mutations (or of primary injuries to the genetic apparatus). The rules governing induction of mutations have been studied sufficiently well, the principles of calculation of the increase in frequencies of cytogenetic disturbances and of determination of the maximal effect after exposure to chemical mutagens have been suggested [5], but the principles and methods of elimination of mutations in the living organism have not been adequately studied.

The aim of this investigation was to study induction and elimination of chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in monkeys over a long period after administration of thiophosphamide, and to create a mathematical model describing the time course of these processes.

EXPERIMENTAL METHOD

Experiments were carried out on four mature male rhesus monkeys given thiophosphamide by intravenous injection in a dose of 3 mg/kg body weight. For 6 months after injection of the compound, blood samples were taken at various time intervals for lymphocyte culture in the presence of 10 μg/ml of 5-bromodeoxyuridine, followed by analysis of frequencies of SCE in the second, and of CA in the first mitoses. The concentration of thiophosphamide was determined in blood samples obtained during the first 4 h after injection, by the nitrobenzylpyridine test [2].

To construct a mathematical model the approach suggested previously [1] was used. In accordance with this approach, induction and elimination of cytogenetic disturbances are described by curves of probability functions. In this work we used probability functions of a Weibull distribution. When the parameters of the model were defined, the time, expressed in days, was plotted on a logarithmic scale.

EXPERIMENTAL RESULTS

The results are evidence that the blood thiophosphamide concentration in monkeys falls exponentially after intravenous injection. Calculations show that during 10 h the thiophosphamide concentration fell approximately by 90%, and its elimination was virtually complete

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