MAPPING APOLIPOPROTEIN A-I AND CERULOPLASMIN GENES ON HUMAN AND MURINE CHROMOSOMES BY IN SITU HYBRIDIZATION WITH SPECIFIC HUMAN DNA PROBES

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Mapping human and animal genes by in situ hybridization on preparations of metaphase–chrometaphase chromosomes is one of the main activities on the borderline between modern cytogenetics and molecular biology. The number of investigations of this kind is rising rapidly with the intensive growth in the number of cloned genes and of anonymous DNA sequences in mammals and man [6, 9].

An advantage of this method is that it is possible not only to map both multicopy and unique structural genes on a particular chromosome, but also to localize them adequately, purely along the length of this chromosome, i.e., to find their position relative to the differential banding pattern of the corresponding chromosome. The writers showed previously that both homologous and heterologous hybridization is effective, i.e., that unique genes can be mapped with the aid of specific DNA probes of animals of a different species [1-3].

The aim of this investigation was, by the use of specific human DNA probes, to map by in situ hybridization unique structural genes of man and laboratory rats and mice: apolipoprotein A-I (Apo A-I), the main protein of the blood plasma high-density lipoproteins [4], and ceruloplasmin (CP), a glucoprotein of the α_2-globulin fraction of blood plasma, which plays a decisive role in the transport, metabolism, and elimination of copper [5].

EXPERIMENTAL METHOD

Experiments were carried out on preparations of metaphase (prometaphase) chromosomes from cultures of phytohemagglutinin-stimulated human blood lymphocytes and from bone marrow cells of laboratory rats and mice. Mice homozygous for one [Rb (9.19) 163H] or two different translocations of Robertsonian type [Rb (8.17) 1IEM, Rb (2.6) 4IEM] [1, 3] were used. Marker products of Robertsonian translocations are easily identified on chromosome preparations even without differential staining. Chromosome preparations were made by the standard method [1-3]. The following hybridization probes were used: two fragments of cDNA of human Apo A-I: I) plasmid pB-AI with a 900 bp cDNA insert, generously presented to V. S. Baranov by Dr. S. Humphries (England); II) plasmic pAI-113 with a 600 bp cDNA fragment of Apo A-I, generously presented to Academician of the Academy of Medical Sciences of the USSR A. N. Klimov by Dr. D. Breslaw (USA), and a cloned cDNA fragment of CP, isolated by the writers by immunoscreening from the human liver cDNA bank of λgt 11 expression vector (size of insert 2100 bp). The DNA probes were labeled in the nick translation reaction with ^{3}H-dTTP and ^{3}H-dATP. Specific activity of the labeled DNA preparations varied from $1.6 \cdot 10^6$ to $4 \cdot 10^6$ cmp/μg DNA. Hybridization in situ was carried out by the technique in [8] with some modifications [1-3]. After hybridization, M2 emulsion in a dilution of 1:1 was applied to the preparations, which were dried and exposed for 20-25 days. After development of the autoradiographs the chromosomes were differentially stained by the trypsin-Giemsa method. The number of grains of silver above the entire metaphase plate and above each visually identified chromosome separately was recorded. The relative number of grains of silver per unit length of chromosome was calculated on the basis of the ratio of the length of the chromosome to the total length of the female haploid set of chromosomes [1, 3].

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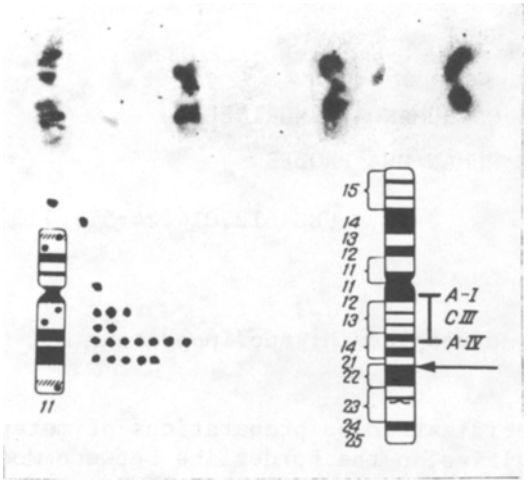

Fig. 1. Metaphase chromosome 11 (above) and diagram of differential banding pattern of this human chromosome (below) with typical arrangement of grains of silver (arrow). Hybridization with cDNA of human Apo A-I gene. Hypothetical location of cluster of apolipoprotein genes A-I, C-III, and A-IV after [6] is indicated. Here and in Figs. 2 and 3, staining by trypsin-Giemsa method.

EXPERIMENTAL RESULTS

The results of hybridization with the two Apo A-I DNA probes are in good agreement and they will therefore be examined together. Altogether 119 human methaphase plates, above which 1146 grains of silver were recorded (on average 9.6 per plate) were analyzed; 625 grains were immediately above chromosomes, and half of them (276) were linked with chromosomes of the C group ($X^2 = 9.2$). Much of the label (190 grains) in this group was accounted for by three small submetacentrics: 10, 11, and 12 ($X^2 = 129.7$). As may be judged from the chromosomes which preserved their ability to undergo differential staining, chromosome 11 was most strongly labeled. Another essential fact is that the distribution of label along its length was nonrandom. The presence of grains in the middle part of the long arm of this chromosome, or more precisely in the region between disks 14 and 22 between two blocks of intercalated heterochromatin (Fig. 1), was a characteristic feature. According to data in the literature, the Apo A-I gene is located at q13-ter on the map of chromosome 11 [6]. On

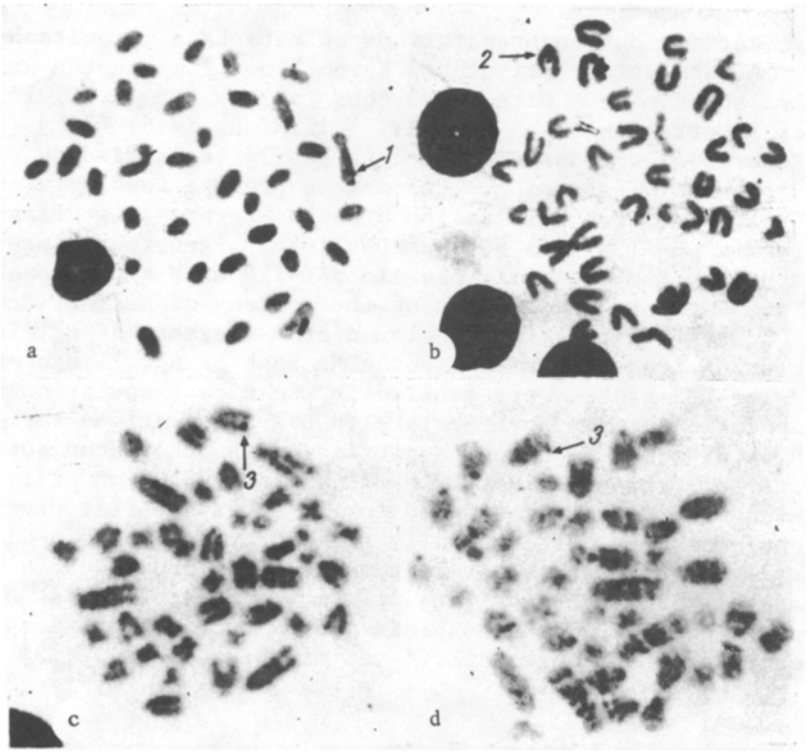

Fig. 2. Metaphase plates of mouse (a, b) and rat (c, d) bone marrow cells. Hybridization with cDNA of human Apo A-I gene. 1) Submetacentric 9/19 Ro 163H; 2) clusters of grains of silver; 3) label on rat chromosome 5.

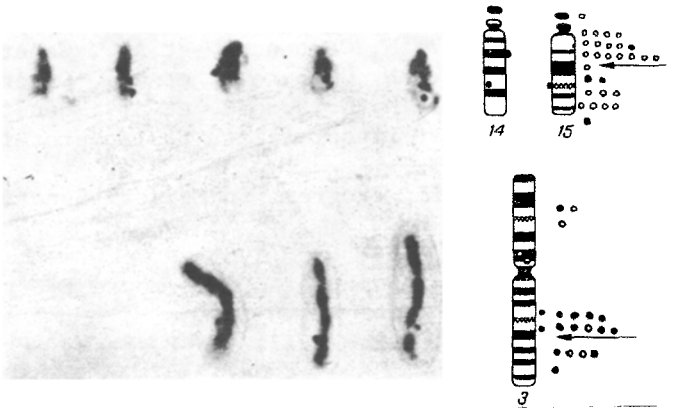

Fig. 3. Labeled metaphase chromosomes 15 (14) and chromosome 3 (above) and diagram of differential banding pattern of these chromosomes (below) with typical arrangement of grains of silver (arrow). Hybridization with cDNA of human CP gene.

the basis of our own data this gene and, consequently, the whole cluster of apolipoprotein genes, can be located not at 11 q 13, but rather more distally, at 11 q 14-22.

The mouse of Apo A-I gene was mapped previously on chromosome 9 [7], but its precise location was unknown. We analyzed 108 metaphases, above which 494 grains were counted. Along all identified chromosomes involved in the Robertsonian translocations, the largest number of grains (95) was observed above chromosomes 9 and 19, involved in the Rb 163H translocation (Fig. 2a). Most grains were concentrated in the juxtacentromeric zone of submetacentric 9-19 and in the upper third of chromosome 9 (Fig. 2b). These data suggest that the Apo A-I gene is located in the proximal part of chromosome 9 (9 A2-4).

The Apo A-I gene in rats has not previously been mapped. We analyzed 104 metaphases and counted 334 grains of silver, most of which (253) were linked with chromosomes. A highly significant excess of label above the background level was observed above chromosomes of the 4-6-X group ($X^2 = 28.7$). The level of label above chromosome 5 of this group was twice that expected theoretically ($X^2 = 8.1$). The distribution of label in the distal part of this chromosome (5 q 36) was typical (Fig. 2c, d).

Let us examine the data on CP. Altogether 119 human metaphases were studied, with 453 grains of silver counted above them. The distribution of label by karyotype was nonrandom. A significant excess of label above the background level was observed only for chromosome 3 ($X^2 = 11.4$) and chromosomes of the D-13 group ($X^2 = 25.5$) and 15 (14). Chromosome 3 was easily identified without differential staining, whereas chromosomes of the D group were much more difficult to distinguish from one another after the hybridization procedure. The location of the label in the middle part of the long arm of chromosome 3 (3 q 23-25), in the proximal part of chromosome 15 (14) and (15 q 13-20; Fig. 3), and in the distal part of chromosome 13 (13 q 23-25) was characteristic. These data agree with the results of mapping the CP gene by other methods of the 3 21-ter region [10, 11]. Meanwhile the results demonstrate high affinity of the DNA probe of human CP for acrocentrics of the D group. The same relationship was discovered previously by the writers for DNA probes of rat CP [3]. These data confirm the existence of a DNA sequence homologous to the CP gene on chromosomes of the D group and, in particular, on chromosome 15 (15 q 13-20).

In experiments on mice and rats 64 and 53 metaphases were studied and the location of 324 and 307 grains of silver respectively on the chromosomes was analyzed. No significant increase in the level of labeling above the background was found for any chromosome. Consequently, the specific human DNA probe used in this investigation was unsuitable for mapping the CP gene of rats and mice.

The investigations thus enable the Apo A-I gene to be located in region 11 q 14-22 in man, 9 A2-4 in mice, and 5 q 36 in rats. The most probable location of DNA sequences homologous to the CP DNA probe in man is 3 q 23-25, 3 q 24-24, and 15 q 13-20. Meanwhile, as our results show, the method of in situ hybridization with heterologous DNA probes does not always give reliable results for the location of individual structural genes on metaphase chromosomes.

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SIMULTANEOUS ANALYSIS OF SISTER CHROMATID EXCHANGES AND CELL CYCLE DELAY

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Analysis of sister chromatid exchanges (SCE) is a highly sensitive method of evaluating the mutagenic action of environmental factors [8]. The degree of indeterminacy associated with this method is due to uncertainty about the mechanism of SCE formation. One approach to the elucidation of the mechanisms of SCE formation is to use various chemical modifiers of DNA synthesis, replication, and repair. However, the use of such substances as a rule causes cells to be held up in a particular phase of the cell cycle. Meanwhile, we know that the level of inducible SCE depends strongly on the phase of the cell cycle and on the number of cell cycles elapsing between application of the agent and fixation [6]. This may perhaps explain why data on the effect of modifiers on the level of induced SCE at the end of the S and G_2 stage differ [7]. To avoid mistaken conclusions on the action of modifiers of DNA synthesis, replication, and repair, affecting changes in the duration of the cell cycle, during SCE analysis in these particular variants of the experiment delay of the cell cycle must be estimated. An appropriate technique for this may be the connection found previously between the phase of the cell cycle at which the cells are treated with 5-bromodeoxyuridine (5-BUdR) and the type of differential staining of the sister chromosomes in mitosis [5].

The aim of this investigation was to develop a method of simultaneous analysis of the frequency of SCE and evaluation of cell cycle delay under the influence of the mutagen and of modifiers of DNA synthesis, replication, and repair.

EXPERIMENTAL METHOD

Experiments were carried out on a transplantable culture of Chinese hamster cells (clone 237). Culture medium (Eagle's medium containing 10% bovine serum and 0.03% glutamic acid) in a volume of 1 ml, containing 200,000 cells, was introduced into each well of 24-well plastic dishes (Nune, Denmark). The cells were incubated in an atmosphere of CO_2 (5%). 5-BUdR in a dose of 10 μg/ml was added to the culture 24 h after seeding and 24 h before fixation. To induce SCE the cells were treated with $1.32 \cdot 10^{-6}$ M thiophosphamide (TP), which was added 24 h before fixation and allowed to remain until the end of culture. To evalu-

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