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Genetics

Chromosomal localization of the human apolipoprotein B gene and detection of homologous RNA in monkey intestine

(molecular hybridization/sorted chromosomes/metaphase cells/tissue specificity)

SAMIR S. DEEB*†, CHRISTINE DISTECHE†‡, ARNO G. MOTULSKY*†§, ROGER V. LEBO¶, AND YUET W. KAN¶

Departments of *Medicine, ‡Pathology, and §Genetics and †Center for Inherited Diseases, University of Washington, Seattle, WA 98195; and ¶Howard Hughes Medical Institute and Division of Genetics and Molecular Hematology, Department of Medicine, University of California, San Francisco, CA 94143

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ABSTRACT **A cDNA clone of the human apolipoprotein B-100 was used as a hybridization probe to detect homologous sequences in both flow-sorted and *in situ* metaphase chromosomes. The results indicate that the gene encoding this protein is on the distal end of the short arm of chromosome 2 (2p23–2p24). RNA isolated from monkey small intestine contained sequences (6.5 and 18 kilobases) homologous to the cDNA of apolipoprotein B-100. These results are consistent with the hypothesis that one gene codes for both the intestinal (B-48) and the hepatic (B-100) forms.**

Apolipoprotein B (apo B), the major protein of the human serum low density lipoproteins (LDLs), exists in two forms, designated apo B-100 and B-48. Apo B-100 is a glycoprotein of $M_r \approx 450{,}000$, synthesized primarily by the liver, whereas B-48 is about one half that molecular weight and is synthesized in the small intestine (1, 2). The two forms are antigenically related, and analysis of common epitopes suggested that apo B-48 represents the portion of apo B-100 that is not involved in binding to the LDL receptor (3). A cDNA clone encoding a part of the apo B-100 protein was recently isolated and characterized (4). The mRNA encoding this protein in the liver was shown to be approximately 18 kilobases long (4).

The availability of a cDNA clone made assignment and regional localization of this gene possible and allowed us to examine whether the two forms of apo B are encoded by one or two genes. In this report we demonstrate that the apo B gene is located on the distal end of the short arm of chromosome 2, at 2p23–2p24 and present evidence to support the hypothesis that the same gene codes for both forms of apo B.

MATERIALS AND METHODS

Preparation of Radiolabeled DNA Probe. Recombinant plasmid pHApoB44 (4) DNA was isolated and purified by centrifugation to equilibrium in cesium chloride/ethidium bromide density gradients (5). The probe used for *in situ* hybridization was labeled by nick-translation using [^{3}H]dCTP (60 Ci/mmol; 1 Ci = 37 GBq), [^{3}H]dATP (40 Ci/mmol), and [^{3}H]dTTP (80 Ci/mmol) (radioactive dNTPs from New England Nuclear), to a specific activity of 1.7×10^7 cpm/μg. The probe used for hybridization with blots of sorted chromosomes was labeled by nick-translation using [α-^{32}P]dCTP, to a specific activity of 1.5×10^8 cpm/μg.

Chromosomal *in Situ* Hybridization. Mitotic chromosome preparations were prepared from human peripheral blood lymphocyte cultures. Hybridization was carried out by a modification of the method described by Harper and Saunders (6). Chromosome preparations were treated with pancreatic ribonuclease A (100 μg/ml) at 37°C for 1 hr, denatured in 75% (vol/vol) formamide in 2× standard saline citrate (1× SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7) at 70°C for 2.5 min, and incubated overnight at 42°C with ^{3}H-labeled probe at concentrations of 2–12 ng/ml in 10% dextran sulfate/2× SSC/50% formamide/0.2% Ficoll/0.2% polyvinylpyrrolidone/0.2% bovine serum albumin/yeast tRNA (100 μg/ml) at pH 7.0. The slides were washed three times for 2 min in 2× SSC at room temperature, three times for 2 min in 50% formamide in 2× SSC at 39°C, and twice for 2 min in 2× SSC at 39°C. The slides were coated with nuclear track emulsion NTB2 (Kodak), exposed for 8–13 days at −4°C, and then stained with quinacrine mustard (500 μg/ml for 20 min). Photographs were taken under both fluorescent and transmitted visible light.

Analysis of RNA. RNA was isolated, by the guanidinium isothiocyanate method (7), from the small intestine of the monkey *Macaca nemestrina* (obtained from the Regional Primate Research Center at the University of Washington) within 20 min of death. Electrophoresis of RNA in agarose containing formaldehyde, transfer to nitrocellulose, and hybridization to radiolabeled apo B cDNA were as described (4, 5). HepG2 cells were grown in minimal essential medium supplemented with 5% fetal bovine serum. RNA was prepared from a culture, grown to about 75% confluence, by the above method.

RESULTS

Chromosomal Location of the apo B Gene. Chromosomal assignment of the apo B gene was done by hybridization to DNA from individual human chromosomes sorted by a dual-laser flow sorter (8). The cloned apo B cDNA hybridized solely to chromosome 2 (Fig. 1). The very faint hybridization to chromosome 1 is due to a slight contamination of this fraction by chromosome 2.

In situ hybridization was performed using the same DNA probe. Thirty-two percent of the 205 metaphase cells examined had between one and four grains, the rest had no grains. Forty-one out of 147 (28%) informative sites localized the apo B gene to the distal end of the short arm of chromosome 2 (Fig. 2). The other grains were scattered over the genome. Of the 41 grains on the distal end of the short arm, 21 (51%) were on band 2p23 and 11 (27%) were on band 2p24. When 10–50 times higher concentrations of labeled probe were used, a second hybridization site was observed on the long arm of chromosome 2 at band 2q21. Out of a total of 94 grains, 9 were on 2p23–2p24, 5 were on 2q21, and the rest were randomly distributed on other chromosomes.

Analysis of Intestinal apo B mRNA. RNA was prepared from the small intestine and brain of an adult female monkey (*M. nemestrina*), electrophoretically fractionated in agarose gels containing formaldehyde, and analyzed by hybridization

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Abbreviation: apo, apolipoprotein.

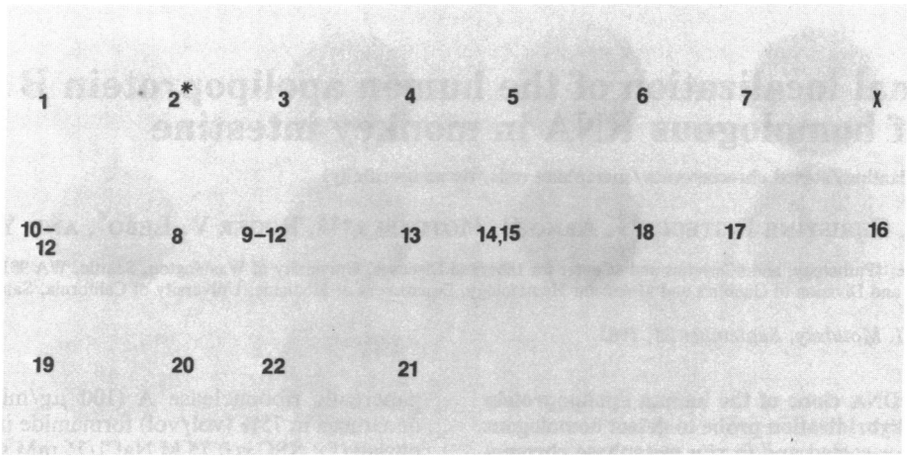

FIG. 1. Autoradiogram of dot blot filters of sorted chromosomes. For experimental details, see ref. 8. The cDNA probe used was ^{32}P-labeled HApoB44 insert DNA with specific activity 1.5×10^8 cpm/μg (4). Note a single dot on chromosome 2. A very faint dot could be discerned on chromosome 1, which was caused by contamination with chromosome 2.

to a radiolabeled cDNA clone (pHApoB44) previously isolated from a human cDNA library and presumed to code for part of apo B-100. The results given in Fig. 3 show clearly that whereas the human hepatoma cell line HepG2 produces the typical hepatic 18-kilobase mRNA species (lane 1), the monkey small intestine produces two apo B-specific molecular species, approximately 6.5 and 18 kilobases long (lane 2). The monkey brain produces no apo B RNA (lane 3).

DISCUSSION

The human apo B-100 gene was mapped, by molecular hybridization to sorted human chromosomes and by *in situ* hybridization to human chromosome preparations, to the distal end of the short arm of chromosome 2 (2p23–2p24). A second weakly hybridizing site on the same chromosome (2q21) was detected with higher probe concentrations. This could indicate the presence of either a pseudogene or another gene with partially homologous sequences.

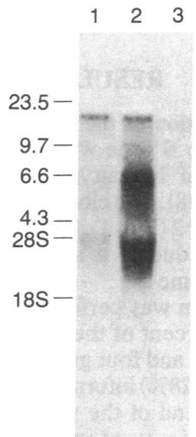

FIG. 3. Blot analysis of electrophoretically separated RNA. ^{32}P-labeled pHApoB44 insert DNA (10^7 cpm, specific activity 1.5×10^8 cpm/μg) was hybridized to RNA blotted onto nitrocellulose after electrophoresis in a formaldehyde-containing 1.0% agarose gel. Unhybridized probe was removed by washing in 0.2× SSC/0.1% $NaDodSO_4$ at 68°C. The filters were exposed to x-ray film (Kodak XAR-2) with an intensifying screen at −70°C for 16 hr. Lanes 1–3 contained, respectively, 27 μg, 48 μg, and 36 μg of total cellular RNA from the human hepatoma cell line HepG2, small intestine of *M. nemestrina*, and brain of *M. nemestrina*. Note that the probe hybridized with both the hepatoma cell line RNA and the intestinal RNA. The positions to which human 18S and 28S RNA migrated are indicated. The other size markers (given in kilobases) were *Hin*dIII-digested bacteriophage λ DNA (Bethesda Research Laboratories).

Results of recent experiments aimed at defining the role of primate liver and small intestine in the synthesis of the two forms of apo B (B-48 and B-100) suggest a high degree of organ specificity. Organ cultures of monkey and human liver (10, 11) and cell cultures of the human hepatoma line HepG2 (12, 13) all produce only apo B-100. Monkey intestine, on the other hand, has been shown to synthesize only apo B-48 (10). The availability of a cDNA clone of apo B-100 has provided other approches to studying the relationship between the two forms of apo B. The following observations support the hypothesis that one gene codes for both forms and that organ specificity is due to posttranscriptional events: A cDNA clone of apo B-100 (pHApoB44, isolated from a liver cDNA library) hybridizes to RNA from both liver and small intestine. By use of the same clone, the gene for apo B was mapped to a single locus on chromosome 2 by *in situ* hybridization. Single hybridization bands were detected on Southern blots of genomic DNA digested with either *Hin*dIII, *Bgl* II, or *Cla* I (data not shown). In most cases of deficiencies of apo B, both the intestinal and hepatic forms are absent, a condition referred to as abetalipoproteinemia (14), which is the result of a single autosomal recessive gene defect. Three individuals have been described who were unable to synthesize hepatic apo B but did produce the intestinal form of the protein (apo B-48) (15–17). To explain our finding of a single gene for the hepatic (apo B-100) and intestinal (apo B-48) forms of the protein, differential gene processing in the liver and intestine would have to be postulated. The genetic defect that allowed preservation of intestinal apo B-48 synthesis (15–17) might affect a domain of the apo B gene that is not involved in synthesis of the smaller intestinal form.

The small intestine was shown to synthesize appreciable quantities of 6.5- and 18-kilobase mRNA species that presumably code for apo B-48 and apo B-100, respectively. The absence of apo B-100 in monkey intestine (9) would suggest that the 18-kilobase mRNA is not translated in that organ. Alternatively, one could postulate that apo B-100 is synthesized in the small intestine but that its cellular steady-state level is too low to be detected by the method used (9).

We thank D. Adler for the computer graphics of the *in situ* hybridization data. This work was supported by National Institutes of Health Grant HL30086 and Center Grant GM15253.

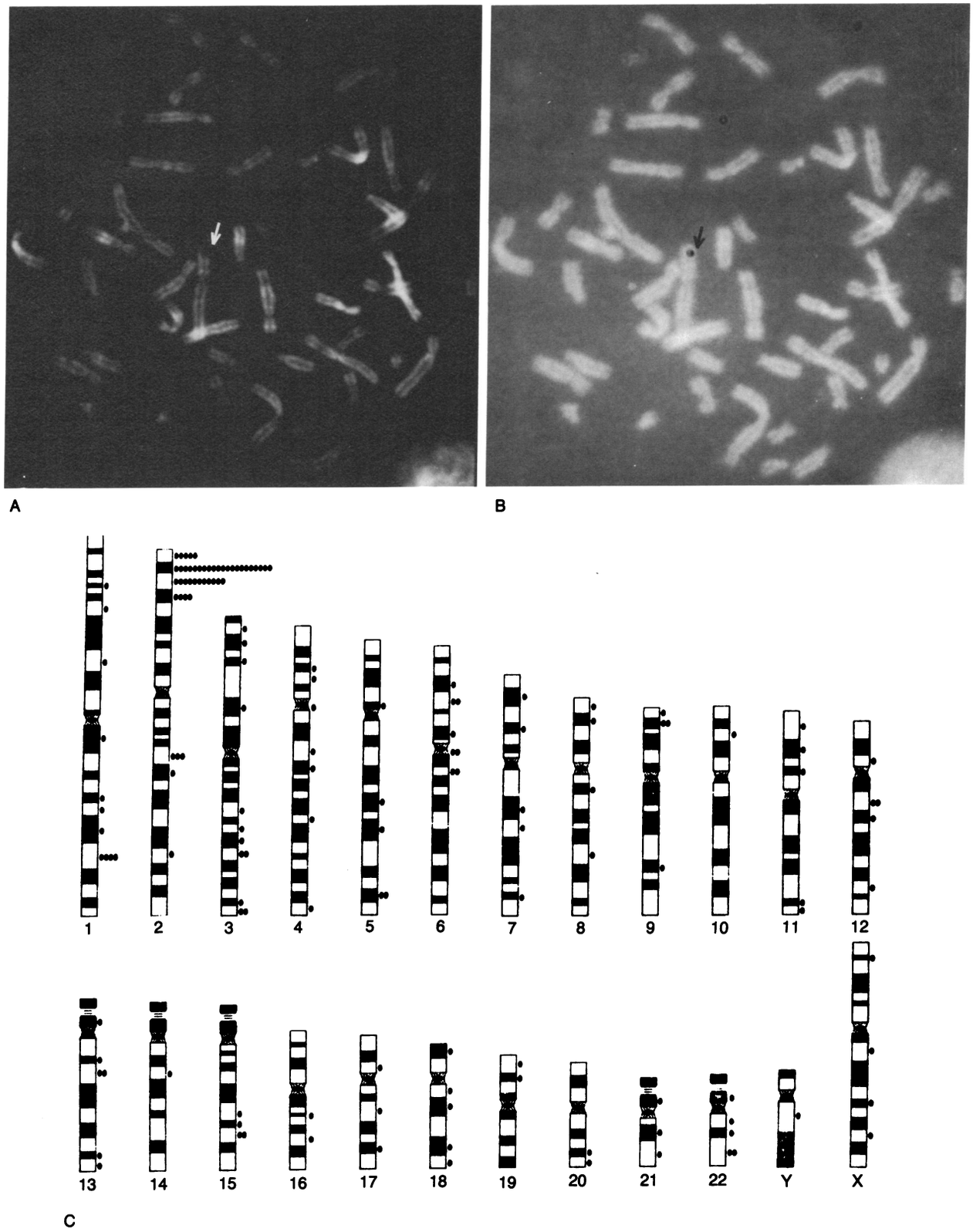

FIG. 2. *In situ* hybridization of human metaphase chromosomes to ^{3}H-labeled pHApoB44. (*A*) Metaphase spread visualized with incident ultraviolet light. (*B*) The same spread visualized with transmitted visible light (arrow indicates silver grain). (*C*) Distribution of silver grains over metaphase chromosomes (chromosome idiograms from ref. 9). Note maximal accumulation of grains at 2p23–p24.

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