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The Gene Encoding the Large Subunit of Human RNA Polymerase II Is Located on the Short Arm of Chromosome 17

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SUMMARY

We have used chromosomal in situ hybridization and Southern blot analysis of DNA from somatic cell hybrids to determine the chromosomal localization of the subgenomic DNA fragment that encodes part of the large subunit of human RNA polymerase II. The results of our analysis demonstrate localization of the human RNA polymerase II large subunit gene to the short arm of chromosome 17.

INTRODUCTION

Cytological mapping of genes involved in functions required for cellular housekeeping and growth is essential to our further understanding of the significance of chromosomal aberrations. For example, localization of oncogenes in close proximity to chromosomal breakpoints has provided exciting new insights into the regulation of cell growth and differentiation [1]. DNA-dependent RNA polymerase II is the enzyme responsible for the synthesis of mRNA precursors in all eukaryotes [2]. The enzyme is ubiquitous and essential in all eukaryotic cells. The fungal toxin α-amanitin that binds stoichiometrically to RNA polymerase II results in cell death in vitro or death of the animal ingesting the toxin [3].

We have isolated genomic and cDNA sequences encoding the large subunit of human RNA polymerase II [4]. This DNA hybridizes to mRNA large enough to encode the 220-kd large subunit and produces a translation product of that

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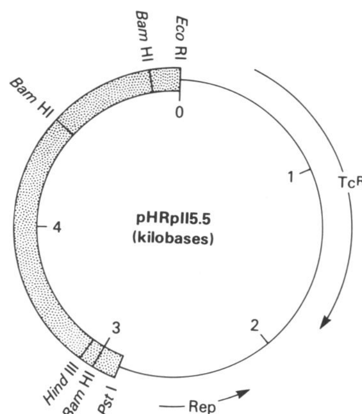

FIG. 1.—The pHRpII 5.5 probe. The 2.4-kb *Eco*RI-*Pst*I genomic DNA fragment used as a probe was inserted into pBR328 as described [4]. This DNA fragment is free of repeated sequences and reveals a single 5.5-kb *Eco*RI DNA fragment when hybridized to human DNA on Southern blots [4].

size in vitro. The sequences code for a polypeptide that reacts with anti-RNA polymerase II antisera. Furthermore, antisera against a fusion protein containing part of this polypeptide react with crude and purified RNA polymerase II, specifically with the largest subunit. The antiserum inhibits the activity of purified RNA polymerase II and inhibits specific transcription by the enzyme present in crude extracts.

We used human DNA probes derived from this recombinant for in situ hybridization to normal human chromosomes and for hybridization to somatic cell hybrid DNA to establish that the sequences encoding the large subunit are located on the short arm of chromosome 17.

MATERIALS AND METHODS

DNA Probes

All methods are described in detail in [4–7]. Human genomic sequences from the phage λHRpIIA were subcloned in pBR328 as shown in figure 1. The *Eco*RI to *Pst*I 2.5-kilobase (kb) DNA is free of human repeated sequences and contains protein coding information for the large subunit of human RNA polymerase II [4]. The plasmid DNA was labeled in vitro with [^{3}H]dCTP (ICN, 20 Ci/mmol) by nick-translation to a specific activity of 3.4×10^7 cpm/μg for the in situ hybridization experiments or with α[^{32}P]dCTP (Amersham, 3,000 Ci/mmol) to a specific activity of 2×10^8 cpm/μg for hybridization against Southern blots. Unincorporated labeled nucleotides were removed by chromatography on Sephadex G-50 fine (Pharmacia).

In Situ Hybridization

Metaphase chromosome spreads were prepared on glass slides from peripheral blood lymphocytes of normal 46,XY males and air-dried. Chromosomally bound RNA was removed by RNAse treatment, washed, and dehydrated through an alcohol series. Chromosomal DNA was denatured by immersing the slides in 2× SSC/70% formamide at 70°C followed by rapid transfer to dehydration series with ethanol.

The ^{3}H-labeled probe mixed with 100-fold excess of salmon sperm DNA was resus-

pended in the hybridization mix, which consists of 25% formamide (Fluka)/2 × SSC/10% dextran sulfate, pH 7.0 [5]. Probe DNA was denatured for 5 min at 70°C, quickly chilled in ice, and incubated with the slides at 37°C for 18 hrs at a final concentration of 0.07 μg/ml [6]. Slides were washed extensively at 37°C, dehydrated, and coated in liquid nuclear track emulsion (Kodak NTB-2). Slides were developed at appropriate intervals, stained with a modified Giemsa procedure [7], and analyzed under the microscope.

Genomic Blot Hybridization

Cellular DNA (15 μg) was digested with the restriction enzyme *Apa*I (New England Biolabs) in the conditions recommended by the manufacturer. The DNA fragments were separated in a 0.8% agarose gel, transfered to nitrocellulose, and baked at 80°C for 2 hrs. Hybridization was carried out in 5 × SSC, 50% formamide, 1 × Denhardt's solution, 0.1% SDS, 0.1 mM EDTA, and 100 μg/ml salmon sperm DNA for 12–16 hrs at 37°C. Washes were in 0.5 × SSC at 50°C, followed by autoradiography.

RESULTS

The recombinant plasmid used for these studies derived from sequences contained in λHRpIIA [4] is briefly described in figure 1. These human genomic sequences were isolated by homology with the *Drosophila* RNA polymerase II large subunit gene [8] and shown to contain coding information for the human enzyme [4]. In particular, the human DNA fragment between *Bam*HI and *Hin*dIII restriction enzyme sites has extensive sequence homologies with the *Drosophila* and yeast large subunit genes (Cho and Weinmann, unpublished observations, 1986). It is a 2.4-kb *Eco*RI-*Pst* DNA fragment, free of repeated sequences, inserted into the pBR328 vector at the *Eco* and *Pst* sites. This *Alu*-free insert recognizes a single unique 5.5-kb *Eco*RI band under stringent hybridization conditions to Southern blots of genomic human DNA. The intensity of hybridization and the simplicity of the pattern of hybridization obtained with this probe indicates that there is a single cellular homologous gene [4].

Two in situ hybridization experiments were performed on normal human peripheral blood lymphocytes with the pHRpII 5.5 probe (fig. 2*A*). The predominant site of hybridization in each experiment was chromosome 17, with 18% and 19% of the total grains over its entire length and 12% of the total grains concentrated over the short arm region.

The chromosomal distribution of grains for the two experiments is shown in figure 2*B*. A total of 175 metaphases were analyzed, and 254 grains were on morphologically identifiable chromosomes. Of the 254 grains, 30 (12%) were on the short arm extending from 17p11→17p13 region. Of these grains, 27 (90%) were localized to the subregion 17p12→17p13 (fig. 3). These results suggest that the pHRpII 5.5 RNA polymerase gene is regionally mapped to the distal portion of the chromosome 17 short arm.

To confirm the results of the in situ hybridization experiments, we analyzed DNA from somatic cell hybrids containing single intact chromosomes 17 or translocated portions of chromosome 17. The hybrids used for these studies contain human chromosomes on a C127 mouse background [9]. The hybrids were selected for the presence of thymidine kinase, which is located on the long arm of chromosome 17. Cytogenetic and isozyme analysis of the hybrids indi-

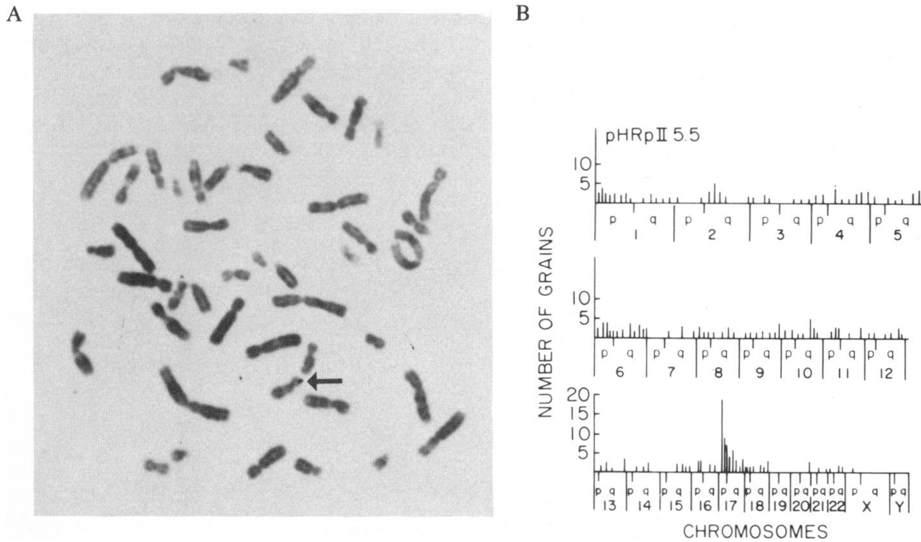

FIG. 2.—In situ hybridization of the RNA polymerase probe pHRpII 5.5 to normal human lymphocytes [5, 6]. (*A*) Representative autoradiograph of Wright-Giemsa-stained chromosomes [7] showing localization of ^{3}H-labeled pHRpII 5.5 probe to the short arm of chromosome 17. (*B*) Diagram showing grain distribution in 175 metaphases. *The abscissa* represents the chromosomes in their relative sizes; *the ordinate* shows the number of silver grains. The distribution of 254 grains on 175 metaphases was scored; 30 grains were found over 17p11→p13 with the majority of grains located on the 17p12-p13 region.

cated that they do contain chromosome 17. The *Apa*I restriction enzyme was found to give distinct restriction enzyme patterns for mouse and human *Rpo*II DNA sequences. Shown in figure 4 are restriction enzyme digests of human, mouse, and hybrid DNAs that were separated on agarose gels, transferred to nitrocellulose, and analyzed with radiolabeled HRpII 5.5 probe. The higher molecular weight human *Apa*I restriction enzyme DNA fragment is clearly

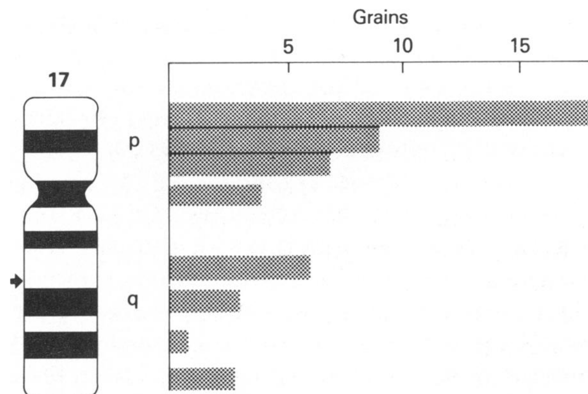

FIG. 3.—Chromosome 17. A detailed idiogram of chromosome 17, showing the grain distribution from our in situ hybridization analysis. The breakpoint of the translocation in CMC 275S is in q21-q22.

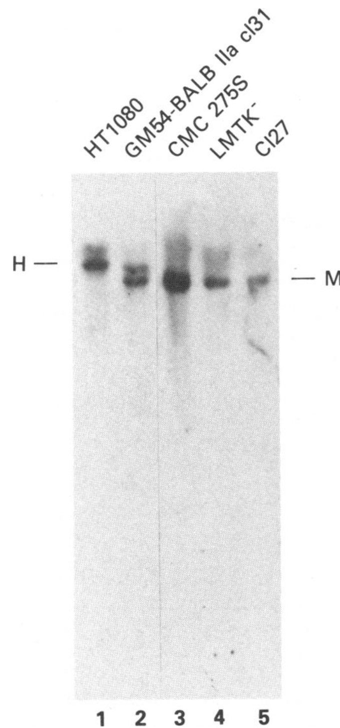

FIG. 4.—Hybridization of pHRpII 5.5 probe against human, mouse, and hybrid DNA. Genomic DNA from human fibrosarcoma cell line HT 1080 (*lane 1*) or from mouse LMTK$^-$ or C127 cell lines (*lanes 4* and *5*) were digested with *Apa*I (New England Biolabs) under the conditions recommended by the manufacturer. *Lanes 2* and *3* contained the *Apa*I-digested DNA from the GM-54-BalbIIa c131 and CMC 275S hybrids. The digested DNA was separated by electrophoresis in 0.8% agarose gel, blotted onto nitrocellulose paper, and hybridized with *Alu*-free pHRpII 5.5 DNA (5×10^8 cpm/μg). The filters were washed in 0.5× SSC at 50°C. The autoradiogram shown has markings corresponding to the human (*H*) and mouse (*M*) RNA polymerase II homologous sequences.

evident in lane 1, and the smaller mouse fragment is shown in lanes 4 (for LMTK$^-$) and 5 (for C127). In lane 2, the DNA that corresponds to the hybrid, GM-54-BalbIIa c131, shows a band corresponding to the human parent and one corresponding to the C127 mouse parent. This hybrid contains all of chromosome 17, as determined by cytogenetic examination [9].

A second hybrid, CMC 275S (lane 3), contains only a part of chromosome 17 translocated to chromosome 21. This hybrid was derived from a fusion with t(17;21) (q21 − q22; q22) cells from a patient with acute undifferentiated human leukemia. The hybrid retains only the der(21) chromosome that contains the long arm of 17 (q21→qter), as determined both by karyotypic analysis and by the cosegregation of the human chromosome 21 isozyme marker for soluble superoxide dismutase (SOD 1) and thymidine kinase when the cells are grown in HAT-selective medium [9]. The results of hybridization clearly indicate that there are no detectable human *Rpo*II sequences in this hybrid. The restriction pattern for the mouse parent of this hybrid is shown in lane 4 (LMTK$^-$). This

result supports the localization of the large subunit of the *Rpo*II gene in the short arm of chromosome 17.

DISCUSSION

Other human genes located on chromosome 17 are the *c-erbA* oncogene [9], homeotic genes [10, 11], and MYH1, MYH2, and MYH3 skeletal myosin heavy-chain genes. The arrow indicates the breakpoint of the translocation used for these studies (fig. 4). The *c-erbA* oncogene is proximal to this breakpoint [10], and the pair of human homeotic genes [11, 12] seem to map to the long arm of chromosome 17, but their location in relation to this breakpoint remains to be determined. The myosin heavy chain is localized in the short arm [13].

Miller-Dieker syndrome (MDS) is characterized by lissencephaly, smooth brain lacking convolutions [13], and has been associated with deletion of the distal short arm of chromosome 17 [13]. Six MDS patients studied had, in addition to lissencephaly, microcephaly, bitemporal hollowing, long philtrum, mild micrognathia, abnormal ears, anteverted nares and growth retardation, and a deletion of 17-p. It is interesting to note that *Drosophila* RNA polymerase II large subunit mutants (C4 locus and *Ubx*1; ultrabithorax-like) also show developmental abnormalities and pleiotropic interactions with other homeotic genes [14, 15]. The relationship between RNA polymerase II genomic sequences, myosin heavy-chain genes, and the phenotypic and cytogenetic anomalies detected in MDS patients remains to be examined in detail. Preliminary data from our group (K. W. Y. C. and R. W.) indicates that genomic RNA polymerase II large subunit DNA sequences from an α-amanitin-resistant mutant are sufficient to transfer toxin resistance by transfection. In *Drosophila,* α-amanitin resistance mutations are located in the gene for the large subunit of RNA polymerase II [8, 14].

Thus, we can conclude that the human α-amanitin-resistant mutation is located on the short arm of chromosome 17.

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