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Assignments of the Tyrosinase Related Protein-1 and -2 Genes to Human Chromosome Bands 9p23 and 13q32.1 by *in situ* Hybridization

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Abstract

To determine the precise chromosomal localization of tyrosine related protein-1 and -2 (TRP-1 and TRP-2) genes by fluorescence *in situ* hybridization, we used DNAs isolated from human bacterial artificial chromosome clones. They contain genomic sequences with approximately 120 kb inserts for TRP-1 and TRP-2. The TRP-1 and TRP-2 genes were assigned to human chromosome bands 9p23 and 13q32.1, respectively. These results confirmed the previously mapped location for the TRP-1 gene and more precisely located the TRP-2 gene, which had previously been mapped to chromosome 13q31-q32.

Key Words: Tyrosine related proteins, bacterial artificial chromosome library, fluorescence *in situ* hybridization

INTRODUCTION

The biosynthesis of melanin is a complex event requiring the expression of many genes.[1] Three of these genes are members of the tyrosinase gene family: tyrosinase (TYR), tyrosinase related protein-1 (TRP-1), and TRP-2. These three proteins have approximately a 40% common amino acid sequence identity among themselves. Although TRP-1 and TRP-2 are thought to arise from the TYR gene by means of gene duplication and subsequent divergence, they have different enzymatic activities. The biosynthetic pathway of melanin begins with the substrate tyrosine. Tyrosinase is responsible for the first two steps of melanin formation, the hydroxylation of tyrosine to dihydroxylphenylalanine (DOPA) and the oxidation of DOPA to DOPAquinone. Two additional enzymes are required for melanin biosynthesis. TRP-2, or DOPAchrome tautomerase, converts 2-carboxy-2,3-dihydroindole-5,6-quinone (DOPAchrome) into 5,6-dihydroxyindole-2-carboxlic acid (DHICA), which is subsequently oxidized to quinone by TRP-1, or DHICA oxidase.

Human oculocutaneous albinism (OCA) is a genetic disorder transmitted as an autosomal recessive trait, and is clinically heterogeneous.[1] To date, three OCA genes have been identified, the tyrosinase of OCA1,[2] the P gene of OCA2,[3] and the TRP-1 of OCA3.[4] The human genetic disorder, if any, caused by the malfunction of the TRP-2 gene has not yet been described.

TRP-1 and TRP-2 have been mapped to human chromosomes chr9p23[5] and chr13q32-q32,[6] respectively. Since more precise chromosomal localization of these genes would be valuable, we isolated the genomic clones of TRP-1 and 2 by screening a human genomic bacterial artificial chromosome (BAC) library and used them for fluorescence *in situ* hybridazation (FISH).

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MATERIALS AND METHODS

Human genomic BAC library

The arrayed human genomic BAC library used for screening in this study consists of 96,000 BAC clones with an average insert size of nearly 140 kbp.[7]

Isolation of BAC DNAs

Of several clones isolated by screening the arrayed human genomic BAC library (4 fold coverage human genomic DNA) using cDNA probes of the TRP-1[8] and TRP-2 genes,[9] two clones, one designated D17 and the other P22, were selected for FISH analyses. The BAC DNAs were purified by using the conventional alkaline lysis method with severe RNase treatment.[7] The insert sizes were around 120 kbp in length for both DNAs.

Chromosome preparations

Phytohemagglutinin-stimulated lymphocytes from a healthy female subject (46, XX) were cultured in RPMI 1640 medium (GibcoBRL Life Technologies, Inc. Gaithersburg, MD, USA) containing 15% fetal bovine serum. Mitotic cells were arrested with colcemid, treated with hypotonic solution of 0.075 M KCl, and fixed in methanol/acetic acid (3 : 1). Metaphase spreads were prepared following the standard procedure.[10]

Fish

The DNAs of BAC clones were labeled with biotin-16-dUTP (Roche Molecular Biochemicals, Mannheim, Germany) using standard nick translation protocols.[11] For hybridization, glass slides with chromosome spreads were denatured in 70% formamide, 2 X SSC at 73°C for 2 mins and dehydrated by immersing in ice-cold ethanol with several changes. Five hundred nanograms of biotinylated BAC clone DNAs and 40 μg of unlabeled human Cot-1 DNA fraction (GibcoBRL Life Technologies, Inc.) were dissolved in 10 μl hybridization solution (50% deionized formamide and 10% dextran sulfate in 2 X SSC). This probe mixture was denatured for 5 mins at 75°C and allowed to preanneal for 1 hr at 37°C. It was then placed on the slides with denatured chromosomes. A coverslip was added and sealed with rubber cement. The slides were incubated for 12 hrs at 37°C for hybridization.

For signal detection, the slides were washed three times in 50% formamide, 2 X SSC at 45°C for 10 mins each, followed by 5 min washes in 0.1 X SSC at 60°C three times. The slides were then preincubated in 4% bovine serum albumin (BSA), 4 X SSC/0.1% Tween 20 at 37°C for 30 mins. Biotinylated DNA sequences were detected using avidin-Cy3 (Cytocell Ltd., UK). The preparations were counterstained with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI) (40 ng/ml) for 10 mins at RT and mounted in PBS/glycerol (1 : 9, v : v) containing the antifade *p*-phenylendiamin-dihydrochloride (1 mg/ml)

The FISH results were analyzed using an Olympus fluorescence microscope (Tokyo, Japan) and the digital image analysis system (Applied Imaging, UK) based on a high-sensitivity integrated monochrome CCD-camera and automated FISH analysis software package.

RESULTS AND DISCUSSION

In an attempt to isolate human genomic clones from

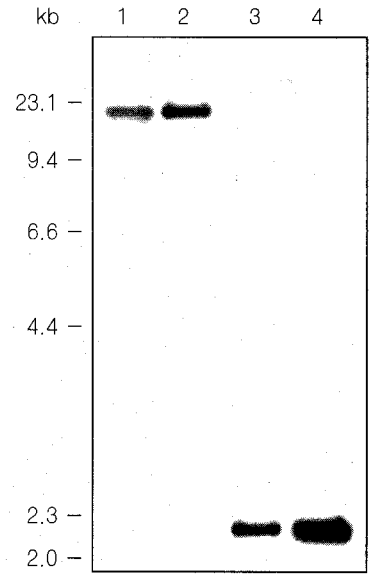

Fig. 1. Southern analysis of the TRP-1 and TRP-2 genes. Human genomic DNAs (lanes 1 and 3) and BAC DNAs (lane 2 for D17 and lane 4 for P22) were digested with Hin dIII, run on a 0.8% agarose gel, transferred to a nitrocellulose membrane, and probed with cDNA inserts of TRP-1 (lanes 1 and 2) and TRP-2 (lanes 3 and 4).

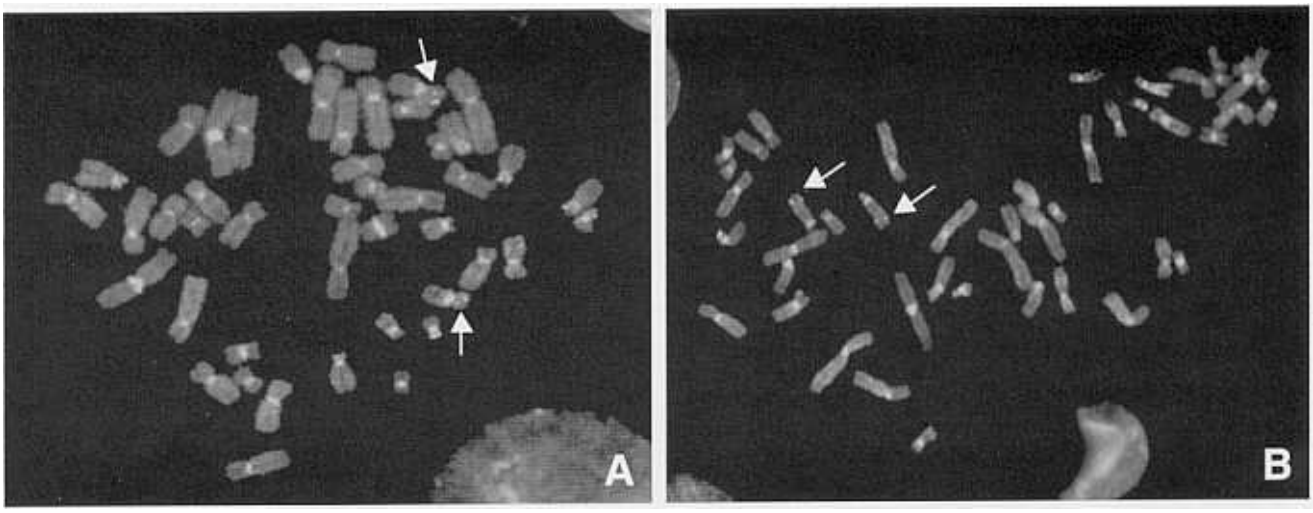

Fig. 2. Chromosome assignments of the TRP-1 and TRP-2 genes by FISH. Metaphase chromosomes 9 and 13 showed specific hybridization of TRP-1 (A) and TRP-2 (B), respectively.

the BAC library, 7 clones of TRP-1 and 8 clones of TRP-2 were obtained. The existence of the corresponding genes in the BAC clones was primarily confirmed by Southern blotting (Fig. 1). Of these, two clones designated D17 and P22, were selected to represent TRP-1 and -2, respectively.

For FISH analysis, a total of 20 cells with no background signals were examined. Approximately three quarters of the cells with a specific signal contained 4 chromatids. The human TRP-1 gene was mapped to chromosome band 9q23 and the TRP-2 gene was mapped to band 13q32.1 (Fig. 2). The position of the TRP-1 gene matched that previously reported[5] and that of the TRP-2 gene was mapped more precisely. Although the authenticity of the probe was proven only by Southern blotting in this study, the BAC DNAs proved to be highly reliable for determining single locations on the chromosome for TRP-1 and -2.

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