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ASSIGNMENT OF THE HUMAN CYTOCHROME P-450 NIFEDIPINE OXIDASE GENE (CYP3A4) TO CHROMOSOME 7 AT BAND q22.1 BY FLUORESCENCE *IN SITU* HYBRIDIZATION

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Summary We have used a full length cDNA clone (2.2 kb) for the human cytochrome P-450 nifedipine oxidase (CYP3A4) enzyme as a probe to determine its chromosome localization by fluorescence *in situ* hybridization. CYP3A4 was mapped on R-banded human prometaphase chromosomes, and the precise localization of CYP3A4 on chromosome 7 was further confirmed by a delineation of G-banded pattern on the same prometaphase chromosomes through a combination of UV-filter. We assigned CYP3A4 to chromosome 7 at q22.1.

Key Words fluorescence *in situ* hybridization, CYP3A4, chromosomal mapping, chromosome 7

INTRODUCTION

Cytochrome P-450 (P-450) is an important enzyme system involved in the oxidative metabolism of drugs and carcinogens as well as endogenous compounds. Nebert *et al.* (1991) have recently reported that the P-450 gene superfamily comprises about 27 gene families with more than 150 genes. The human cytochrome P-450 nifedipine oxidase gene (CYP3A4) encodes a human liver P-450 responsible for the oxidation of many drugs and carcinogens including the calcium-channel blocker nifedipine and related dihydropyridine derivatives (Brian *et al.*, 1990). Using somatic cell hybrids, the gene for a P-450 that encodes the enzyme nifedipine oxidase (CYP3) was localized to human chromosome 7 (Riddell *et al.*, 1987; Gonzalez *et al.*, 1988). Spurr *et al.* (1989) assigned CYP3 to 7q22-qter with the use of

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a panel of human-rodent somatic cell hybrids, and Brooks *et al.* (1988) mapped the gene to the band 7q21.3-q22.1 using multilocus linkage analysis in Centre d'Etude Polymorphisme Humain (CEPH) families. However, no regional mapping of CYP3A4 has been proposed.

In recent years, *in situ* hybridization techniques using biotinylated probes have been used to develop so called fluorescence *in situ* hybridization (FISH) for precise localization of human genes on chromosomes. FISH techniques have simplified and shortened the handling process while increasing the sensitivity for detection and resolution, as compared to original *in situ* hybridization methods using isotopically labeled probes. However, only a few results on the simultaneous localization of short single-copy genes on banded human chromosomes by FISH have been reported (Viegas-Péquignot *et al.*, 1989; Hori *et al.*, 1990).

In this paper, we report the precise and direct localization of CYP3A4 by means of combining FISH on the human R-banded chromosomes, synchronized by treatment with excess-thymidine followed by 5-bromodeoxyuridine (BrdU) incorporation during the late S-phase. This system has made it possible to obtain excellent G-banded chromosomes by changing the filter combination to Nikon filter UV-2A.

MATERIALS AND METHODS

Preparation of high resolution R-banded chromosomes. High resolution R-banded chromosomes were obtained after human lymphocyte cultures, synchronized by excess-thymidine followed by BrdU incorporation during the late S-phase (Viegas-Péquignot and Dutrillaux, 1978; Takahashi *et al.*, 1990). Chromosome preparations were made according to standard procedures, including treatment with a hypotonic solution and fixation with methanol/acetic acid (3:1, vol/vol). The fluorescence staining method of detecting R-banded chromosomes was carried out according to protocols described previously (Tsuji and Kato, 1981).

DNA probes and labeling. A NF-25 cDNA clone (2.2 kb insert) related to the human cytochrome P-450 nifedipine oxidase gene (Beaune *et al.*, 1986) was used. The cDNA clone was labeled by nick translation using biotin-16-dUTP according to the directions of the supplier (Boehringer, Mannheim). After the reaction was terminated by heating at 65°C for 10 min, the biotinylated probe was purified by an ethanol precipitation method using 4 M ammonium acetate (Wako) and sonicated salmon DNA containing *Escherichia coli* tRNA (10 mg/ml). The probe was used at a level of 500 ng per slide.

In situ hybridization. In situ hybridization and rinsing was carried out according to the protocol described by Takahashi *et al.* (1989, 1990) with slight modification (Inazawa *et al.*, 1991). The hybridization mixture consisted of 0.5 μg biotin-labeled cDNA, 50% formamide, 2×SSC, 10% bovine serum albumin (BSA), and 20% dextran sulfate (final concentration). After the slides were incubated overnight at 37°C in a humid chamber, they were washed sequentially with 50% formamide-2×SSC at 37°C and then 2×SSC and 1×SSC at room temperature for 15

min each. The slides were then immersed in 4 × SSC.

Cytochemical detection. The cytochemical detection was done by the protocol described by Pinkel *et al.* (1986) with some modifications. The slides were removed from the 4 × SSC solution and incubated with a blocking solution (4 × SSC containing 4% Block Ace™ powder) for 10 min at 42°C. The slides were then incubated with 4 × SSC containing 5 μg avidin-FITC (Boehringer) per ml and 1% Block Ace™ powder (Dainippon, Osaka) at 37°C for 45 min in a humid chamber. They were then washed by shaking sequentially in 4 × SSC, 4 × SSC containing 0.05% Triton X-100, and 4 × SSC for 10 min each at room temperature. After washing, as described above, the intensity of biotin-linked fluorescence was amplified by adding a layer of biotinylated anti-avidin D (Vector, Burlingame, CA) (1 μg/ml in 4 × SSC containing 1% Block Ace™ powder), followed by washing as above and then incubation with another layer of avidin-FITC in 4 × SSC with 1% Block Ace™ powder at 37°C. After washing by shaking, as above, the slides were counterstained with a solution containing 1 μg propidium iodide (PI, Sigma) per ml and mounted with a fluorescence anti-fade solution containing *p*-phenylenediamine (Johnson *et al.*, 1981). Slides were observed and photographed using a fluorescence microscope (Nikon). The fluorescein and PI were excited at 450–490 nm (Nikon filter combination B-2A). The DNA-specific dye PI was used to stain the chromosomes red, and the greenish-yellow fluorescein signal of the hybridized probe was simultaneously observed. The use of another filter combination, B-2E (exciter 450–490 nm, barrier 520–560 nm), allowed observation of more contrasted yellow signals on green chromosomes. Moreover, excitation in the ultraviolet (Nikon filter combination UV-2A) allowed the observation of the G-banded chromosomes. Fujichrome 400 D color slide film (Fuji) was used for microphotographs.

RESULTS AND DISCUSSION

To determine the chromosomal localization of CYP3A4, we applied FISH techniques allowing simultaneous identification of fluorescent signals on high-resolution R-banding chromosomes. The technique also allows identification of the G-band and location of the signals on the same preparation by changing filters (Fig. 1, A and B). Filter combination UV-2A allowed an optimal observation of G-banded chromosomes with excitation near the wavelength of 365 nm. This mapping was performed directly with a biotinylated 2.2 kb long fragment inserted in bacteriophage λgt11. Because of the small size of 2.2 kb probe it is difficult to detect the hybridization signals in the original system (Viegas-Péquignot *et al.*, 1989). We adopted the procedure of labeling with avidin-FITC, followed by biotinylated anti-avidin D, and then a second labeling with avidin-FITC. The fluorescence intensity was increased significantly by these treatments.

The detailed analysis was performed with 100 metaphase cells, 34 of which showed fluorescent signals on band 7q22. The distribution pattern of the signals on 7q22 was as follows: twin spots on both homologs of chromosome 7 (8.8%),

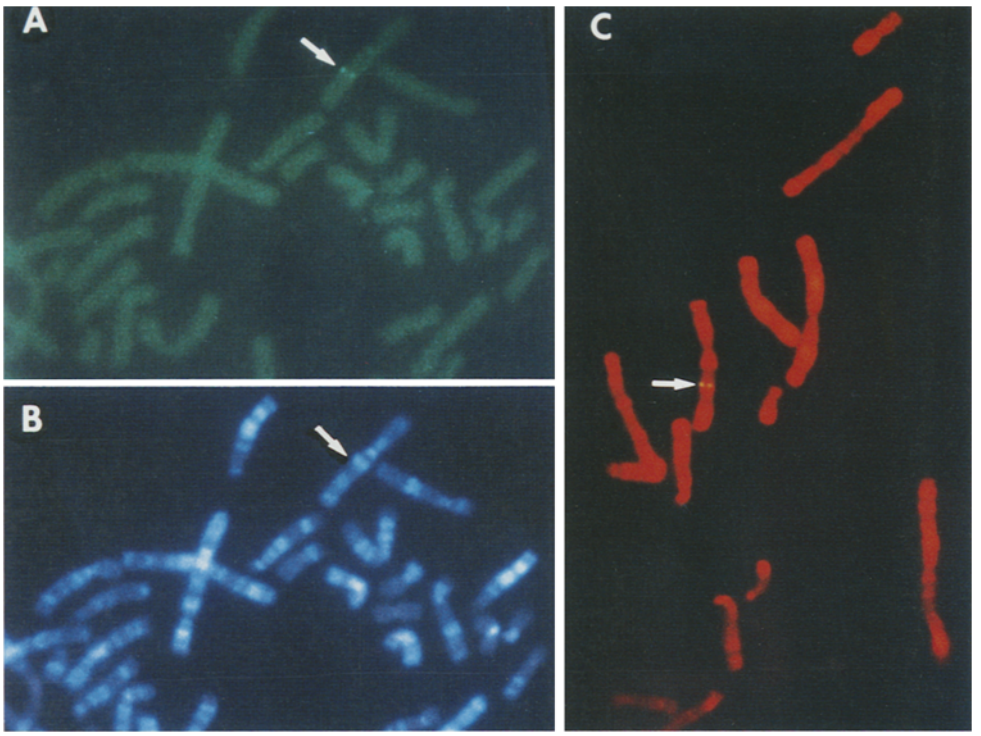

Fig. 1. Chromosomal localization of CYP3A4 gene by fluorescence *in situ* hybridization. (A) Fluorescent signals for CYP3A4 gene were detected on the long arm of chromosome 7 (filter combination B-2E Nikon). (B) The G-banding pattern on the same metaphase was delineated through different filter combination (UV-2A Nikon), showing the signals at 7q22.1. (C) Simultaneous visualization of R-bands and hybridization signal (filter combination B-2A Nikon). Arrow indicates hybridization signals on 7q22.1.

twin spots on one homolog and single spot on the other (11.8%), twin spots on only one homolog (70.6%), and a single spot on only one homolog (8.8%). No such twin fluorescent signals were detected on other chromosomes. CYP3A4 was assigned on typical R-banded chromosomes, and the precise localization of CYP-3A4 on chromosome 7 was further confirmed by a delineation of G-banded pattern on the same metaphase chromosomes through a combination of UV-filter (Nikon UV-2A). Of 34 metaphase cells that showed fluorescent signals in the 7q22 region, 91.2% were located on 7q22.1, 2.9% on the interface between 7q21.3 and 7q22.1, 5.9% on 7q22.2. Thus, CYP3A4 was assigned to the 7q22.1 band of the human karyotype (Fig. 1C).

This mapping system has made it possible to directly identify the localization of signals on the R-banded chromosomes and to delineate the G-banded pattern

on the same metaphase chromosome through a different filter combination (UV-2A, wavelength 365 nm).

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