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ISOLATION AND MAPPING OF A COSMID CLONE CONTAINING THE HUMAN NAT 2 GENE

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The NAT2 gene encodes for a polymorphic arylalkylamine N-acetyltransferase and thus accounts for the human N-acetylation polymorphism. By a NAT2-specific primer set we have screened a human chromosome 8-specific cosmid library. A positive cosmid clone was mapped by fluorescence in situ hybridization to 8p22. The polymerase chain reaction followed by restriction analysis of the PCR product was used to identify allele 2 to be contained in the cosmid clone.

The human NAT2 gene belongs to a group of three similar DNA sequences, two of which (NAT1,2) are encoding for genes while the third has been reported to be a pseudogene (1). The N-acetyltransferase (E.C.2.3.1.5.) accounts for one of the most intensively investigated pharmacogenetic traits, the N-acetylation polymorphism (for review, see Weber, (2)). The molecular polymorphism of the NAT2 gene is caused by different combinations of four alleles reported so far (3,4). Recently, a somatic human x hamster cell hybrid panel was used to assign the DNA region containing NAT 1-3 to the chromosomal segment 8pter-> 8q12 (1). However, neither of the NAT-related sequences has precisely been mapped yet. It was the aim of this study to isolate a cosmid clone containing NAT2 from a human chromosome 8-specific cosmid library and to map it to a band of this segment. In addition, the positive cosmid clone used for mapping was genotyped.

MATERIALS AND METHODS

Screening of the cosmid library:

The chromosome 8 library used (LA08NC01) was prepared at the Los Alamos National Laboratory from flow-sorted human chromosomes 8 (5,6) and kindly provided by L.L.

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Deaven. Fragments are ligated in the vector cCos1 transfected in E. coli DH5alphaMCR cells. The library was plated on Hybond N-membranes (Amersham) placed on LB plates containing kanamycin and grown overnight at 37°C. Preparation of replicas from the master filters was performed according to standard procedures. The filters were prehybridized for at least 3 hours at 42°C in a solution (0.2 ml/cm^2 filter) of 50 % formamide (Merck), 5 x SSPE, 5 x Denhardt's solution, 0.1 % SDS, and 200 μg/ml heparin. To obtain the DNA probe for hybridization, a primer set specific for the NAT2 locus (1) was designed (sense (5'): ATCAGGAGAGAGCAGTAT, antisense (5'): AGTTTTAAACTCGACCAG) and used to amplify a 281 bp DNA fragment by PCR. The PCR product was run on a 2 % TAE agarose gel, isolated using the Magic DNA Clean-Up System (Promega), labeled with alpha^{32}-pdATP by random primed labeling (7), and purified by running through a push column. Then, the probe was used for hybridization of the filters according to standard protocols. A secondary screening round was performed to assure that pure positive clones were picked up.

Isolation of cosmid DNA:

DNA was isolated by maxi-prep (Quiagen). To get a rough estimation of the insert size, DNA was digested by BamHI, KpnI, and TaqI.

Fluorescence in situ hybridization:

Cosmid DNA was nick translated/labeled in the presence of Biotin-14-dATP (BioNick Labeling System, BRL). For cytogenetic preparations we used peripheral blood lymphocytes from a healthy donor and a human x hamster hybrid cell line (WORxAd64) recently established in our laboratory retaining human chromosome 8. Slides for FISH were prepared following routine methods and G-banded as described by Rommel et al. (8) except for the trypsin concentration which was reduced to 0,06 μg/ml buffer. FISH was performed according to Kievits et al. (9).

Genotyping of the NAT2 gene:

The primer set specific for the NAT2 gene (1) (sense (5'): GAGGATATCTGATAGCACATA; antisense (5'): TCCCTCCAGTTAACAAATAC) was used to amplify a 668 bp fragment which was then digested by BamHI, KpnI, and TaqI, respectively.

RESULTS

Screening of the human chromosome 8-specific cosmid library with a NAT2-specific PCR product resulted in 5 positive clones. Of these, one was used for further analyses. The insert size determined by restriction analysis was found to be approximately 38 kb. Cosmid DNA was used for FISH studies after previous demonstration of G-bands. The intensity of FISH signals was rather low. Of 124 lymphocyte metaphases, only 15 showed signals. These were double signals on both chromatids of the short arm of chromosome 8. In addition, the cosmid probe was hybridized to metaphases of a human x hamster hybrid cell line containing an apparently normal chromosome 8. 62 metaphases were analyzed resulting in an assignment of the NAT2 cosmid to 8p22 (Figure 1a,b) thus confirming the results obtained by hybridizing the lymphocyte metaphases. In addition, the NAT2 allele contained in the cosmid was genotyped. By PCR and restriction enzyme analyses we determined the fragment pattern of the allele S2 (Figure 2) as initially described by Hickman and Sim (3).

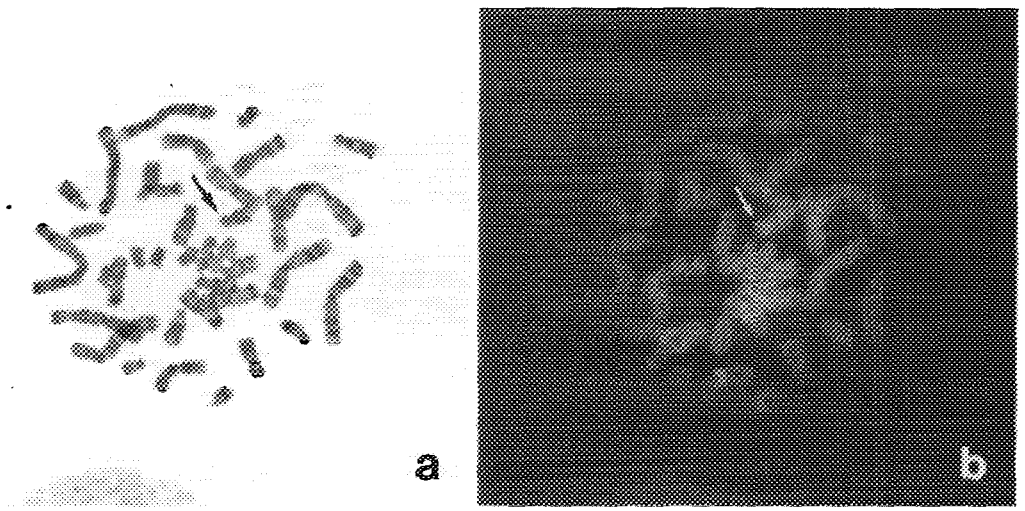

Figure 1. **(a)** G-banded metaphase spread of the human x hamster cell line WORxAd64. *Arrow* indicates human chromosome 8. **(b)** Same metaphase as in **a** after fluorescence in situ hybridization with biotinylated NAT2 cosmid DNA and detection with avidin-FITC. Chromosomes were counterstained by propidium iodide. *Arrow* marks the hybridization signal.

DISCUSSION

N-acetylation is one of the pharmacogenetically best investigated reactions. It is one of the major routes in detoxification of carcinogenic substances, several drugs, e. g. isoniazid, sulfamethazine, procainamide (2), and is also involved in the melatonin pathway (10). In

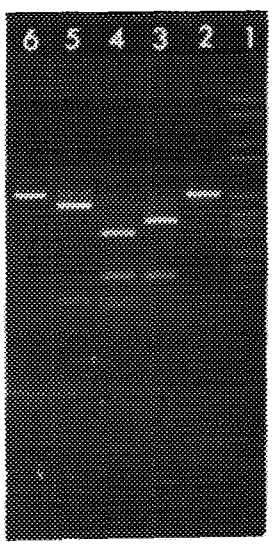

Figure 2. Fragment pattern of the allele S2. Lane 4: TaqI digest of the PCR product showing that there is no restriction site for TaqI resulting in a fragment of 396 bp. Lane 3: KpnI digest. Lane 5: BamHI digest. Lane 2 and 6: Controls (undigested PCR product). Lane 1: DNA molecular weight marker VI (Boehringer; 2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, 220, 154 bp).

human, the NAT2 gene encodes for an important enzyme catalyzing this reaction. Herein we have mapped this gene to a single band of the short arm of chromosome 8 using a NAT2 positive cosmid clone. Together with four other positive clones this will be the starting point for the establishment of a cosmid contig of the particular region of chromosome 8 harboring NAT2 and also to determine the physical distance between NAT2, NAT1, and the NAT pseudogene.

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