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COSMIDS AND TRANSCRIBED SEQUENCES FROM CHROMOSOME 11q23

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Summary To obtain cosmid markers and transcribed sequences from a specific chromosome region, a series of radiation-reduced hybrids (RHs) containing various regions of human chromosome 11 was prepared from microcell hybrid A9 (*neo*11) cells containing a normal human chromosome 11 tagged with pSV2*neo* at 11p11.2. Among 15 radiation hybrid clones isolated, RH(11)-9 which contains a q23 fragment in addition to the *neo* integration site, was used for the construction of a cosmid library. Cosmid clones having human DNA sequences were screened, and localized by Southern hybridization with the radiation hybrid panel. Fifty-nine cosmids were assigned to 11q23 and 6 cosmids to 11p11.2. Exon amplification proceeded with 23 of the 59 cosmids and 16 putative exons were cloned. Three of them were identical to those constituting a known gene which locates on q23 (ATDC), and the others were unknown. Thus, the RHs containing various subchromosomal fragments of chromosome 11 were useful for constructing region-specific DNA markers. The RH-(11)-9 cells and putative exons also facilitate the positional cloning of genes in the 11q23 region.

Key Words chromosome 11q23, radiation-reduced hybrid, cosmids, exon amplification, transcribed sequences

INTRODUCTION

The 11q23 region reportedly harbors the genes responsible for hereditary dis-

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orders such as Ataxia-telangiectasia (Gatti, 1991; Sobel *et al.*, 1992) and paraganglioma, which is potentially related to genomic imprinting (Heutink *et al.*, 1992; Devilee *et al.*, 1994). Chromosome deletions and/or allelic losses on 11q22-q24 have been found in breast, ovalian, colorectal, and cervical carcinomas and malignant melanomas (Keldysh *et al.*, 1993; Tomlinson *et al.*, 1993; Foulkes *et al.*, 1993; Hampton *et al.*, 1994; Carter *et al.*, 1994). Three genes whose defect by chromosome translocations cause some leukemias, have been identified (Ziemin *et al.*, 1991; Akao *et al.*, 1992; Chen *et al.*, 1993; reviewed by Rabbits, 1994). Chromosome deletions or translocations on q23 possibly distinct from that including the three genes have been found in leukemias (Cherif *et al.*, 1992; Kobayashi *et al.*, 1993). Thus, the accumulation of cosmid clones and transcribed sequences from this region will facilitate the cloning of genes related to the disorders described above and to construct a transcriptional map of this defined region.

To clone human genes and to construct a human genome map by means of the positional approach, human DNA segments must be isolated from a specific human chromosome or chromosomal region. Interspecies somatic cell hybrids containing a single human chromosome have been used as a donor of human chromosome specific DNA (Emi *et al.*, 1992; Hori *et al.*, 1992; Kugoh *et al.*, 1995). An irradiation and fusion procedure has reduced the size of human chromosomes in hybrid cells (Leach *et al.*, 1989; Tamari *et al.*, 1992; Gerhard *et al.*, 1992). This allows DNAs to be more efficiently isolated from a specific region by PCR, utilizing interspersed repetitive sequences (Zoghbi *et al.*, 1991; Gillet *et al.*, 1993). The cloned DNA segments using PCR are however, relatively short, therefore subsequent screening of genome library is inevitable to analyze genome structure of chromosome region in interest.

In this study, we generated radiation-reduced hybrids (RHs) containing various regions of chromosome 11, including the 11q23 region, constructed a cosmid library from this region and selected transcribed sequences by exon amplification (Buckler *et al.*, 1991). These should be valuable resources for the genome and transcribed mapping of chromosome 11.

MATERIALS AND METHODS

Generation of radiation-reduced hybrids. A9(*neo*11) is a mouse A9 cell hybrid containing a single human chromosome 11, upon which the selectable marker pSV2*neo* is integrated at p11.2 (Koi *et al.*, 1989). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, 100 units/ml penicillin and 100 μl/ml streptomycin. The antibiotic G418 sulfate (Gibco) at 800 μg/ml was added to the growth media. A9(*neo*11) cells were irradiated using a cobalt source set at 100 rad/sec for periods calculated to deliver radiation doses of 2,000 and 3,000 rads, and fused with A9 cells. Irradiation and fusion were performed following the described protocol (Tamari *et al.*, 1992) and

G418-resistant colonies were cloned and expanded for subsequent analyses.

Inter-Alu PCR. High molecular weight genomic DNAs were prepared by proteinase K digestion and phenol/chloroform extraction. Genomic DNA (20 ng) from RH cells were used in inter-*Alu* PCR amplification using the primer 559 (Ledbetter *et al.*, 1990) following the described protocol (Kurimasa *et al.*, 1994). Products were visualized by staining with ethidium bromide after electrophoresis in 0.8% agarose gels.

Southern blot analysis of RHs. High molecular-weight DNAs extracted from RHs were digested with *Eco*RI for Southern blotting, which was performed using the following 27 chromosome 11-specific markers (Tokino *et al.*, 1991; Hori *et al.*, 1992) and pSV2*neo*; D11S483, D11S459, cCI11-546, cCI11-489, D11S431, cCI11-421, D11S466, cCI11-370, D11S455, cCI11-382, D11S441, cCI11-393, cCI11-496, D11S436, D11S427, D11S426, D11S429, cCI11-362, cCI11-279, D11S464, D11S448, D11S428, D11S456, D11S473, D11S425, cCI11-276, and D11S439. The loci whose symbol names have not yet been defined are designated by the probe names in the text (Hori *et al.*, 1992).

FISH analyses. The FISH procedure was as described (Takahashi *et al.*, 1990, 1992; Kugoh *et al.*, 1995). Total human DNA was used as the probe for FISH painting. FISH for cosmid mapping was carried out using normal human lymphocytes.

Cosmid library construction and mapping. A cosmid library was constructed from the genomic DNA of the radiation-reduced hybrid cell line, RH(11)-9, as described (Tokino *et al.*, 1991), using the cosmid vector pWEX15. Clones carrying human DNA inserts were selected by plaque hybridization with the total human DNA as a probe, then by Southern hybridization with total human and mouse DNAs. The human DNA clones were mapped by Southern hybridization with the radiation hybrid panel.

Exon amplification. Exon amplification was performed using cosmids as described (Buckler *et al.*, 1991). Briefly, cosmids were completely restriction-digested by *Bam*HI/*Bgl*II and ligated to the splicing vector pSPLI at the *Bam*HI site. Constructs were transfected into Cos7 cells by the electroporation (Bio-Rad, Gene pulser; 1.2 kV, 25 μF). Cytoplasmic RNAs were recovered 48 hr after transfection and reverse transcribed followed by two steps of amplification using the primers SD2/SA2 and SD1/SA1. Second PCR products were cloned into the *Bam*HI/*Sal*I site of plasmid vector pBluescript SK(−).

Analysis of cloned exon candidates.

Preparation of probes for Southern/Northern hybridization: Cloned PCR products were excised by restriction digestion with *Bam*HI/*Sal*I, and isolated by agarose gel electrophoresis. Isolated sequences ^{32}P-labeled by means of random priming, were used as probes for Southern/Northern blot analyses.

Southern blot analysis: Cosmid clones were restriction-digested with *Bam*HI/*Bgl*II, and Southern blots were prepared. Genomic DNAs were isolated from

human lymphocytes and calf, rat, mouse, and guinea pig tissues. Twenty micrograms of each sample was digested with *Eco*RI and Southern blots were prepared. Prehybridization and hybridization were performed overnight at 65°C in solution containing 10% SDS, 7% polyethylene glycol 8000, and 200 μg/ml human placental DNA. ^{32}P-labeled probes described above were used in hybridization. After hybridization, filters were washed at 65°C with 0.1×SSC and 0.1% SDS and exposed for autoradiography to X-ray films at −70°C.

Northern blot analysis: Total RNA was extracted from rat tissues by the AGPC method (Chomczynski and Sacchi, 1987). RNA samples (20 μg) were then resolved by electrophoresis in a 1% agarose, 6% formamide gel and blotted onto Nylon membranes (Amersham). These were prehybridized and hybridized in 50% formamide, 0.5% SDS, 5×SSPE, 100 μg/ml shared denatured salmon sperm DNA. ^{32}P-labeled probes described above were used in hybridizations. After overnight hybridization at 42°C, the membranes were washed with 0.1×SSC, 0.1% SDS at 50°C.

Sequencing and sequence analysis: Sequences were demonstrated by means of dideoxy chain termination method (Sanger *et al.*, 1977), using the BcaBEST sequencing kit (TAKARA). Sequences were analyzed using the FASTA network service of DDBJ.

RESULTS AND DISCUSSION

Sixty radiation-reduced hybrids (RHs) were constructed from A9(*neo*11) cells, and the human DNA content in them were estimated by the banding patterns using inter-*Alu* PCR (data not shown). Of these, 15 RHs containing various amounts of human DNA were selected, and the retained chromosome regions were determined by Southern blotting with 28 DNA markers on chromosome 11. An RH panel consisting of 15 hybrids was prepared to localize the DNA sequences to 20 regions on chromosome 11 (Fig. 1). Among 15 RHs, RH(11)-9 retained 11q23 in addition to p11.2, in which the selection marker pSV2*neo* has been integrated. FISH analysis (painting) using total human DNA was performed to investigate the integrity of the q23 and *neo*-tagged fragments in RH(11)-9 (Fig. 2A). A human chromosome fragment was inserted into the mouse chromosome in most of the metaphase plates analyzed. Thus, human chromosome fragments including the 11q23 and *neo*-tagged regions were stably retained as an insertion into mouse chromosomes.

A cosmid library was constructed from the RH(11)-9. Cosmid clones carrying human DNA were screened by plaque hybridization with total human DNA and subsequent Southern blot hybridization with total human and mouse DNA probes. Ninety-seven selected clones were hybridized to the RH panel to localize on the chromosome 11 (data not shown). Of these, 59 (60%) were localized on 3 regions in 11q23, represented by the cosmid markers D11S428(cCI11-6), D11S425(cCI11-1),

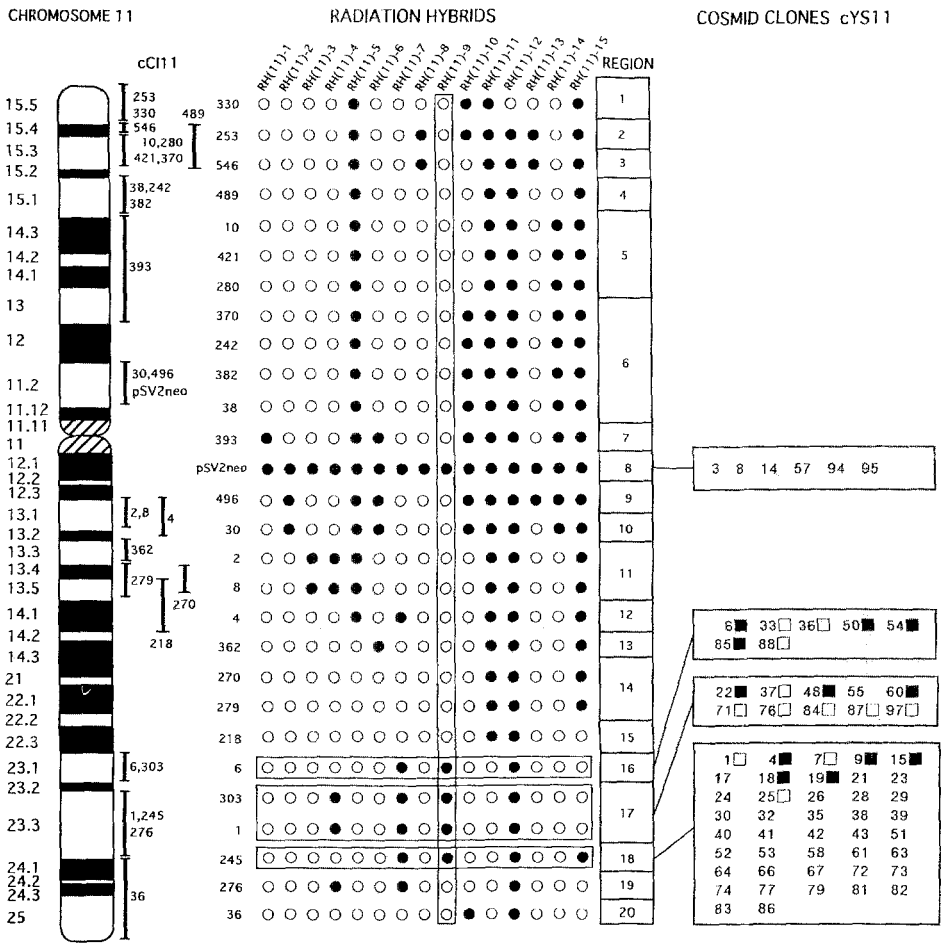

Fig. 1. An ideogram of human chromosome 11 (left) and a summary of the results of Southern blot hybridization showing the presence (solid circle) or absence (open circle) of specific DNA sequences on human chromosome 11 (cosmid marker cCI11s; Tokino *et al.*, 1991) in radiation-reduced hybrids. Only the numbers of cosmid markers are shown. The numbers of cloned cosmids (cYS11s) located on 11q23 and p11.2 are represented on the right. The square following the number indicates identification (solid) or lack (open) of putative exons.

and D11S456(cCI11-245). Six were localized on 11p11.2 represented by the integrated pSV2*neo* (Fig. 1). Three selected cosmids (cYS11-4, 6, 9) were confirmed their map positions on 11q23 by FISH analyses (Fig. 2B). The remaining 32 were not localized using the RH panel. An alternative study such as FISH is required to localize these clones. We noted that 6 (cYS11-29, 35, 36, 54, 82, 83) of the 59 cosmids mapped on 11q23 cross-hybridized to mouse DNA under highly stringent conditions. Whether these cosmids are chimeras of human/mouse DNA or con-

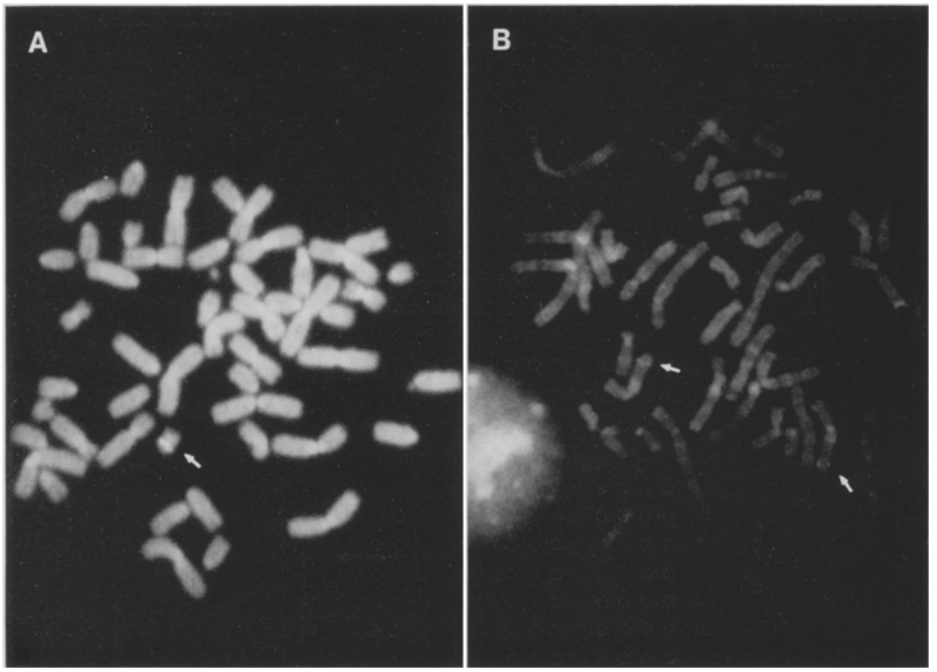

Fig. 2. A: A metaphase spread of RH(11)-9 after *in situ* hybridization with total human DNA. The arrow indicates a human chromosome fragment inserted into a mouse chromosome. B: Mapping of cosmid cYS11-6 on chromosome 11. Arrows indicate the specific hybridization signals of twin spots on 11q23.

taining conserved sequences throughout species, remains to be investigated.

Exon amplifications were performed using the 23 cosmids localized on 11q23, to isolate region-specific transcribed sequences. First PCR products using the primer set SD2/SA2 were recovered from agarose gels as fractions from 400 to 1,400 bp. Second PCR products using the primer set SD1/SA1 corresponding to the major PAGE bands were independently recovered and cloned into the plasmid vector. As a first screening, cloned DNAs corresponding to the major bands on PAGE were selected and their origin was ascertained by Southern hybridization with cosmids. Sixteen clones were derived from the original cosmids. The following analyses were performed using the 16 exon candidates. Searching for cross-species sequence conservation has been adopted as an approach to identifying a gene in a stretch of genomic DNA (Monaco *et al.*, 1986; Page *et al.*, 1987; Rommens *et al.*, 1989; Call *et al.*, 1990). To characterize the cloned exon candidates, we first performed Southern blot analysis using genomic DNA derived from selected mammals (Fig. 3). Eleven clones were conserved throughout human, calf, mouse, rat, and guinea pig samples, whereas the other 5 clones were single copy in the human genome. This may be due to the relatively stringent hybridization conditions.

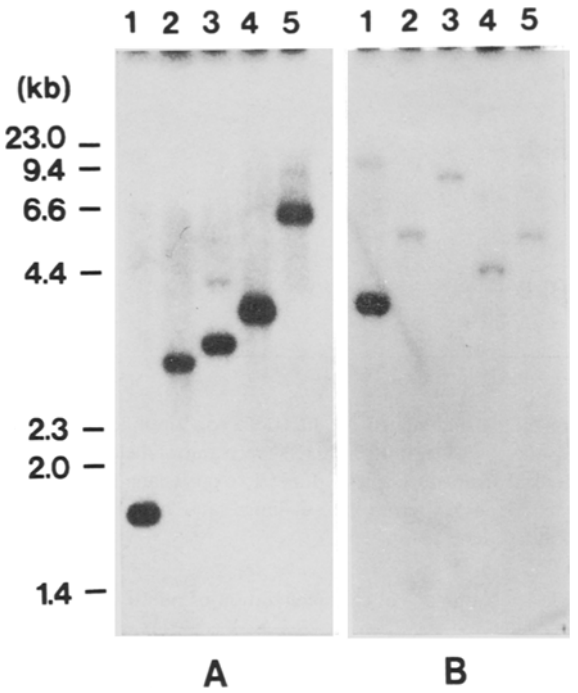

Fig. 3. Southern blots of cloned products of exon amplification. 4A-3 (A) and 15B-18 (B) were excised, gel purified, radiolabeled and hybridized to DNA samples from various species. Lane 1, human; lane 2, calf; lane 3, rat; lane 4, mouse; lane 5, guinea pig.

The transcription of the eleven conserved sequences were subsequently investigated by Northern blotting using total RNAs derived from rat tissues (Fig. 4). Of these, 6 clones detected transcripts in rat tissues. The probes used for hybridization experiments included the common 93 bp sequences of the HIV *tat* exon derived from splicing vector. The additional sequences did not significantly influence the experiments. For the other 5 clones which did not detect transcripts by Northern blotting using total RNA, more sensitive studies, such as RT-PCR are required to ascertain whether or not they are actually transcribed.

To characterize 16 exon candidates, we also performed sequence analyses. All the exon candidates had the correct splicing junction with the 5′ and 3′ splice sites and were flanked on both sides by the *tat* sequences. A homology search with the FASTA program revealed that 3 clones 60D-1, 54B-1, and 54D-1 were identical to exons 5, 5&6, and 7, constituting a human cDNA ATDC, which is localized on 11q23 and partially complements the radiosensitivity of fibroblasts from a patient with Ataxia-telangiectasia group D (Kapp *et al.*, 1992; Leonhardt *et al.*, 1994). The other sequences did not show significant similarities to known genes at both the nucleic acid and amino acid level.

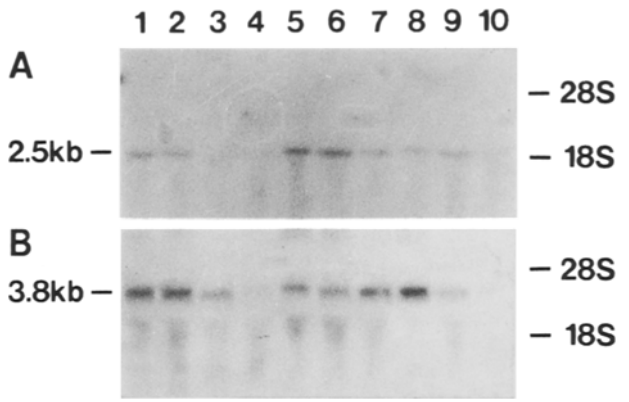

Fig. 4. Northern blots using cloned products of exon amplification. Gel purified inserts of clones 4A-3 (A) and 15B-18 (B) were radiolabeled and hybridized to total RNA derived from rat tissues. Lane 1, ovary; lane 2, brain; lane 3, liver; lane 4, stomach; lane 5, uterus; lane 6, lung; lane 7, heart; lane 8, skeletal muscle; lane 9, kidney; lane 10, spleen.

Table 1. Summary of characterization of putative exon clones.

Clone name	Fragment length (bp)	Zoo Southern	Northern	Sequence homology
4A-3	98	C	+	–
9A-5	68	C	–	–
15B-18	118	C	+	–
18A-13	135	S	N.T.	–
19C-1	139	S	N.T.	–
19D-1	53	C	+	–
22C-1	134	S	N.T.	–
22D-1	150	S	N.T.	–
48D-1	149	C	–	–
60D-1	104	C	+	ATDC exon 5
6A-1	61	C	–	–
6D-1	123	S	N.T.	–
50D-3	124	C	–	–
54B-1	195	C	+	ATDC exon5+6
54D-1	99	C	+	ATDC exon 7
85D-2	101	C	–	–

The results of Southern and Northern blot analyses are presented. C, conserved; S, single copy in human genome; N.T., not tested. The similarity of DNA and predicted amino acid sequences with DNA and protein databases was assesed by means of FASTA. The sequences have been deposited in DDBJ (accession numbers D63746 through D63758).

We selected transcribed sequences from restricted regions of a human chromosome, to help construction of a transcriptional map. Each step in reducing part of the genomic DNA from an intact chromosome 11 to exon candidate sequences, has generated valuable materials for the genome analysis. This includes an RH panel for mapping cloned DNA probes on chromosome 11, q23 region specific cosmid clones as genome DNA resources for positional cloning of genes related to diseases and exon sequences for screening cDNAs from q23. Once a cDNA is cloned, the gene structure can be efficiently analyzed using the original cosmid.

Reduction of the human chromosome fragment in hybrid cell increased regional specificity but decreased the fraction of cloned human DNA in the cosmid library. Since we wished to screen human DNA clone from a hybrid cosmid library, we overcome this problem by stepwise hybridization, which is laborious but simple. The cloned DNA segment described here has not been ordered on the basis of a physical map. Integration of the resources into physical maps will facilitate the construction of a region specific transcriptional map of 11q23.

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