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COSMID CLONES FROM MICRODISSECTED HUMAN CHROMOSOMAL REGION 15q11-q13

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Summary A human chromosomal region, 15q11-q13, was microdissected, its DNA was amplified with the primer-linker PCR method, and the PCR products were cloned into a plasmid vector to construct a microclone library. Of 193 microclones analyzed with Southern blot hybridization on hybrid cell panels, 26 (13.5%) were either single-copy (unique) or low-repetitive fragments. By screening of a cosmid library of human genomic DNA using the 26 microclones as probes, 47 positive cosmids were obtained and underwent regional mapping with chromosome fluorescence *in situ* hybridization (FISH). Sixteen cosmids gave FISH signals at 15p-cen, 5 at 15q11-q13, 6 at 15q22-q26, 3 at other chromosomes, and 17 no signal. These 27 cosmids mapped to chromosome 15 are useful additions to the inventory of DNA markers of this chromosome including the much interested Prader-Willi/Angelman syndrome region.

Key Words cosmid clone, microdissection, FISH, mapping, DNA marker

INTRODUCTION

A human chromosomal region, 15q11-q13, has a unique cytogenetic property. Since deletions, translocations, and inverted duplications have frequently been

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observed at 15q11-q13, the region is considered to be a hot-spot for rearrangements, and this cytogenetic instability may be explained by the presence of highly repetitive sequences, *e.g.*, inverted repeats, in the region (Mattei *et al.*, 1984; Donlon *et al.*, 1986). In addition, chromosome 15 contains highly methylated DNA much more than other acrocentric chromosomes (Okamoto *et al.*, 1981), and the DNA methylation is assumed to play a role in the genomic imprinting process. Actually, abnormalities at 15q11-q13 are causally associated with Prader-Willi syndrome (PWS) and Angelman syndrome (AS), which are contiguous gene syndromes and good examples of genomic imprinting in the human (Nicholls *et al.*, 1989; Hamabe *et al.*, 1991a, b). A number of DNA markers and at least 6 genes or transcripts have been assigned to this region: the $GABA_A$ receptor β3 subunit gene (*GABRB3*) (Wagstaff *et al.*, 1991), $GABA_A$ receptor α5 subunit gene (*GABRA5*) (Knoll *et al.*, 1993), DN10 (human homologue of the mouse pink-eyed dilution gene, *P*) (Gardner *et al.*, 1992), DN34 (*ZNF127*) (Waters *et al.*, 1992), the small nuclear ribonucleoprotein polypeptide N gene (*SNRPN*) (Özcelik *et al.*, 1992), and *MN7* (human homologue of a unique gene in the mouse) (Glenn *et al.*, 1993). YAC contigs have also been constructed in this region (Kuwano *et al.*, 1993), but several gaps still remains between the contigs (Donlon, 1992).

Chromosome microdissection is a simple and rapid means to construct a genomic DNA library from a defined chromosomal region. Among region-specific DNA libraries constructed with this method (Lüdecke *et al.*, 1989; Buiting *et al.*, 1990; Hirota *et al.*, 1992; Seki *et al.*, 1993), there has been such a library specific for 15q11-q13 (Buiting *et al.*, 1990), from which several microclones were mapped at a segment involved in deletions among PWS patients (Buiting *et al.*, 1992). However, since a microclone library does not completely cover the DNA at a defined region and the gaps among the YAC contigs at the 15q11-q13 region have not been bridged (Donlon, 1992), more number of cosmid clones are necessary to analyze the genomic structure of the region. Here, we report the results of our microdissection-microcloning at the 15q11-q13 region and the isolation of corresponding cosmid clones.

MATERIALS AND METHODS

Microdissection and amplification of chromosomal DNA. The procedure of chromosome preparation for microdissection was a modification of the method described previously (Deng *et al.*, 1992; Hirota *et al.*, 1992; Ohta *et al.*, 1993). In short, metaphase spreads were prepared from a human lymphoblastoid cell line. After treatment with a hypotonic solution consisting of 0.2% NaCl and 0.2% KCl, cells were fixed with absolute methanol. A fixed cell suspension (30 μl) was mixed with acetic acid (10 μl), and then immediately spread on a glass slide. GTG-banding was performed on non-aged chromosomes with 0.025% trypsin, and then chromosomes were stained with 3% Giemsa. Procedures of microdissection and enzymatic

amplification (polymerase chain reaction, PCR) of chromosomal DNA were those described previously (Hirota *et al.*, 1992; Jinno *et al.*, 1992) with a slight modification. A chromosomal region, 15q11-q13, was microdissected with a micromanipulator. One hundred pieces of dissected chromosomal fragments were transferred to a siliconized moist chamber. By lighting the moist chamber which was covered with a cap plugged with wet paper, dewdrops were formed on the collected chromosomal fragments. The dewdrops were covered with paraffin oil saturated with *Sau*3AI buffer (10 mM Tris-HCl, pH 7.5/7 mM $MgCl_2$/10 mM NaCl), then fused with 2–3 nl of proteinase K solution (0.5 mg/ml proteinase K/0.1% SDS/10 mM Tris-HCl, pH 7.5/10 mM NaCl) and incubated at 37°C for 4 hr. DNA extraction, *Sau*3AI digestion, DNA amplification with the primer-linker PCR method were all the same as described previously (Jinno *et al.*, 1992). Sequences of the linker, MboL1, and the primer, NlaM1, were 5′-GATCCATGTC-3′ and 5′-CGGGAATTCTGGCTCTGCGACATG-3′, respectively. The DNA was amplified for 30 cycles of PCR with denaturation at 93°C for 2 min, annealing at 62°C for 2 min and extension at 72°C for 2 min.

Confirmation of localization of PCR product. To confirm the chromosomal derivation of the PCR products, fluorescence *in situ* hybridization (FISH) was performed on metaphase chromosomes from a karyotypically normal individual as described previously (Deng *et al.*, 1992; Ohta *et al.*, 1993). A 1/100 portion (4 μl) of the first PCR products was PCR-amplified again to be biotin-labeled for 12 cycles in a reaction mixture containing biotin-16-dUTP (100 μl) (Boehringer-Mannheim, Germany) and dTTP (100 μl). Human Cot-1 DNA (GIBCO BRL, USA) was used as a competitor. FISH signals were detected with FITC-conjugated avidin (Vector Laboratories Inc., USA), and chromosomes were counterstained with propidium iodide. Photomicroscopy was done under a fluorescence microscope equipped with filter combinations, B-2A and B-2E (Nikon).

Microcloning of PCR products. The first PCR products were ethanol-precipitated and digested with *Eco*RI (50 U) at 37°C for 4 hr. Five microliter of the DNA, dissolved in 100 μl of 1 × TE buffer, was incubated at 16°C for 4 hr with 100 ng of dephosphorylated *Eco*RI-cut pUC19 in a 10 μl reaction mixture. The ligation mixture (5 μl) was transformed into competent DH5α cells.

Southern blot analysis. Inserts of the pUC recombinants (microclones) were digested with *Eco*RI and isolated by electrophoresis on a 4% polyacrylamide gel. The insert DNA was used as a probe for Southern blot hybridization of *Eco*RI- or *Hin*dIII-digested genomic DNA from normal human leukocytes, a human-mouse hybrid cell line (GM10500, Coriell Institute, USA) containing a human der(15), t(15;17)(q22;q11.2), and a hybrid cell line (A9Neo8, kindly provided by Dr. M. Oshimura, Tottori University) containing two human chromosomes 8. Inserts, which hybridized only to both total human DNA and DNA from GM10500 and contained no highly repetitive sequences, were used as probes for screening of a human cosmid library.

Screening of a cosmid library. A cosmid library from partial *Sau*3AI-digests of total human DNA was constructed using a cosmid vector, pWEX15. A mixture of 2 or 3 microclone inserts, having single-copy or low repetitive sequences, was used for screening of the library which was equivalent to 4-fold genomes. DNA on replica nylon filters was prehybridized with 200 μg/ml human placental DNA at 64°C for 8 hr. Hybridization was performed with ^{32}P-labeled microclone inserts at 65°C for 10–12 hr, the filters were washed twice in 0.1×SSC/0.1% SDS at 65°C for 15 min, and positive cosmid clones were isolated. To find overlapping cosmid clones, *Eco*RI digests were electrophoresed on a 0.8% agarose gel, transferred on nylon filters and hybridized with each of the microclones. Cosmids independent for their inserts were used as probes for further FISH studies.

Regional mapping of isolated cosmid clones. The isolated cosmids were labeled with biotin-16-dUTP by the use of a nick translation labeling kit (Boehringer-Mannheim). FISH was performed on R-banded normal metaphase chromosomes as described above.

RESULTS

Characterization of microclones

From 100 chromosomal fragments dissected at 15q11-q13, PCR products of a 150–700 bp size range were obtained. FISH using the PCR products as a probe pool painted the 15q11-q13 region, indicating that the PCR products were rich specifically for the DNA at the region. By cloning of the PCR products, a total of 341 pUC-recombinants were obtained. From these microclones, 193 inserts (MC1–MC193) with sizes larger than 150 bp (mean size, approximately 290 bp) were selected and analyzed. Southern blot analysis revealed that 26 (13.5%) were either single-copy (unique) fragments or low-repetitive sequences, which were all different in size and hybridized to both human genomic DNA and GM10500 DNA but not to A9Neo8 DNA (Fig. 1), 105 (54.4%) highly repetitive or smear-appearing sequences, and 62 (32.1%) the fragments not hybridized to human or mouse DNA (Table 1).

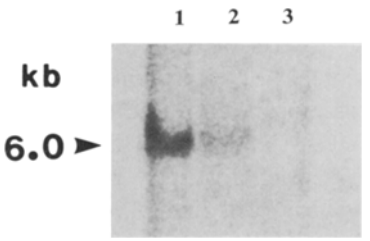

Fig. 1. Hybrid cell panel analysis with a microclone pM15C17, showing a single-copy signal in both total human DNA (1) and DNA from a human mouse hybrid cell-line containing a human der(15) chromosome (2), but no signal in DNA from the other hybrid cell without human chromosome 15 (3).

Table 1. Results of Southern blot analysis of microclones from 15q11-q13.

Signals	Number of microclones	
Single-copy or low-repetitive	26	(13.5%)
Highly repetitive or smeary	105	
None	62	
Total	193	

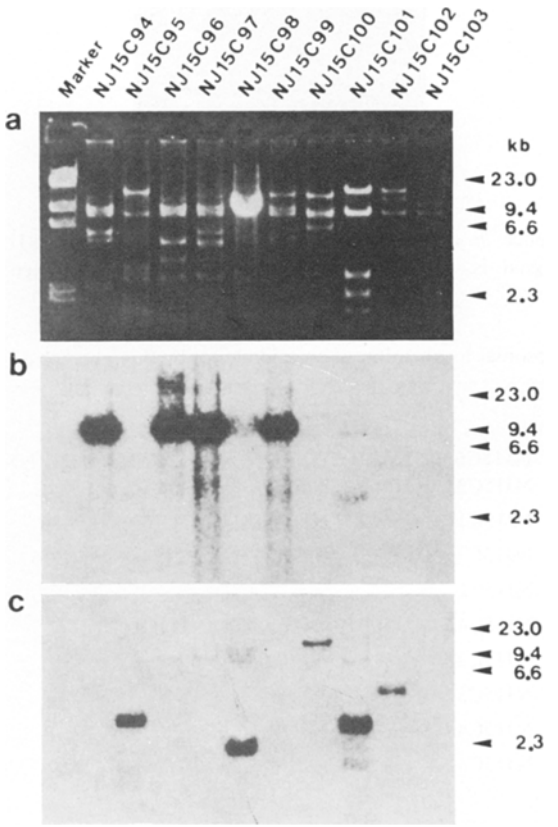

Fig. 2. Ethidium bromide staining of *Eco*RI digests of cosmid clones (NJ15C94-NJ15-C103) after electrophoresis on a 0.8% agarose gel (a), and Southern blot hybridization of the same digests using two microclone probes, pM15C292d (b) and pM15C293a (c). Four cosmid clones, NJ15C98, NJ15C100, NJ15C102, and NJ15C103, are independent, while others are overlapping, because NJ15C94, NJ15C96, NJ15C97, and NJ15C99 have an identical 9.4 kb fragment and common *Eco*RI digestion patterns, similarly NJ15C95 and NJ15C101 as well.

Cosmid library screening and regional mapping by FISH

By the cosmid library screening using the 26 unique or low-repetitive microclones as probes, 66 cosmid clones were isolated. Restriction patterns and Southern blot hybridization revealed that all but 19 cosmids were independent (Fig. 2). Of

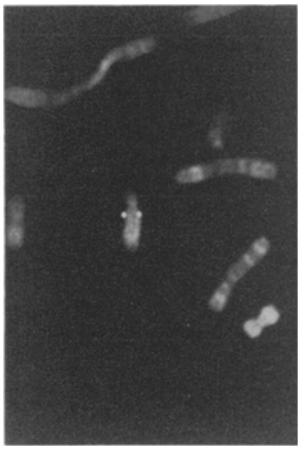

Fig. 3. Fluorescence *in situ* hybridization using a cosmid clone, NJ15C3, as a probe. FITC signal is observed at 15q12. Photograph was taken with filter B-2A (Nikon).

Table 2. Chromosomal localization of cosmid clones by fluorescence *in situ* hybridization.

Mapped regions	Cosmid clones
15p	NJ15C6, NJ15C10, NJ15C16, NJ15C17, NJ15C21, NJ15C28, NJ15C30, NJ15C31, NJ15C32, NJ15C108, NJ15C109
15cen	NJ15C11, NJ15C13, NJ15C14, NJ15C15, NJ15C24
15q11-q13	NJ15C3, NJ15C22, NJ15C39, NJ15C112, NJ15C115
15q22	NJ15C1
15q24	NJ15C42, NJ15C113, NJ15C114, NJ15C117
15q26	NJ15C37
3p14	NJ15C5
4p16	NJ15C4
1cen, 4cen, 5cen and 19cen	NJ15C41

the 47 independent cosmids used as probes for FISH, 16 were assigned to 15p-cen, 5 to q11-q13 (Fig. 3), 6 to other regions of chromosome 15, and 3 to 4p16, 3p14, and centromeres 1, 4, 5, and 19, respectively (Table 2). The remaining 17 cosmids gave either no FISH signals or multi-signals on many chromosomes.

DISCUSSION

A 15q11-q13 region may contain many copies of instable, repetitive sequences. In order to try to obtain unique clones efficiently, we collected a relatively large

number (100 pieces) of chromosomal segments dissected from the region. Nevertheless, the proportion (13.5%) of unique microclones obtained was smaller than that (20–50%) from the microdissection of other chromosomal regions with the same methods, although their mean size was well comparable to that from other regions (Hirota *et al.*, 1992; Seki *et al.*, 1993; Karakawa *et al.*, 1993). There has been only a similar 15q11-q13 specific microlibrary that was constructed by means of microdissection and subsequent PCR (Lüdecke *et al.*, 1990; Buiting *et al.*, 1990), where unique clones took 39%, the cloning efficiency being comparable to that (39%) in 22q12-q13.1 specific microclones, but lower than that (63–80%) in their other microclone libraries (Lüdecke *et al.*, 1990). Thus, it is conceivable that the fewer unique clones obtained from 15q11-q13 in the present study are attributable to a particular structure of DNA in this chromosomal region and/or in the paracentromeric regions of acrocentric chromosomes.

Among the cosmid clones screened from a cosmid library, 16 were assigned to 15p-cen, 5 to 15q11-q13, 6 to 15q22-q26, and 3 to other chromosomes. All together, 21 (44.7%) cosmids were mapped to paracentromeric regions of chromosome 15, the localizations corresponding to the dissected region. The 16 cosmids mapped to 15p-cen also gave FISH signals at the centromeres of other acrocentrics. These findings may have indicated that some of the original microclones shared low-repetitive sequences with other chromosomal regions. In fact, a cosmid clone, NJ15C4, mapped at 4p16 was detected by two microclones, MC16 and MC62, while MC16 detected NJ15C3 that was mapped at 15q12, and MC62 detected both NJ15C1 and NJ15C5 which were mapped at 15q22 and at 3p14, respectively. Corresponding sequences between chromosomes 15 and 4 were observed by a recent study by Youngman *et al.* (1992) that the telomeric 60 kb of 4p is homologous to a telomeric region on 15p.

Chromosome 15q11-q13 region is causally associated with PWS and AS. In order to know whether our 5 cosmid clones mapped at 15q11-q13 would be located within the AS critical region, FISH were performed on the metaphase chromosomes of an AS patient who deleted only two PWS/AS region markers, *D15S10* and *GABRB3* (Saitoh *et al.*, 1992). Since none of the cosmids were deleted in this patient, they are located outside a segment between *D15S10* and *GABRB3*. Further characterization of these cosmids as well as others is in progress.

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