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Mapping and Ordering Cosmid Clones Obtained from Human Chromosome Region 11q13.4-q25 by Fluorescence *in situ* Hybridization

Rumiko KATO

Department of Human Genetics, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan

A cosmid library was screened with 40 sequence-tagged sites (STSs) designed previously on the basis of DNA sequences of microclones that were generated by chromosome microdissection on two different human chromosomal regions, 11q14-q22 and 11q23-q25. Seventeen cosmid clones that corresponded to the STSs were successfully isolated. Single-color fluorescence *in situ* hybridization (FISH) using the 17 cosmids as probes, 11 were mapped to the expected regions of 11q. Then, the order of localization of the 7 cosmids (cG20, cG38, cG38R, cG50, cG51, cG75 and cR54) at the 11q21-q22.3 region were determined by two-color FISH on the extended prometaphase chromosomes, as cen-cG50-cG75-cG51-cG38-cG38R-cG20-cR54-tel. The result not only confirmed that microdissection is useful in the isolation of region-specific DNA clones, but also indicated that subsequent single- or two-color FISH is a valuable tool to map novel clones and marker DNAs, especially their order in the human genome.

Introduction

Chromosome microdissection is a powerful technique to isolate DNA clones from defined chromosomal regions[1-4]. Although these region-specific microclones have proven to be useful, their size (about 300 bp) is too small to confirm their chromosomal locations. To overcome this problem, larger genomic clones corresponding to microclones, such as cosmids, yeast artificial chromosomes (YACs) or bacterial artificial chromosome (BAC), have to be isolated[3,5-8]. Nevertheless, the small size of microclones are easy to sequence, so they can easily be converted into sequence-tagged sites (STSs), i. e., short DNA sequences that can be specifically detected by the polymerase chain reaction (PCR) in the presence of two short oligodeoxynucleotide primers[9-11]. Thus, STSs are useful landmarks for physical mapping of the human genome. For example, using STSs as markers, a YAC contig of the whole human chromosome 21 has been constructed[12]. Yoshiura et al.[4] obtained microclones from two human chromosomal regions, 11q14-q22 and 11q23-q25, with the microdissection-microcloning method as described previously[3,13,14]. Then, from these microclones, Soejima et al.[11] generated 50 STSs, and mapped them to five different regions by means of PCR on a somatic cell hybrid panel. However, the precise locations of these microclones has remained elusive. The purposes of this study were isolation of cosmids corresponding to the 50 STSs and their detailed assignments by means of single- and/or two-color fluorescence *in situ* hybridization (FISH).

Materials and Methods

Screening of a human genomic DNA cosmid library

Two sets of cosmid libraries were constructed from total human genomic DNA by standard methods. In brief, genomic DNA from normal human leukocytes was partially digested with *Sau3*AI and fractionated by sucrose-density gradient centrifugation to yield 35- to 42-kb fragments. The fragmented DNA was partially filled in, and ligated to the cosmid vector pWEX15. The ligated products were then packaged with *in vitro* packaging extracts, Gigapack II Gold (Stratagene, USA). The two libraries were composed of 2.2×10^5 and 2.3×10^5 clones respectively, each being equivalent to three haploid genomes.

To obtain probes for a cosmid library screening, human genomic DNA was PCR-amplified using 40 of the 50 STS primers designed by Soejima et al.[11] These 40 primers were those amplifying DNA fragments of 120 bp or more in size. PCR products showing a single-band by electrophoresis on a 4% polyacrylamide gel were cut out from the gel, and extracted DNA was labeled with [α -^{32}P] dCTP by random priming and used as probes for the screening. After overnight hybridization at 65℃, membranes were washed twice at 65℃ for 15 min in 0.1 × SSC/0.1% SDS, and positive cosmid clones were isolated. Hybridization stringencies and washing conditions were varied with the length of the probes. Prehybridization with sonicated

human placental DNA was occasionally employed to diminish background signals. To find overlapping clones, *Eco*RI digests were electrophoresed on a 0.8% agarose gel. Colonies with positive signals were recultured and cosmid DNA was extracted.

Fluorescence in situ hybridization (FISH)

For single-color FISH, metaphase chromosomes from a karyotypically normal individual were prepared by standard techniques. In brief, cultured peripheral blood lymphocytes were treated with an excess amount of thymidine (300 μg/ml) for 16 hr, followed by 6-hour bromodeoxyuridine (BrdU) incorporation. Metaphase chromosomes were collected by adding 0.02 μg/ml Colcemid (Difco, USA) 1 hr prior to harvest. Cells were then treated with 0.075 M KCl at room temperature for 20 min, fixed in 1 : 3 acetic acid/methanol, and chromosomes were spread onto a glass slide. For two-color FISH experiments, extended prometaphase chromosomes were prepared by treating cells with a topoisomerase II inhibitor, ICRF154 (Zen-yaku Kogyo, Japan), 1 hr prior to harvest, according to Inazawa et al.[15] Chromosome slides were made by 0.05 M KCl-hypotonic treatment, followed by flame-drying and baking at 65℃ for 5 hr before FISH.

Cosmid clones isolated were labeled with biotin-16-dUTP or digoxigenin-11-dUTP using a nick-translation labeling kit (both agents from Boehringer-Mannheim, Germany), and precipitated together with sonicated salmon sperm DNA, and then resuspended in formamide. Single-color FISH with biotin-labeled probes was performed as described previously[16] with a slight modification. Two-color FISH was also done according to the method of Inazawa et al[15]., using a mixture of the biotin- and digoxigenin-labeled probes at a v/v ratio of 7 : 3. After adding human Cot-1 DNA (GIBCO BRL, USA) as a competitor, the labeled probes were denatured at 75℃ for 10 min, and mixed with a 4 × SSC solution containing 20% dextran sulfate. Chromosomal DNA was also denatured at 80℃ for 3 min in 70% formamide/2×SSC, and immediately dehydrated by passing through 70% and 99% ethanol series for 5 min each and air dried. After hybridization at 37℃ overnight, chromosome slides were washed at 37℃ in 50% formamide/2×SSC, and at room temperature in 2×SSC and 1×SSC each for 15 min. Then, they were incubated at 37℃ for 1 hr in 4×SSC/1% BSA containing avidin-FITC (Boehringer-Mannheim) with or without anti-digoxigenin-rhodamine (Boehringer-Mannheim), and rinsed in 4×SSC, 4×SSC containing 0.02% Tween 20, and in 4×SSC. Chromosomes for single-color FISH were counterstained with propidium iodide (PI) containing an antifade agent, 1, 4-diazabicyclo-(2, 2, 2)-octane (DABCO) (Sigma, USA), and those for two-color FISH with 4', 6'-diamino-2-phenylindole (DAPI) containing DABCO and *p*-phenylenediamine (Sigma). Photomicroscopy was performed under a fluorescence microscope (Nikon, Japan). FITC signal was observed with a B-2E filter (Nikon). Two-color FISH signals were simultaneously visualized through a FITC/Texas-red dual-band-pass filter (Omega Optical, USA), and chromosomal contours were recognized with a UV-2A filter (Nikon).

Results

Isolation of cosmid clones

Of the 40 STS primers, 30 yielded PCR products showing a clear single electrophoretic band, while the products with the remaining 10 primers exhibited multiple bands. Thus, these 30 PCR products were radiolabeled and used as probes for the cosmid library screening. By the screening, 39 cosmid clones were isolated. Restriction analysis revealed that 19 of the 39 cosmids were independent and corresponded to 17 probes (Table 1). Thirteen STSs failed to detect any cosmid clones.

Table 1. Number of STSs and cosmid clones isolated

Chromosomal region	STS	STS used as primer	Cosmid clone obtained
11q13.4-q21	1	1	1
11q21-q22.3	28	17	10
11q23.1-q23.2	1	1	1
11q23.2-q23.3	10	6	3
11q24-q25	10	5	2
total	50	30	17

Localization of cosmid clones and their ordering

With single-color FISH, 11 of the 17 cosmid clones were assigned to expected regions of the long arm of chromosome 11 (Fig. 1a). They included one clone (cG73) mapped to 11q13.4-q21, seven (cG20, cG38, cG38R, cG50, cG51, cG75 and cR54) to 11q21-q22.3, one (cR56) to 11q23.1-q23.2, and two (cR82 and cR96) assigned to 11q24-q25 (Table 2). Four cosmids mapped to other chromosomes, but the remaining 2 gave no FISH signals. Since the 7 clones (cG20, cG38, cG38R, cG50, cG51, cG75 and cR54) gave FISH signals at one fine region, their order on chromosome 11 was analyzed in pairs by two-color FISH. An example of the results is as follows : the two-color FISH for biotin-labeled cG20 and digoxigenin-labeled cG38 probes simultaneously gave two pairs of twin-signals in 42 of 60 prometaphase cells analyzed, one twin-pair and one single-pair signal in 13, and gave overlapping two-pair signals in the other 5 cells. Analysis of relative positions of the probes on the chromosome revealed that the localization of cG20 was centromeric to cG38 (Fig. 1b and 1c). Likewise, relative locations of other loci were detected, and thus the order of the 7 loci was determined to be cen-cG50-cG75-cG51-cG38-cG38R-cG20-cR54-tel.

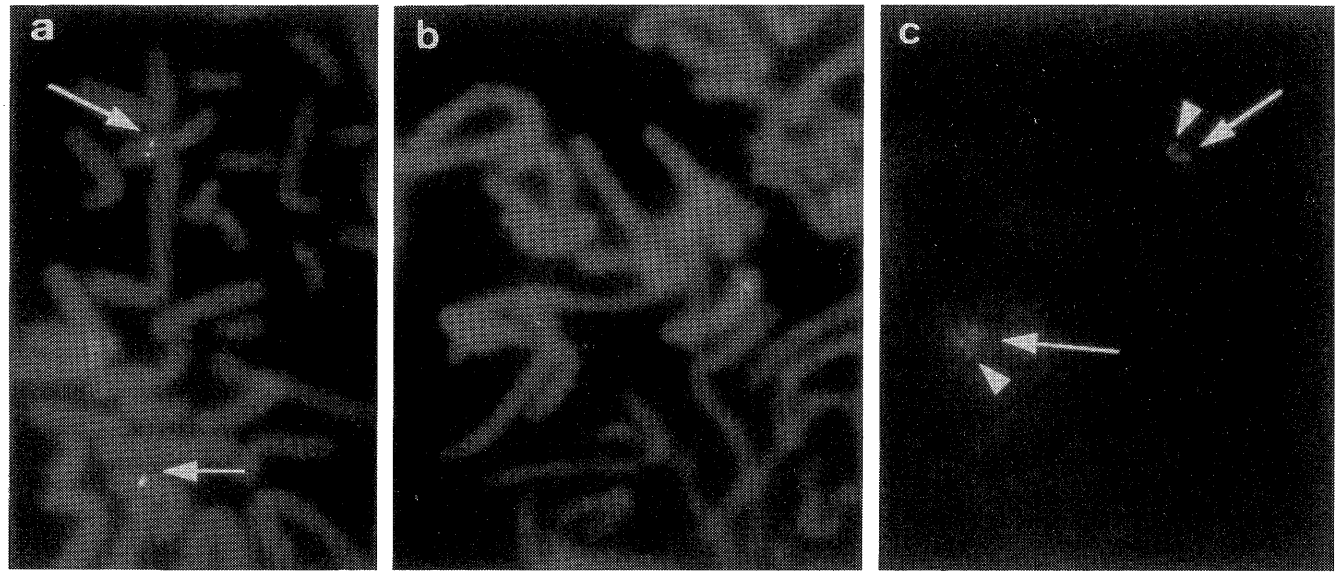

Fig. 1. Localization of cosmid clone cG38 on normal human metaphase chromosomes by single-color fluorescence *in situ* hybridization (FISH). FISH signal is located on the expected region of chromosome 11q (arrows) (a). Two-color FISH on ICRF154 treated prometaphase chromosomes using two cosmids, cG20 and cG38 as probes (b and c). DAPI-stained prometaphase chromosomes observed through a UV-2A filter (b) and the same prometaphase chromosomes through a dual band-pass filter (c). FITC signal corresponding to biotin-labeled cG20 is shown with arrowheads. Rhodamine signal corresponding to digoxigenin-labeled cG38 is shown with arrows.

Table 2. Assignment of 17 cosmid clones by FISH

STS	Original location of STS	Cosmid clone	Location detected by FISH
G73	11q13.4-q21	cG73	11q14-q22
G6	11q21-q22.3		
G8	11q21-q22.3		
G20	11q21-q22.3	cG20	11q14-q22
G26	11q21-q22.3		
G38	11q21-q22.3	cG38	11q14-q22
G38R	11q21-q22.3	cG38R	11q14-q22
G50	11q21-q22.3	cG50	11q14-q22
G50R	11q21-q22.3		
G51	11q21-q22.3	cG51	11q14-q22
G51R	11q21-q22.3		
G61	11q21-q22.3		
G65	11q21-q22.3	cG65	no signal
G75	11q21-q22.3	cG75	11q14-q22
G76	11q21-q22.3	cG76	distal long-arm of group D
R40	11q21-q22.3		
R54	11q21-q22.3	cG54	11q14-q22
R55	11q21-q22.3	cG55	short-arm of group E
R56	11q23.1-q23.2	cG56	11q23-q25
G58	11q23.2-q23.3	cG58	distal long-arm of group B
R23	11q23.2-q23.3	cR23	no signal
R25	11q23.2-q23.3	cR25	distal long-arm of group A
R30	11q23.2-q23.3		
R84	11q23.2-q23.3		
R86	11q23.2-q23.3		
R27	11q24-q25		
R62	11q24-q25		
R76	11q24-q25		
R82	11q24-q25	cR82	11q23-q25
R96	11q24-q25	cR96	11q23-q25
Total		17	11*

*Number of clones mapped to 11q

Discussion

One of the major objectives of the human genome project was to construct a physical map of human genomes. International effort has achieved an STS-based physical map that contains 15,086 STSs with an average interval of 199 kb[17]. Human chromosome 11 comprises about 4% of the genome and consists of approximately 1.3 -1.4 $\times 10^8$ bp of DNA. Because several important disease genes are located on this chromosome, extensive mapping information on chromosome 11[18-20] has been accumulated. Smith et al.[21] reported 370 STSs of human chromosome 11 which were produced by direct sequencing of cosmid clones isolated from a chromosome 11-specific library. Miwa et al.[22] also identified 50 STSs from cosmid clones mapped on chromosome 11. The distributions of these two large series of STSs show a slight bias with a paucity in the 11q14-q22 region (G-band-rich region). This bias may have been due either to the clone-selecting procedures used or to difficulty cloning from R-band-rich regions. Since cosmid clones were usually screened by colony hybridization with radiolabeled human genomic DNAs which contain *Alu* repeats abundantly, clones with many such repeats may have been selected predominantly from *Alu*-rich R-band regions. On the contrary, STSs identified from microdissection-generated microclones were localized almost evenly between G- and R-band regions : 29 were in G-band regions and the other 21 in R-band regions[11]. Thus, it is expected that a microclone library generated from either of G- or R-bands has an advantage as a source of evenly distributed STSs. Nevertheless, microclone-derived STSs also had a biased distribution within a given band. Most of the G-band-derived microclones were concentrated in a distal half of the microdissected region, 11q21-q22.3, while only one clone was localized to the 11q14-q21 region. This paucity in 11q14-q21 region has also been observed in the STS distribution reported by Miwa et al[22]., and in a microsatellite-based index map of human chromosome 11[19]. It is conceivable that DNA at this region is resistant to cloning and/or PCR amplification. By PCR on a hybrid cell panel using STS primers, some microclones were mapped to regions different from their original microdissected regions[11]. A similar phenomenon was also experienced by La Pillo et al.[9] who generated STSs from an 8q24.1-specific microclone library. These errors may be attributable to technical overdissection of adjacent chromosomal regions or to complex DNA loops or three-dimensional DNA structures in the condensed chromatin. Alternatively, the error may have come from contamination of other chromosomal fragments.

Based on STSs from the microclones, we isolated 17 cosmid clones and mapped 11 of them to the 11q13.4-q25 region. In addition, since we assigned 7 of the 11 clones to one fine region (11q21-q22.3), we could put them in order, as cen-cG50-cG75-cG51-cG38-cG38R-cG20-cR54-tel. These mapping and ordering data are useful and add to the knowledge of the human physical map. However, difficulties had arisen when cosmid clones are isolated with STS-based PCR. Of 30 STSs to be selected as PCR primer sets, only 17 detected cosmid clones. Thus, it is likely that our cosmid library was incomplete and did not contain all of the expected genomic fragments. There was also a biased distribution in location of the isolated cosmids. Eight clones were mapped to the expected G-band regions (11q13.4-q21 and 11q21-q22.3), while only three were obtained from the R-band region (11q23.1-q23.2 and 11q24-q25), though the total number of clones analyzed was small. An explanation that the bias reflected the degree of *Alu* repetitive sequences between two-band regions is less likely, because more STSs had been identified from an R-band region in previous studies[21,22]. Since microclones themselves are the PCR products and may occasionally have rearrangements, designing of STS primers from the microclones may result in inappropriate PCR products for library screening. This explanation was most plausible and was supported by the finding that some isolated cosmid clones were mapped to other chromosomes by FISH.

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