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Painting of defined chromosomal regions by in situ suppression hybridization of libraries from laser-microdissected chromosomes


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Abstract. "Painting" of defined chromosomal regions provides a powerful tool for cytogenetic analyses. Here, we demonstrate that chromosome in situ suppression (CISS)-hybridization of DNA libraries derived by microcloning laser-microdissected chromosomal regions can be applied to achieve this goal. As an example, we used unbanded metaphase spreads from a female patient carrying a balanced translocation, t(1;7)(p36;q11). Fragments from the long arms of 130 translocation chromosomes were microdissected. After microcloning, human insertions with an average size of about 3 kb were pooled from 400 recombinant bacteriophage DNA clones and used as a complex probe set in CISS-hybridization experiments. This resulted in painting of the translocation chromosome along the region 7q35 to 1p31. Painted chromosome subregions in normal chromosomes 1 and 7 were consistent with this finding. This approach may be used to perform painting of any chromosome regions for which microlibraries can be established. Possible applications include the definition of marker chromosomes in clinical and tumor cytogenetics and studies of chromosomal evolution, as well as studies of nuclear chromosome topography in animal and plant species.

Chromosomal in situ suppression (CISS)-hybridization of biotinylated libraries from sorted human chromosomes, in conjunction with fluorescent or colorimetric detection procedures, has provided a new tool for the specific "painting" of complete individual chromosomes in both mitotic and interphase cells (Lichter et al., 1988a, b; Pinkel et al., 1988). CISS-hybridization has also been useful for the mapping of genomic DNA sequences (e.g., cloned in cosmid vectors) which contain interspersed repetitive sequences, such as Alu sequences, in addition to chromosome site-specific sequences (Landeget al., 1987; Lichter et al., 1990a, b). Still, the generation of complex probe sets for the selective staining of desired chromosomal subregions, such as chromosome arms or chromosome bands, has remained a time-consuming task. In addition, the lack of sorted chromosome libraries from nonhuman species has, until now, constituted another limiting factor in the general use of the CISS-hybridization technique.

This study was carried out to investigate whether probe sets useful for the painting of chromosomal subregions could be established by microcloning of laser-microdissected chromosomes. As a model case, a marker chromosome (1qter→1p36.37q11→7qter) (Fig. 1) from a healthy female carrying a balanced translocation, t(1;7)(p36.37q11), was microdissected using an excimer laser microbeam. Chromosomes were not stained to facilitate microcloning procedures, but the marker chromosome could be easily recognized as the largest chromosome of the complement. Laser microdissection was aimed to include the region 7q22→32 (Eckelt et al., 1989). Accordingly, two laser cuts were performed, one to remove the chromosome 1 material and the other to remove the distal region of 7q. In the absence of banding, it was difficult to reproduce the localization of the laser cuts precisely in marker chromosomes that had undergone various amounts of condensation. While a preliminary investigation of the resulting microlibrary indicated the presence of clones from the desired region (Eckelt et al., 1989), CISS-hybridization should answer the question of the actual extent of the microdissected region.

Materials and methods

Cell material. Phytohemagglutinin (PHA)-stimulated human lymphocyte cultures were established from healthy (46,XY; 46,XX) donors. Metaphase spreads were prepared for subsequent use in CISS-hybridization experiments (see below) using standard procedures and stored at 4°C. For laser microdissection, Epstein-Barr virus-transformed cells of a female patient carrying a balanced translocation, t(1;7)(p36.37q11), were kindly provided by K. Miller (Medizinische Hochschule Hannover) (Eckelt et al., 1989). Metaphase spreads were prepared and fixed as described by Martinsson et al. (1989).

Laser microdissection. The details of the procedure have been described previously (Monajembashi et al., 1986; Eckelt et al., 1989; Ponelies et al., 1989). Briefly, pulses of an excimer pumped dye laser (Lambda Physik, Göttingen, FRG), at a repetition rate of about 10 Hz, were directed into an inverted IM 35 microscope via the fluorescence illumination path. In the focus, power densities of up to 10¹¹ W cm⁻² were obtained. Laser cutting of the translocation chromosome, t(1;7)(qter→1p36.37q11→7qter), was achieved by moving the marker chromosome at preselected sites through the laser focus. A total of 130 fragments were collected for microcloning.

Microcloning. Microcloning was performed essentially as described by Scalenghe et al. (1981) and Martinsson et al. (1989), with several modifications described below. Micromanipulation work was done in a paraffin oil-filled...
chamer under a phase-contrast microscope. After laser dissection, fragments wereaken up by a glass needle, using a de Fonbrune micromanipulator, and transnioned into a 0.6-nl aqueous droplet containing 1 mg/ml proteinase K and 0.2% SDS. After digestion for 2 h at 55°C, proteins in the microdrop were extracted three times with four volumes of phenol saturated with TE buffer, followed by a wash with 0.5 μl of chloroform. Restriction digestion was carried out in a 1-ml reaction volume containing 10 mM Tris HCl (pH 7.5), 5 mM NaCl, 0.5 mM MgCl2, and 280 units/μl EcoRI. After incubation for 2 h at 37°C, the enzyme was removed by extraction with phenol. The digested DNA was ligated for 2 h at 12°C to 0.56 ng EcoRI-cut dephosphorylated λ phage NM 1149 DNA (Murray et al., 1982), using 0.1 units T4 ligase in a total volume of 2.4 ml of ligation buffer (50 mM Tris HCl; pH 7.5, 10 mM MgCl2, and 1 mM ATP). The ligation product was packaged in vitro as described by Scherer et al. (1981). Phags were plated on plates with the hft mutant NM514 as bacterial host (Murray et al., 1983).

A total of 450 recombinant phage clones from the microlibrary (containing 478 cones) were picked onto LB-agar plates with an overlayer of 3 ml of soft agar containing the NM514 bacterial host and incubated at 37°C overnight. Plaque hybridization was carried out with32P-labeled, sheared human genomic DNA (Church and Gilbert, 1988). After autoradiography, 230 (51%) of the 450 phags showed signals of varying intensity, indicating the presence of highly or moderately repetitive human sequences. Clones from the remaining fraction were not further tested individually but would likely contain single or low-copy human sequences. The average insert size of 22 randomly picked clones (11 “single copy” clones and 11 clones containing highly repetitive sequences) was 3 kb range, 0.55–8.2 kb.

DNA probes and preparation. As a probe set for CISS-hybridization experiments, 400 recombinant phages from the microcloned phage-DNA library were pooled at random and amplified in liquid culture using E. coli LE 392 as bacterial host. Purification of the phages and extraction of phage DNA were carried out as described by Maniatis et al. (1982). After digestion with EcoRI, human inserts were recovered from a 0.8% agarose gel by electrotelution into dialysis bags and concentrated with phenol (Maniatis et al., 1982).

Hasmid DNA clone pu7tl containing the alphoid sequence specific for human chromosome 7 was established by Wayne et al. (1987) and kindly provided by Peter Devilee (Leiden). Plasmid transformation and DNA preparation were carried out following standard procedures (Birnboim and Doly, 1979).

Probe labeling, CISS-hybridization, and probe detection. Plasmid DNA pu7tl and human inserts from the pooled phages of the microlibrary established from the microdissected chromosome region were labeled with biotin-11 dUTP as described by Pinkel et al. (1986). Preparations were counterstained with 0.2 μg/ml 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI) and 1 μg/ml propidium iodide and mounted in fluorescence antilushing buffer (1 mg n-phenylenediamine in 1 ml of glycerin buffer, pH 8.0). Cells were evaluated with a Zeiss Photomicroscope III equipped for epifluorescence. Photographs were taken on Agfachrome 1000 RS color slide film. Alternatively, digitized images were obtained using a laser-scanning confocal microscope as described by Lichter et al. (1990b). In the latter case, photographs were taken from a video monitor.

Fig. 1. Schematic drawing of the translocation chromosome, t(1;7)(qlter→Ip36::7ql1→7qter). The bracket on the right indicates the extension of the painted region, as obtained by fractional length measurements of 10 marker chromosomes (see text).

Results

Figure 2a (left side) shows the marker chromosome, t(1;7)(qlter→Ip36::7ql1→7qter), after painting with the probe set derived from the microlibrary. Fractional length measurements (Lichter et al., 1990b) of 10 marker chromosomes, with the telomere of the long arm and the centromere serving as boundaries, made it possible to determine the extension of the painted region from 7q35 to Ip31 (Fig. 1). This result indicates that the microcloned chromosome fragments frequently included chromosome Ip3 material in addition to 7q material. Consistent with this conclusion was the observation in normal chromosomes 1 that Ip3 was painted except for the telomeric region (Fig. 3a). This finding
CISS-hybridization of libraries from microdissected chromosomes

The painted region is estimated to include 100–150 Mb of DNA from the marker chromosome—equivalent to the length of a medium-sized chromosome of the human complement. Provided that all 400 microclones used for probe preparation carry human inserts from different sites of the microdissected fragments, the maximum complexity of sequences contained in the probe pool could equal up to 1.2 Mb of DNA, which might possibly include some 600 kb of unique sequences. The actual complexity of the probe may be lower, however, depending on the number of clones that carry identical inserts.

The banded pattern seen on the long arms of more extended chromosomes 7 after CISS-hybridization of the microlibrary was observed, although Alu banding of unpainted chromosomes due to the presence of biotinylated Alu sequences in the probe set was efficiently suppressed. The presence of some clustered, 7q-specific, repetitive sequences may provide a possible explanation. Other explanations, such as a chance spatial clustering of the cloned sequences in the microlibrary or a nonuniform distribution of EcoRI restriction sites (used for digestion of the microdissected DNA) along 7q, may be taken into consideration. Notably, such a banded pattern was not obvious in CISS-hybridization experiments using a library from sorted chromosomes 7 where the DNA sequence complexity of this chromosome was represented much more completely (Cremer et al., 1988; Lichter et al., 1988a).

We expect that the approach exemplified here will open intriguing new possibilities in basic and applied cytogenetics. These possibilities include the evaluation of unidentified marker chromosomes or translocated chromosome material contained, for example, in tumor cells. Furthermore, microlibraries derived from individually collected complete or microdissected chromosomes may provide a universal tool for chromosome painting in any desired species. Recently, the elegant work of Lüdecke et al. (1989) showed that banded chromosomes can be used for microcloning. Since the collection of banded chromosomes by micromanipulation can be precisely controlled (Senger et al., 1990), impurities in a microlibrary caused by undesired chromosomes or fragments can be avoided. In contrast, libraries from sorted chromosomes may not only be difficult to achieve for many species, but even the best sorts inevitably contain a certain fraction of unwanted chromosomes or fragmented chromosome pieces.

At present, microdissection and microcloning of chromosomes are highly specialized procedures which may not be easy to establish in all clinical cytogenetic laboratories. We believe, however, that probe sets useful for the painting of chromosome arms and bands, once they are established in a few specialized laboratories, could provide an important service to the cytogenetic community in general. Furthermore, polymerase chain reaction-based methods, when used to amplify DNA from microdissected chromosome material, have the potential to facilitate or even replace elaborate microcloning procedures (Nelson et al., 1989; Ledbetter et al., 1990; Lengauer et al., 1990).

Discussion

In this study we demonstrate that a microlibrary established from laser-microdissected fragments of a translocation chromosome, t(1;7)(1qter→1p36::7q11→7qter), is sufficient for specific painting of the microdissected chromosome region. In addition to most of the long arm of the normal chromosome 7, a region of the short arm of the normal chromosome 1 (1p3, except for the telomeric region) was also painted, proving that the microdissected fragments included material from both chromosomes.

The painted region is estimated to include 100–150 Mb of DNA from the marker chromosome—equivalent to the length of a medium-sized chromosome of the human complement. Provided that all 400 microclones used for probe preparation carry human inserts from different sites of the microdissected fragments, the maximum complexity of sequences contained in the probe pool could equal up to 1.2 Mb of DNA, which might possibly include some 600 kb of unique sequences. The actual complexity of the probe may be lower, however, depending on the number of clones that carry identical inserts.

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References