Hum Genet (1987) 76:290–292

Human Genetics

A method for nucleic acid hybridization to isolated chromosomes in suspension

Gertrud Dudin[1], Thomas Cremer[2], Margit Schardin[2], Michael Hausmann[1], Frank Bier[1], and Christoph Cremer[1]

[1]Institut für Angewandte Physik I der Universität, Albert-Ueberle-Strasse 3–5, D-6900 Heidelberg 1, Federal Republic of Germany
[2]Institut für Anthropologie und Humangenetik der Universität, Im Neuenheimer Feld 328, D-6900 Heidelberg 1, Federal Republic of Germany

Summary. A procedure was developed to provide differential fluorescent staining of metaphase chromosomes in suspension following nucleic acid hybridization. For this purpose metaphase chromosomes were isolated from a Chinese hamster × human hybrid cell line. After hybridization with biotinylated human genomic DNA, the human chromosomes were visualized by indirect immunofluorescence using antibodies against biotin and fluoresceine-isothiocyanate-(FITC)-labeled second antibodies. This resulted in green fluorescent human chromosomes. In contrast, Chinese hamster chromosomes revealed red fluorescent staining only when counterstained with propidium iodide. Notably, interspecies chromosomal rearrangements could be easily detected. After hybridization and fluorescent staining, chromosomes still showed a well-preserved morphology under the light microscope. We suggest that this procedure may have a useful application in flow cytometry and sorting.

Introduction

Hybridization of chromosome-specific DNA probes to isolated chromosomes in suspension may offer a new possibility to classify and separate specific chromosomes with high purity (Gray and Langlois 1986). In this approach chromosome-specific DNA probes are biotinylated and then hybridized to denatured chromosomal DNA (Langer-Safer et al. 1982; Cremer et al. 1986). The hybridized probe is visualized by indirect immunofluorescence using antibodies against biotin and fluoresceine-labeled second antibodies (Langer-Safer et al. 1982). In rodent × human hybrid cell lines, human genomic DNA can serve as the human chromosome-specific DNA probe due to differences of highly abundant repetitive DNAs in the different species (Manuelidis 1985; Schardin et al. 1985; Hopman et al. 1986; Pinkel et al. 1986). So far this technique has been successfully applied to chromosomes fixed on slides. To adapt this procedure to suspended chromosomes as a prerequisite for further use in flow cytometry and sorting, the chromosome integrity has to be maintained under hybridization conditions. As described by Trask (1985), chromosomes isolated in the Hepes/$MgSO_4$ isolation buffer fall apart under DNA denaturation conditions.

In the present study we used suspended metaphase chromosomes from a Chinese hamster × human hybrid cell line isolated in a hexylene glycol buffer for nucleic acid hybridization in suspension. The human chromosomes were labeled with biotinylated human genomic DNA and visualized with fluoresceine-isothiocyanate (FITC). The Chinese hamster chromosomes remained unlabeled but became visible by counterstaining with propidium iodide.

Offprint requests to: G. Dudin

Materials and methods

The Chinese hamster × human hybrid cell line A1wbf2 (with mainly four human chromosomes), kindly provided by Dr. P. Pearson (Leiden), was grown in 750-ml Falcon flasks in Ham's F10 medium supplemented with 10% fetal calf serum, 1% hypoxanthine/thymidine solution (HT Konzentrat 50×, Boehringer Mannheim), and 1% penicillin/streptomycin solution (10000 U, 10000 µg/ml). Mitotic cells were synchronized by a colcemid block of 8 h (0.25 µg colcemid/ml medium). Upon a medium change the cells were allowed to grow for another cell cycle and then harvested in mitosis by a shake off with no or a short (up to 1 h) second exposure to colcemid. The mitotic cells were pelleted by centrifugation (350 *g*; 15 min), and a hypotonic treatment with a solution of 10 m*M* Tris, 5 m*M* $MgCl_2$, and 10 m*M* NaCl at pH 7.5 was applied for 15 min at room temperature. In the hypotonic solution the amount of mitotic cells was determined. After centrifugation (350 *g*; 10 min), to remove the hypotonic solution, 1 ml hexylene glycol isolation buffer, described by Stoehr et al. (1982), with pH 3.2 and modified by adding 0.5 m*M* $MgCl_2$, was added. In the same buffer the mitotic cells were sonicated to disrupt the cell membrane and get a suspension of dispersed chromosomes. This suspension was stored at 4°C.

In our experiments, chromosome preparations up to 3 months old were successfully used for hybridization. Before hybridization, the isolation buffer was removed by centrifugation (350 *g*; 15 min), and the chromosome pellet (ca. 4×10^5 chromosomes) was washed in 1 ml 2 × SSC (1 × SSC is 0.15 *M* NaCl/0.015 *M* sodium citrate) and transferred to a Falcon tube No. 2054. In this tube, chromosome sticking is minimized (R. Lebo, personal communication).

After centrifugation the chromosome pellet was resuspended in 0.5 ml hybridization mixture containing 40% deionized formamid, 4 × SSC, 2 × Denhardt's solution, 10% dextran sulfate, and 1 µg biotinylated human genomic DNA that has been nick translated with biotin-dUTP as described by Langer-Safer et al. (1982) and Schardin et al. (1985).

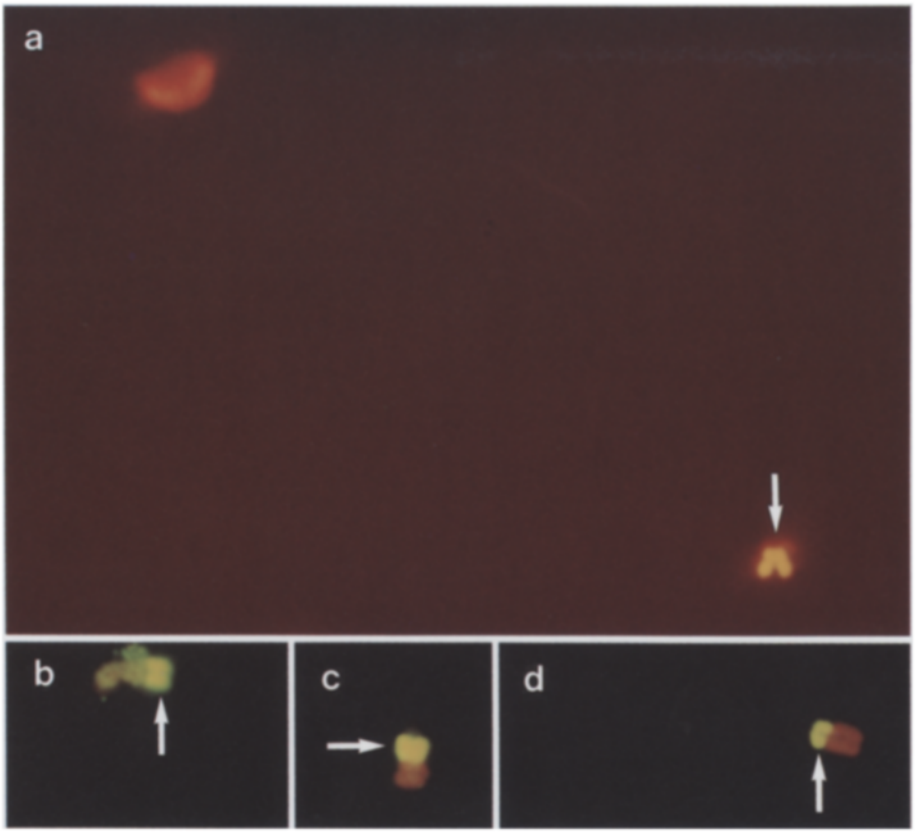

Fig. 1a–d. Isolated metaphase chromosomes of the Chinese hamster × human hybrid cell line A1wbf2 after fluorescence hybridization with biotinylated human genomic DNA in suspension. The chromosomes were counterstained with propidium iodide and then photographed with a 515-nm filter upon ethanol fixation on a slide. Human chromosomes and parts of human chromosomes in interspecies rearrangements show a yellow-green FITC fluorescence *(arrows)*, while Chinese hamster chromosomes display red fluorescence. For further details see text

The chromosomes were denatured at 73°C for 6 min and transferred to a water bath at 42°C for hybridization overnight. After hybridization the chromosomes were pelleted by centrifugation at 350 *g* for 10 min, washed in 1 ml prewarmed 2 × SSC for 30 min at 42°C and again centrifuged. After another wash in 1 ml 0.1 × SSC for 30 min at the same temperature and centrifugation, the chromosome pellet was resuspended in 2 × SSC. To visualize the biotinylated DNA after hybridization, the double antibody fluorescent detection system supplied by ENZO (Neckargemünd, FRG) was used. The chromosomes were centrifuged and resuspended in 1 ml IB + M buffer (50 m*M* KCl, 5 m*M* Hepes pH 8.0, 10 m*M* $MgSO_4$; Trask et al. 1985) and then treated according to the ENZO protocol for staining chromosomes fixed on slides. The chromosome suspension was incubated for 5 min at room temperature in 1 ml IB + M buffer containing 0.1% (v/v) Triton X-100, centrifuged, washed twice in 1 ml IB + M buffer, centrifuged again, and incubated for 2 h at 37°C in 300 µl IB + M buffer containing 3% (w/v) bovine serum albumin (BSA) and 1% (v/v) rabbit-antibiotin antibody solution. After centrifugation the chromosomes were then washed twice in 1 ml IB + M buffer, centrifuged, and incubated for 1 h at 37°C in 300 µl IB + M buffer containing 3% (w/v) BSA and 1% (v/v) FITC-goat-antirabbit-IgG solution. The chromosomes were centrifuged again, washed once more in 1 ml IB + M buffer and after centrifugation resuspended in 0.5 ml IB + M buffer. Centrifugation always took place at 350 *g* for 15 min. The labeled and FITC-stained chromosomes could be stored at 4°C for about 2 weeks without apparent loss of quality in chromosome morphology or FITC-staining as judged by light microscopic observation.

For photography, 5 µl of this chromosome suspension was either directly dropped on a slide cleaned in absolute ethanol and then counterstained with 5 µl propidium iodide solution (75 µ*M*) (Fig. 1a), or the chromosome suspension was first stained with 0.5 ml 75 µ*M* propidium iodide solution for 20 min at room temperature, then centrifuged at 350 *g* for 10 min and washed once in 1 ml IB + M buffer followed by centrifugation. The chromosome pellet was resuspended in 0.5 ml IB + M buffer, and a 5-µl sample dropped on a slide (Fig. 1b–d). The same amount of fluorescence antifading buffer (1 mg p-phenylenediamine in 1 ml glycerine buffer, pH 8) was added, and the cover slide closed with rubber solution. Photography was done with a Zeiss Photomicroscope III and Kodak Ektachrome ASA 200 color slide film type R. Fluoresceine and propidium iodide were excited at 450–490 nm and photographed with a 515-nm long pass filter.

Results and discussion

Metaphase chromosomes of the Chinese hamster × human hybrid cell line A1wbf2 were isolated according to the hexylene glycol method described by Stoehr et al. (1982) and hybridized to biotin-labeled human genomic DNA. After fluorescence detection, chromosomes were photographed upon ethanol fixation on the slide (Fig. 1a–d). Figure 1 shows isolated metaphase chromosomes visualized with FITC and counterstained with propidium iodide. Human metaphase chromosomes appeared yellow-green, indicating the specific hybridization with biotinylated human DNA (shown by arrow in the figure). The yellow-green appearance is due to overlapping of the emission spectra of FITC and propidium iodide. In contrast, Chinese hamster chromosomes showed the red fluorescence of propidium iodide only. In interspecies

translocations the human part was correspondingly stained yellow-green (indicated by arrow in the figure) while the hamster part appeared fluorescent red (Fig. 1b–d).

The morphology of the chromosomes, as judged by microscopic observation, is so well preserved that the chromosomes can still be identified by size and centromere position. This seems to be an advantage of the hexylene glycol isolation procedure over the Hepes/$MgSO_4$ method, which was studied by Trask (1985). Chromosomes isolated in the latter buffer fall apart when denatured in suspension.

We are presently investigating possible applications of our procedure in flow cytometry. Such applications might include the characterization and sorting of complete and rearranged human chromosomes.

Acknowledgements. We thank Miss B. Hitzelberger for technical assistance and Dr. P. Pearson from Leiden for providing the hybrid cell line A1wbf2. We also thank Dr. D. Ward and Dr. P. Lichter (Yale University, New Haven) for reading the manuscript and for their comments. The study was supported by the Deutsche Forschungsgemeinschaft.

References

Cremer T, Landegent J, Brückner A, Scholl HP, Schardin M, Hager HD, Devilee P, Pearson P, Van der Ploeg M (1986) Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: diagnosis of trisomy 18 with probe L 1.84. Hum Genet 74:346–352

Gray JW, Langlois RG (1986) Chromosome classification and purification using flow cytometry and sorting. Annu Rev Biophys Biophys Chem 15:195–235

Hopman AHN, Wiegant J, Raap AK, Landegent JE, Van der Ploeg M, Van Duijn P (1986) Bi-color detection of two target DNAs by non-radioactive in situ hybridization. Histochemistry 85:1–4

Langer-Safer PR, Levine M, Ward DC (1982) Immunological method for mapping genes on Drosophila polytene chromosomes. Proc Natl Acad Sci USA 79:4381–4385

Manuelidis L (1985) Individual interphase chromosome domains, revealed by in situ hybridization. Hum Genet 71:288–293

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934–2938

Schardin M, Cremer T, Hager HD, Lang M (1985) Specific staining of human chromosomes in Chinese hamster × man hybrid cell lines demonstrates interphase chromosome territories. Hum Genet 71:281–287

Stoehr M, Hutter KJ, Frank M, Goerttler KL (1982) A reliable preparation of mono-dispersed chromosome suspensions for flow cytometry. Histochemistry 74:57–61

Trask B (1985) Studies of chromosomes and nuclei using flow cytometry. Thesis, Leiden University

Trask B, Van den Engh G, Landegent J, In de Wal NJ, Van der Ploegh M (1985) Detection of DNA sequences in nuclei in suspension by in situ hybridization and dual beam flow cytometry. Science 230:1401–1403

Received September 17, 1986 / Revised February 9, 1987