GENOMICS 8, 347–350 (1990)

Molecular Cytotaxonomy of Primates by Chromosomal *in Situ* Suppression Hybridization

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Received April 27, 1990

A new strategy for analyzing chromosomal evolution in primates is presented using chromosomal *in situ* suppression (CISS) hybridization. Biotin-labeled DNA libraries from flow-sorted human chromosomes are hybridized to chromosome preparations of catarrhines, platyrrhines, and prosimians. By this approach rearrangements of chromosomes that occurred during hominoid evolution are visualized directly at the level of DNA sequences, even in primate species with pronounced chromosomal shuffles. © 1990 Academic Press, Inc.

INTRODUCTION

Comparative studies of chromosome morphology have made considerable contributions to the interpretation of human phylogeny and mammalian evolution (Dutrillaux, 1979; Seuánez, 1979; O'Brien *et al.*, 1988; Yunis and Prakash, 1982). However, establishing interspecific chromosomal or band comparisons can be difficult or speculative and should be confirmed by direct mapping of DNA sequences (Lalley *et al.*, 1989). In this report we present a new strategy for analyzing chromosome homologies in comparative evolutionary studies by use of chromosomal *in situ* suppression (CISS) hybridization (Lichter *et al.*, 1988a; Cremer *et al.*, 1988; Pinkel *et al.*, 1988). Biotin-labeled DNA libraries from flow-sorted human chromosomes (Van Dilla *et al.*, 1986) were hybridized to chromosome preparations of catarrhines, platyrrhines, prosimians, and several nonprimate mammals. Detection of labeled DNA was performed with fluorescein-conjugated avidin. Hybridization of repetitive sequences to nontargeted chromosomes was suppressed by a preannealing step with titrated amounts of human genomic DNA (Lichter *et al.*, 1988a). This procedure allows the direct delineation of individual primate chromosomes or chromosome segments homologous to a single human chromosome and thus will considerably facilitate comparative mapping.

MATERIALS AND METHODS

Standard chromosome preparations were obtained from PHA (great apes) or Con A (lower primates) stimulated peripheral lymphocytes (Figs. 1c, d, e, f, g, r), from fibroblast cultures (Figs. 1a, b, j, m, n), or EBV-transformed lymphoblastoid cell lines (Figs. 1h, i, k, l, o, p, q, s).

CISS hybridization was performed as described (Cremer *et al.*, 1988) using the following libraries from the American Type Culture Collection: X (LA0XNL01), Y (LL0YNS01), 1 (LA01NS01), 2 (LL02NS01), 3 (LA03NS02), 4 (LA04NS02), 5 (LA05NS01), 7 (LA07NS01), 9 (LL09NS01), 17 (LL17NS02). Total phage DNA from libraries was labeled with biotin-11-dUTP and detected following CISS hybridization with fluorescein-labeled avidin. Amplification of signals was carried out as described (Pinkel *et al.*, 1988).

RESULTS AND DISCUSSION

A human X-chromosome library was used to probe the chromosomes of hominoids (*Pan troglodytes, Gorilla gorilla, Pongo pygmeus,* and *Hylobates syndactylus*) and lower primates (*Macacca fuscata, Cercopithecus talapoin, Papio ursinus* (Cercopithecidae), *Lagotrix lagotrix* (Platyhrrinae), and *Galago demidovii* (Prosimians). In all species the X chromosome was specifically stained (Figs. 1a–e). No translocation of X material was detectable. While the arms of hominoid X chromosomes were rather uniformly labeled, the hybridization patterns of lower primates resembled an R-band-like pat-

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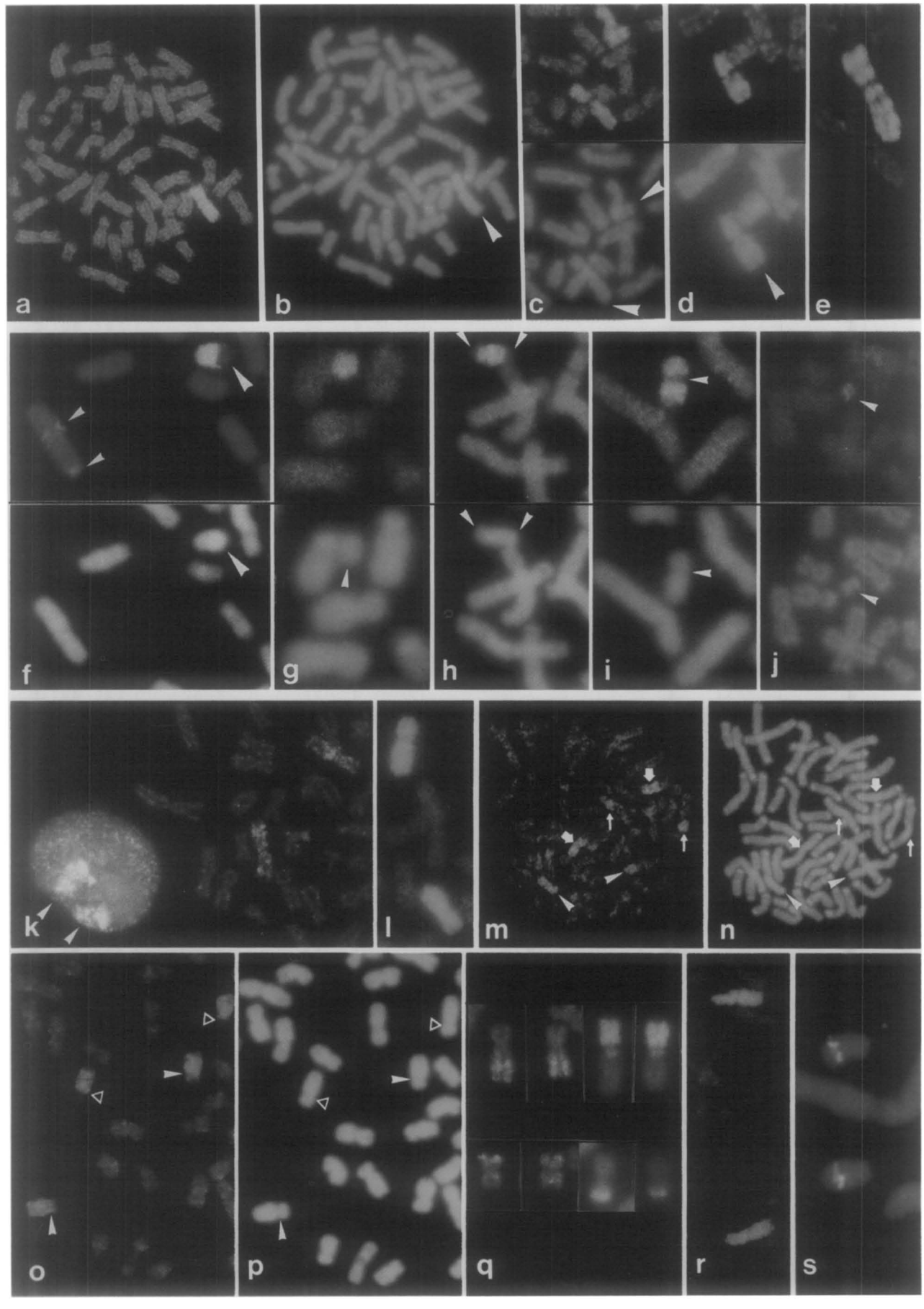
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tern (Fig. 1e). This may support the recent findings that R-bands contain more conserved DNA sequences than G-bands (for reviews, see Holmquist, 1989; Bickmore and Sumner, 1989). These data further confirm Ohno's hypothesis that the X chromosome has been highly conserved during mammalian evolution (Ohno, 1973). Still, with our present protocols we were not able to achieve detectable hybridization signals with the human X-chromosome library on the X chromosome of rodents (*Mus musculus, Microtus savii*) and a carnivore (*Felis catus*), even when hybridization was performed under less stringent conditions. This suggests the possibility that selective staining of the X chromosome in man and other primates was due mainly to a chromosome-specific DNA fraction restricted to these species. This fraction would then have been conserved for at least 40 million years.

CISS hybridization of the human Y-chromosome library exclusively stained the DA-DAPI-negative regions of the Y chromosomes both in man and in the great apes (Figs. 1f–i). In addition, significant labeling was obtained with this library both on the pseudoautosomal region and Xq1.3 of the human X chromosome (Fig. 1f) and on homologous regions of the gorilla X chromosome (not shown). A similar labeling pattern was expected on the X chromosomes of the other great apes (Weber *et al.*, 1987), but was not distinguishable in our present experiments.

In addition to the sex chromosomes, a series of DNA libraries derived from sorted human autosomes, including Nos. 1–5, 7, 9, and 17, was hybridized to chromosomes of hominoid species. Figures 1k–r present examples. These libraries were previously shown to stain the respective human chromosomes under suppression conditions (Lichter *et al.*, 1988a) and to delineate structural aberrations (Cremer *et al.*, 1988, 1990). Background staining of nontargeted chromosomes seen with these and gonosome libraries was due to incomplete suppression of repetitive sequences. In addition, specific cross-hybridizations to certain subregions of other chromosomes, such as centric heterochromatin, were occasionally seen (Lichter *et al.*, 1988a). These were also considerably weaker in intensity than the signals on target chromosomes and not considered further in the present study.

The human chromosome 1, 3, 4, 7, and 9 libraries hybridized to individual gorilla and chimpanzee homologs, indicating that these chromosomes have been conserved in their entirety in all three species (e.g., Figs. 1k and l). Hybridization was restricted mainly to the euchromatin of primate chromosomes. In particular, staining was entirely lacking in the telomeric C-bands of various chimpanzee, gorilla, and gibbon chromosomes (e.g., Figs. 1a,b,h,o,p) and also often in the centromeric heterochromatin (Lichter *et al.*, 1988a).

The examples below demonstrate that this approach allows the analysis of structural chromosomal changes during hominoid evolution at the level of DNA homologies even in primate species with pronounced chromosome shuffles (see O'Brien *et al.*, 1988, for review).

The human chromosome 2 was derived by fusion of two acrocentric chromosomes (de Grouchy *et al.*, 1972). This fusion is responsible for the change in the diploid number in humans compared to the great apes. As expected, CISS hybridization of the human chromosome 2 library delineates the respective chromosomes in the gorilla (Figs. 1o and p).

Chromosomes 4 and 19 of the gorilla were supposedly derived from a reciprocal translocation between homologs to human chromosomes 5 and 17 (Yunis and Prakash, 1982), while this translocation is not present in the chimpanzee. Accordingly, the human 17 library stained the entire homologous chimpanzee chromo-

FIG. 1. *In situ* suppression (CISS) hybridization of human flow-sorted chromosome DNA libraries to primate metaphase chromosomes fixed on slides. Biotinylated library sequences were detected with FITC-conjugated avidin and chromosomes were counterstained with propidium iodide (upper row, **f–j; k,o,q,s**) and DAPI (**b**; lower row, **c,d,f–j; n,p**). Slides were viewed with a Zeiss microscope III equipped with epifluorescence. Photographs were taken with Kodak Ektachrome 1000 films. (**a–e**) Primate chromosomes hybridized to a human X library. Arrowheads point to the DAPI-stained X chromosomes in **b–d.** (**a**) Gibbon (*Hylobates syndactylus*, male); selective labeling of the X and background hybridization to nontargeted chromosomes is restricted to the euchromatin. (**b**) DAPI staining of the same metaphase with bright telomeric heterochromatin. (**c**) *Cercopithecus talapoin* (female). (**d**) *Macacca fuscata* (male). (**e**) *Lagotrix lagotrix* (male). (**f–j**) Man and ape male metaphase spreads hybridized to a human Y library. Large arrowhead in **f** and small arrowheads in **g–j** point to the Y chromosomes. (**f**) Specific labeling of the human Y chromosome, Ypter–q11; large arrowhead points to the unlabeled, DAPI-stained heterochromatic region Yq12; small arrowheads indicate cross-hybridization to the pseudoautosomal region and Xq13 on the human X chromosome. (**g**) *Pan troglodytes*, labeling of the entire Y chromosome. (**h**) *Gorilla gorilla* and (**i**) *Pongo pygmeus;* arrowheads indicate unlabeled terminal heterochromatin in the gorilla and centric heterochromatin in the orangutan. (**j**) *Hylobates syndactylus*. (**k–r**) Primate chromosomes hybridized to human autosome libraries. Human 3 (**k**) and human 7 (**l**) libraries stain entire gorilla homologs. In **k** labeled chromosomes occupy distinct territories in an adjacent interphase nucleus (arrowheads). (**m, n**) Gibbon metaphase chromosomes stained with the No. 7 library show rearrangements of three chromosomes (arrows). (**o, p**) Human 2 library stains two submetacentric gorilla chromosomes; in one chromosome (triangles) labeling is restricted to the long arm; in the other (arrowheads) labeling extends to the short arm. (**q**) Gorilla chromosomes 4 (upper row) and 19 (lower row) are partially stained with either a library of human chromosome 5 (**left**) or 17 (**right**) delineating a reciprocal translocation (see text). (**r**) In contrast, the human 17 library stains an entire homolog in the chimpanzee. (**s**) Fluorescence *in situ* hybridization of gorilla chromosomes with a pool of 32 single-copy sequences cloned from the terminal subband of human chromosome 21 (21q22.3) (9). Note the shift of the hybridization signal due to terminal heterochromatin in the gorilla homolog.

some (Fig. 1r). When the human 5 and 17 DNA libraries, respectively, were hybridized to gorilla chromosomes, the presumed rearrangement was demonstrated (Fig. 1q). In particular, our data indicate that the centromere of human chromosome 17, the entire long arm, and a small part of the short arm contribute to gorilla chromosome 4, while most of the short arm was translocated to gorilla chromosome 19 (Yunis and Prakash, 1982; Wienberg and Stanyon, 1988).

The lesser apes provide a striking exception from the high extent of cytological conservation in primates. Due to the rapid chromosomal evolution in this group, it has been exceedingly difficult to establish chromosome homologies both between particular gibbon species and between other primates by conventional banding techniques (Stanyon and Chiarelli, 1983). CISS hybridization of human chromosome libraries provides a new tool for comparative chromosome mapping especially in lesser apes. For example, the human chromosome 7 library stains three different chromosome pairs in *H. syndactylus,* with two pairs showing intercalary hybridization signals, while a third pair shows a terminal translocation (Figs. 1m and n). This evidence supports the proposed importance of translocations as a mechanism of chromosomal evolution in this genus (Stanyon and Chiarelli, 1983).

In situ hybridization of pools of DNA sequences established from specific chromosomal subregions (Lichter *et al.,* 1988b) allow direct band-to-band comparisons between species at the DNA level (Fig. 1s). CISS hybridization of DNA clones established from the chromosome region of interest, such as YAC clones or cosmid clones, will provide a new avenue for regional comparative mapping with high resolution (Lichter *et al.,* 1990).

ACKNOWLEDGMENTS

We thank Dr. Henning Wiesner and Dr. Gila v.Hegel from the Munich Zoo 'Tierpark Hellabrunn' for generous support and for providing most of the primate blood and fibroblast samples, J. Hollatz for excellent photographic work, and Dr. P. Lichter for help in the experiment with the chromosome 21 probes. Single-copy clones for this chromosome were kindly provided by P. Watkins and Integrated Genetics.

REFERENCES

1. BICKMORE, W. A., AND SUMNER, A. T. (1989). Mammalian chromosome banding—an expression of genome organization. *Trends Genet.* **5:** 144–148.
2. CREMER, T., LICHTER, P., BORDEN, J., WARD, D. C., AND MANUELIDIS, L. (1988). Detection of chromosome aberrations in metaphase and interphase tumor cells by *in situ* hybridization using chromosome-specific library probes. *Hum. Genet.* **80:** 235–246.
3. CREMER, T., POPP, S., EMMERICH, P., LICHTER, P., AND CREMER, C. (1990). Rapid metaphase detection of radiation-induced chromosome aberrations in human lymphocytes by chromosomal suppression *in situ* hybridization. *Cytometry* **11:** 110–118.
4. DE GROUCHY, J., TRULEAU, C., ROUBIN, M., AND KLEIN, M. (1972). Evolution caryotypique de l'homme et du chimpanzé: Étude comparative des topographies de bandes après dénaturation ménagée. *Ann. Genet.* **15:** 79–84.
5. DUTRILLAUX, B. (1979). Chromosomal evolution in primates: Tentative phylogeny from *Microcebus murinus* (prosimian) to man. *Hum. Genet.* **48:** 251–314.
6. HOLMQUIST, G. P. (1989). Evolution of chromosome bands: Molecular ecology of noncoding DNA. *J. Mol. Evol.* **28:** 469–486.
7. LALLEY, P. A., DAVIDSON, M. T., GRAVES, J. A. M., O'BRIEN, S. J., WOMACK, J. E., RODERICK, T. H., CREAU-GOLDBERG, N., HILLYARD, A. L., DOOLITTLE, D. P., AND ROGERS, J. (1989). Human Gene Mapping 10—Report of the Committee on Comparative Mapping. *Cytogenet. Cell Genet.* **51:** 503–532.
8. LICHTER, P., CREMER, T., BORDEN, J., MANUELIDIS, L., AND WARD, D. C. (1988a). Delineation of individual human chromosomes in metaphase and interphase cells by *in situ* suppression hybridization using recombinant DNA libraries. *Hum. Genet* **80:** 224–234.
9. LICHTER, P., CREMER, T., CHANG TANG, C. J., WATKINS, P. C., MANUELIDIS, L., AND WARD, D. C. (1988b). Rapid detection of chromosome 21 aberrations by *in situ* hybridization. *Proc. Natl. Acad. Sci. USA* **85:** 9664–9668.
10. LICHTER, P., CHANG TANG, C. J., CALL, K., HERMANSON, G., EVANS, G. A., HOUSMAN, D., AND WARD, D. C. (1990). High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science* **247:** 64–69.
11. O'BRIEN, S. J., SEUÁNEZ, H. N., AND WOMACK, J. E. (1988). Mammalian genome organization: An evolutionary view. *Annu. Rev. Genet.* **22:** 323–351.
12. OHNO, S. (1973). Ancient linkage groups and frozen accidents. *Nature (London)* **244:** 259–262.
13. PINKEL, D., LANDEGENT, J., COLLINS, C., FUSCO, J., SEGRAVES, R., LUCAS, J., AND GRAY, J. W. (1988). Fluorescence *in situ* hybridization with human chromosome specific libraries: Detection of trisomy 21 and translocations of chromosome 4. *Proc. Natl. Acad. Sci. USA* **85:** 9138–9142.
14. SEUÁNEZ, H. N. (1979). "The Phylogeny of Human Chromosomes," Springer, Berlin/New York.
15. STANYON, R., AND CHIARELLI, B. (1983). Phylogeny of the Hominoidea: The chromosome evidence. *J. Hum. Evol.* **11:** 493–504.
16. VAN DILLA, M. A., DEAVEN, L. L., ALBRIGHT, K. L., ALLEN, N. A., AUBUCHON, M. R., BARTHOLDI, M. F., BROWN, N. C., CAMPELL, E. W., CARRANO, A. V., CLARK, L. M., CRAM, L. S., CRAWFORD, B. D., FUSCOE, J. C., GRAY, J. W., HILDEBRAND, C. E., JACKSON, P. J., JETT, J. H., LONGMIRE, J. L., LOZES, C. R., LUEDEMANN, M. L., MARTIN, J. C., MCNINCH, J. S., MEINCKE, L. J., MENDELSON, M. L., MEYNE, J., MOYZIS, R. K., MUNK, A. C., PERLMAN, J., PETERS, D. C., SILVA, A. J., AND TRASK, B. J. (1986). Human chromosome specific DNA libraries: Construction and availability. *Biotechnology* **4:** 537–552.
17. WEBER, B., WEISSENBACH, J., AND SCHEMPP, W. (1987). Conservation of human-derived pseudoautosomal sequences on the sex chromosomes of the great apes. *Cytogenet. Cell Genet.* **45:** 26–29.
18. WIENBERG, J., AND STANYON, R. (1988). DA/DAPI bands in the chromosomes of *Pan paniscus. Amer. J. Primatol.* **14:** 91–96.
19. YUNIS, J. J., AND PRAKASH, O. (1982). The origin of man: A chromosomal pictorial legacy. *Science* **215:** 1525–1530.