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C. Lengauer,
J. Wienberg &
T. Cremer

Institut für Humangenetik und Anthropologie, Universität Heidelberg, Germany

H.J. Lüdecke &
B. Horsthemke

Institut für Humangenetik, Universitätsklinikum Essen, Germany

Comparative chromosome band mapping in primates by *in situ* suppression hybridization of band specific DNA microlibraries

A DNA-library established from microdissected bands 8q23 to 8q24.1 of normal human chromosomes 8 (Lüdecke et al., 1989) was used as a probe for chromosomal in situ suppression (CISS-) hybridization to metaphase chromosomes of man and primates including Hylobates lar and Macaca fuscata. Comparative band mapping as first applied in this study shows the specific visualization of a single subchromosomal region in all three species and thus demonstrates that synteny of the bulk sequences of a specific human chromosome subregion has been conserved for more than 20 million years.

Introduction

Comparisons of chromosome morphology are widely used for phylogenetic reconstructions. Fixation of chromosomal changes are rare events and identical chromosomal rearrangements in different species are considered to reflect a common evolutionary path. Accordingly, ancestral versus derived traits can be established. With few exceptions comparative cytogenetics has indicated a highly conserved chromosome morphology during primate evolution (DUTRILLAUX, 1979; YUNIS & PRAKASH, 1982; STANYON & CHIARELLI, 1983; O'BRIEN *et al.*, 1988). It is widely accepted that chromosome bands whose homology has been suggested on the basis of similar banding patterns reflect synteny of DNA sequences. However, comparative mapping of individual DNA sequences (LALLEY *et al.*, 1990) so far has provided only limited data to unequivocally support this hypothesis. These studies are very time consuming and the mapped sequences only reflect a very small part of a given chromosome band.

Recently, we have introduced a new strategy to analyze chromosomal evolution in primates based on chromosomal *in situ* suppression (CISS-) hybridization (WIENBERG *et al.*, 1990). A series of bacteriophage DNA-libraries established from flow sorted human chromosomes was used to visualize homologies of primate chromosomes directly at the DNA level. However, these chromosome specific DNA libraries did not permit to reconstruct intrachromosomal rearrangements which are supposed to be prominent in the evolution of hominoid karyotypes (DUTRILLAUX, 1979; YUNIS & PRASHAK, 1982; STANYON & CHIARELLI, 1983). An unequivocal demonstration of the homology of individual bands could be based on CISS-hybridization of band specific DNA libraries. Construction of such libraries has become possible by microdissection and microcloning (LÜDECKE *et al.*, 1989).

Here, we have used a micro-DNA-library established from the distal third of band 8q23 and the proximal two-thirds of sub-band 8q24.1 from normal human chromosomes (LÜDECKE *et al.*, 1989) for comparative band mapping of human and primate species. For this comparison we have chosen *Hylobates lar and Macaca fuscata* in the present study. Due to the rapid chromosomal evolution in the lesser apes, it has been exceedingly difficult to establish chromosome homologies both between particular gibbon species and other

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primates by conventional banding techniques. In contrast, *Macaca fuscata* shows a much higher degree of chromosomal conservation (STANYON & CHIARELLI, 1983). In particular, CISS-hybridization of a DNA-library from sorted human chromosomes 8 has shown that this chromosome is rearranged in *Hylobates lar* but has been maintained intact in *Macaca fuscata* (our unpublished data).

Materials and Methods

DNA probes and probe labeling: The microlibrary from chromosome bands 8q23-8q24.1 was established and characterized by Lüdecke et al. (1989). Briefly, 37 dissected chromosome fragments were digested with RsaI and ligated to a SmaI-cut pUC-plasmid. The inserts were than amplified by PCR using the universal M13/pUC sequencing and reverse sequencing primers. In our experiments 100 ng of amplified inserts was again amplified in a 100 ul assay containing 1 uM primer each (see above), 10 µM Tris-HCl (pH 8.4), 50 µm KCl, 1.5 µM $MgCl_2$, 0.001% gelatin, 200 θM of dATP, dGTP, dCTP each, 100 θM of dTTP, 150 θM bio-11-dUTP and 2.5 units of *Thermus aquaticus* DNA polymerase. After initial denaturation at 96°C for 3 min 25 cycles of PCR were carried out with denaturation at 96°C for 1 min, annealing at 47°C for 1 min and extention at 72°C for 2 min. 10 sec.

Chromosome preparations: Metaphase chromosome spreads for *in situ* hybridization experiments were prepared and stored as described by JAUCH *et al.* (1990). Human chromosomes were derived from phytohemagglutinin (PHA) stimulated peripheral lymphocytes. Gibbon chromosomes (*Hylobates lar*) were prepared from a lymphoblastoid cell line kindly provided by T. Ishida (Primate Center, Kyoto, Japan). The karyotype of this cell line was found to be normal diploid (H.lar karyotype: 2n = 44; R. Stanyon, personal communication). *Macaca fuscata* chromosome spreads from primary kidney cultures (STANYON *et al.*, 1990) were kindly provided by R.Stanyon (Istituto di Antropologia, Genoa, Italy).

Chromosome banding: Chromosome banding previous to CISS-hybridization was according to Klever et al. (1991). After routine GTG-banding metaphases were photographed (Agfa-Ortho 25), destained with fixative (methanol/acetic acid 3:1) and postfixed with 4% formaldehyde (Merck) in PBS for 15 min. Postfixation was essential to preserve chromosome morphology in the subsequent CISS-hybridization experiments.

CISS-hybridization: Sixty ng of PCR labeled probe was used for CISS-hybridization (LICHTER *et al.*, 1988; PINKEL *et al.*, 1988). Biotin labeled probes were detected with fluoresceine-conjugated avidine. Signals were amplified once as described by PINKEL *et al.* (1986) and slides were analysed with a Zeiss photomicroscope III equipped for FITC fluorescence. Photographs were taken on AGFA-CHROME 1000 RS or Kodak T-MAX black and white (400 ASA) films.

Results

CISS-hybridization of the microlibrary to human lymphocyte metaphase spreads resulted in a complete delineation of band 8q23 and a partial delineation of band 8q24 (*Figure 1a,b*). The telomeric band 8q24.3 clearly remained unstained (*Figure 2a,b*). This

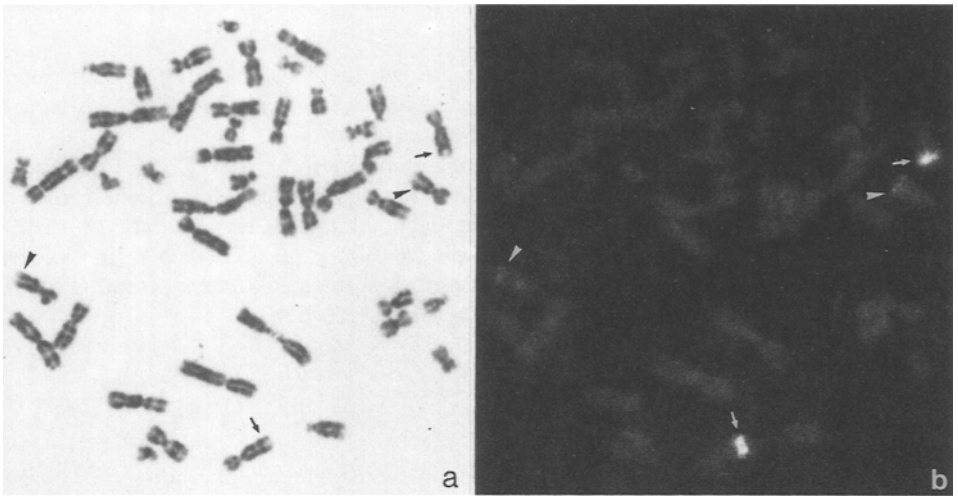

Figure 1 - CISS-hybridization of a microlibrary derived from human chromosome bands 8q23-8q24.1 to a human metaphase spread. a) GTG-banding, b) FITC-detection of hybridized microlibrary sequences. Arrows indicate the labeled subregion on chromosome 8 (bands 8q23-24), arrowheads point to a weak hybridization signal on chromosome 9 (band 9q33).

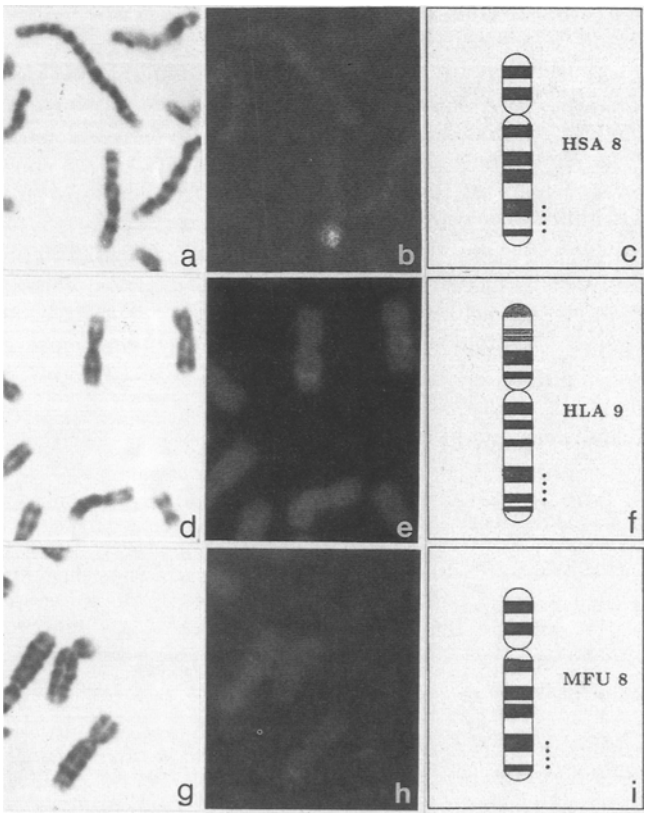

Figure 2 - GTG-banding, CISS-hybridization patterns of the microlibrary and idiograms of labeled chromosomes in man (HSA) (a-c), *Hylobates lar* (HLA) (d-f) and *Macaca fuscata* (MFU) (g-i). The labeled regions established from a series of chromosomes are indicated in the idiograms by black dots.

result is in agreement with the molecular characterization of the microlibrary as previously described (LÜDECKE *et al.*, 1989). Unexpectedly, however, an additional faint signal was also evident on chromosome 9q33 (see Discussion).

In *Hylobates lar* and *Macaca fuscata* a single chromosome subregion was delineated (*Figure 2e,h*), while site specific signals on other chromosomes could not be detected. The chromosomes with the hybridized subregions were identified on the basis of their G-banding pattern as chromosome 9 in *Hylobates lar* (*Figure 2d*) according to the idiogram published by STANYON *et al.*, (1987), and as chromosome 8 in *Macaca fuscata* (*Figure 2g*) according to the numbering system of SMALL *et al.* (1985).

Discussion

CISS-hybridization of DNA microlibraries established from microdissected chromosome regions can be used as a rapid tool to confirm the origin of the bulk DNA sequences from the respective region (Lengauer et al., 1991). This study demonstrates the specific visualization of single chromosome bands using a human microlibrary established after microdissection of bands 8q23-8q24.1. The faint labeling of the additional band 9q33 may be explained by an occassional error in the classification of banded chromosomes used for microdissection. Alternatively, cross-hybridization may indicate the presence of conserved sequences in both bands. This latter explanation seems unlikely, since the decoration of complete chromosomes 8 by CISS-hybridization with DNA-libraries established from sorted human chromosomes 8 did not result in any detectable cross-hybridization events on chromosome 9 and vice versa (our unpublished data).

CISS-hybridization of the microlibrary to a single subchromosomal region in *Hylobates lar* and *Macaca fuscata*, respectively, demonstrates that synteny of the bulk sequences of human chromosome bands 8q23 to 24.1 has been conserved for more than 20 million years. Comparative band mapping as first demonstrated in this study provides new possibilities for cytotaxonomy. As compared to the physical mapping of individual DNA sequences, evolutionary conservation of chromosome bands as well as chromosomal rearrangements with breakpoints in specific bands can be rapidly ascertained. Single copy probes established from such microlibraries may then be used for more detailed studies of possible breakpoints pinpointed within the chromosomal region defined by a microlibrary.

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