Physical Mapping of Human 7q and 14q Subtelomeric DNA Sequences in the Great Apes

Rhea V. Samonte,* Robert A. Conte, and Ram S. Verma*

Division of Genetics, The Long Island College Hospital SUNY Health Science Center, Brooklyn, NY 11201

(Received 8 April 1997)

Abstract

Phylogenetic divergence of the members of the Pongidae family has been based on genetic evidence. The terminal repeat array (T2AG3) has lately been considered as an additional basis to analyze genomes of highly related species. The recent isolation of subtelomeric DNA probes specific for human (HSA) chromosomes 7q and 14q has prompted us to cross-hybridize them to the chromosomes of the chimpanzee (PTR), gorilla (GGO) and orangutan (PPY) to search for its equivalent locations in the great ape species. Both probes hybridized to the equivalent telomeric sites of the long (q) arms of all three great ape species. Hybridization signals to the 7q subtelomeric DNA sequence probe were observed at the telomeres of HSA 7q, PTR 6q, GGO 6q and PPY 10q, while hybridization signals to the 14q subtelomeric DNA sequence probe were observed at the telomeres of HSA 14q, PTR 15q, GGO 18q and PPY 15q. No hybridization signals to the chromosome 7-specific alpha satellite DNA probe on the centromeric regions of the ape chromosomes were observed. Our observations demonstrate sequence homology of the subtelomeric repeat families D7S427 and D14S308 in the ape chromosomes. An analogous number of subtelomeric repeat units exists in these chromosomes and has been preserved through the course of differentiation of the hominoid species. Our investigation also suggests a difference in the number of alpha satellite DNA repeat units in the equivalent ape chromosomes, possibly derived from interchromosomal transfers and subsequent amplification of ancestral alpha satellite sequences.

Key words: chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), orangutan (Pongo pygmaeus), fluorescence in situ hybridization (FISH)

1. Introduction

Classical cytogenetic data based on chromosome banding patterns have suggested similarities between great ape and human chromosomes while molecular data has further strengthened the concept of human descent. In recent years, the structure of several human chromosome ends termed telomeres have been characterized. The telomere repeat unit (T2AG3) has been conserved during vertebrate evolution and is present at all chimpanzee telomeres. A means for cloning human telomeres and their adjacent sequences in yeast artificial chromosomes (YACs) has facilitated the identification of unique physical and genetic markers for a number of human telomeres. Development of genetic markers from DNA in the proteral region adjacent to the human-specific terminal repeat array (T2AG3) has been accomplished for human chromosomes 7q and 14q. In the present investigation, the locations of these subtelomeric sequences have been identified in the chimpanzee, gorilla and orangutan chromosomes using the fluorescence in situ hybridization (FISH) technique.

2. Materials and Methods

Metaphase spreads were prepared from fibroblast cell lines of chimpanzee (AGO 6939A, Pan troglodytes, PTR) and orangutan (AGO 5252, Pongo pygmaeus, PPY) obtained from the Coriell Institute for Medical Research, Camden, NJ, while a lymphoblast cell line of gorilla (CRL 1854, Gorilla gorilla, GGO) was obtained from the American Type Culture Collection (Rockville, MD). Human chromosomes were prepared from PHA-stimulated lymphocyte cultures obtained from healthy individuals. Harvesting of cell cultures was done using standard methods.

The conditions for in situ hybridization were as described by Lichter et al. Briefly, chromosome preparations were dehydrated through an ethanol series and denatured in 70% formamide in 2X SSC (pH 7.0) at 72°C.
Figure 1. [A] Hybridization signals observed on the chromosomes of (a) humans, (b) chimpanzee, (c) gorilla and (d) orangutan using human 7q subtelomeric DNA sequence probe (D7S427) with chromosome 7-specific alpha satellite DNA probe (D7Z1). The chromosome 7-specific alpha satellite DNA (D7Z1) in humans' centromeric region (a) is not conserved in the equivalent apes' chromosomes (see text). [B] The subtelomeric DNA sequence probe specific for human chromosome 14q (D14S308) was hybridized to the chromosomes of (a) humans, (b) chimpanzee, (c) gorilla and (d) orangutan and were also found to be conserved at the equivalent apes' chromosomes.

The DNA probes used were as follows: tel 7q (D7S427) with chromosome 7-specific alpha satellite (D7Z1) and tel 14q (D14S308) (Oncor, Gaithersburg, MD). Both probes employed were digoxigenin-labelled. Overnight hybridization was performed at 37°C. Post-hybridization washes were done using 2X SSPE (pH 7.0) at 70°C. Detection of probes was done using fluorescein-labelled digoxigenin. Chromosomes were counterstained with DAPI/Antifade and observed under fluorescent optics. Digital images were obtained using a cooled CCD camera (Oncor).

3. Results

We have compared hybridization patterns of human 7q and 14q subtelomeric DNA sequence probes on the metaphase chromosomes of the great ape species. At selected hybridization conditions the two probes showed equally strong fluorescent signals at the telomeric positions (Fig. 1). The chromosome arms showing hybridization signals to the 7q subtelomeric DNA sequence probe were identified as HSA 7q, PTR 6q, GGO 6q and PPY 10q, while the chromosome arms showing hybridization signals to the 14q subtelomeric DNA sequence probe were identified as HSA 14q, PTR 15q, GGO 18q and PPY 15q. No hybridization signals to the chromosome 7-specific alpha satellite DNA probe on the centromeric regions of the ape chromosomes were observed.

4. Discussion

Telomeres are important for chromosome integrity and replication. Such functions are mediated by highly conserved short tandem repeats (T\(_2\)AG\(_3\)) at the very ends of telomeres in all vertebrates. High concentrations of genes and transcriptional and recombination activities have been observed in these telomeric regions, and a number of candidate genes for recognizable syndromes are known to be present. A complete set of specific telomeric probes within 100 300 kb of the end of each human chromosome arm has increased the resolution of deletion detection in cytogenetic analysis. Subtelomeric DNA sequences, found adjacent to simple telomere repeats, have been characterized in a number of human chromosomes and have been shown to consist of repetitive elements shared by a subset of chromosomes.

We have used two chromosome-specific subtelomeric DNA sequence probes in this investigation. The 7q subtelomeric DNA sequence probe (D7S127) is a digoxigenin-labelled probe subcloned from a 210-kb YAC
clone containing the human 7q telomere.\(^{18,19}\) It hybridizes to sequences approximately 150 kb of the 7q36.3-qter region of human chromosomes.\(^{20}\) To assist in target chromosome verification in human chromosome preparations, this subtelomeric probe is provided with a chromosome 7-specific alpha satellite DNA probe (D7Z1), which hybridizes to sequences in the centromeric portion of human chromosome 7. The 14q subtelomeric DNA sequence probe (D14S308) is a digoxigenin-labelled DNA probe, subcloned from a 200-kb YAC clone containing the human chromosome the 14q telomere.\(^{21}\) The probe hybridizes to sequences of the 14q32.3-qter region of human chromosomes.

In principle, fluorescence in situ hybridization (FISH) should be able to provide information on the telomere length of individual chromosomes.\(^{22}\) The efficiency of oligonucleotide hybridizations for telomeric repeats has been tested in qualitative studies of T2AG3 repeat sequences in chromosomes of various species.\(^{23,9}\) Our observation of telomeric signals in the great ape chromosomes resulting from the hybridization of the two subtelomeric DNA probes indicates that an analogous number of subtelomeric repeat units exists in these chromosomes and has been preserved throughout the course of differentiation of the hominoid species. Hybridization of the chromosome 7-specific alpha satellite DNA probe (D7Z1) to the great ape chromosomes did not reveal any centromeric signals, possibly due to a difference in the number of alpha satellite DNA repeat units in the equivalent ape chromosomes. Each chromosome-specific alpha satellite DNA probe is composed of a distinct number of repeat units.\(^{24}\) Such disparity observed in our investigation may possibly be derived from interchromosomal transfers and subsequent amplification of ancestral alpha satellite sequences.

Subtelomeric DNA sequences present within D7S427 and D14S308 loci are thus conserved in the equivalent chromosomes of the great apes. The presence of such homologous sequences at equivalent chromosomes in the great apes further support the views of evolutionary history at the DNA sequence level.

References


240 kb of DNA from human chromosome 7q, Genomics, 17, 25-32.


