Short communications

Telomeric repeat [TTAGGG]n sequences of human chromosomes are conserved in chimpanzee (Pan troglodytes)

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Summary. Using a series of genetic parameters, attempts have been made for more than two decades to establish the close kinship of human (Homo sapiens) with chimpanzee (Pan troglodytes). Molecular and cytogenetic data presently suggest that the two species are closely related. The recent isolation of a human telomeric probe (P5097–B.5) has prompted us to cross hybridize it to chimpanzee chromosomes in order to explore convergence and/or divergence of the telomeric repeat sequences (TTAGGG)n. On hybridization, the human probe bound to both ends (telomeres) of chimpanzee chromosomes, suggesting a concerted evolution of tandemly repeated short simple sequences (TTAGGG). Even the terminal heterochromatin of chimpanzee chromosomes was found to be endowed with telomeric repeats, suggesting that evolution of heterochromatin and capping with tandemly repeated short sequences are highly complex phenomena.

Key words: Telomeres – heterochromatin – chromosome – human – chimpanzee – FISH-technique

Introduction

Comparative cytogenetic studies using various banding techniques support the close kinship of human (Homo sapiens) with chimpanzee (Pan troglodytes) (de Grouchy 1987; Miyamoto et al. 1988). The availability of various repetitive DNA probes and application of in situ hybridization methods have opened new vistas in understanding the evolutionary relationship of two species at the molecular level (Durfy and Willard 1990; Baldini et al. 1991; Jörgensen et al. 1987; Luke and Verma 1993a, b). One of the parameters which we have investigated here is the comparison of telomeric tandemly repeated DNA sequences (TTAGGG)n of human with those of chimpanzee. It has been suggested that short telomeric repeats (TTAGGG)n at the chromosomal termini are highly conserved, particularly among species belonging to the same order (Moyzis et al. 1988; Moyzis 1991). This prompted us to cross hybridize chimpanzee chromosomes with a biotinylated human telomeric repeat (TTAGGG)n probe (P50967–B.5). The human probe hybridized to both telomeres of all chimpanzee chromosomes.

Materials and methods

Cell culture and in situ hybridization procedures. Fibroblast culture [cell line No. GM 3450] of chimpanzee was obtained from NIGMS Human Genetic Mutant Cell Repository (Coriell Institute for Medical Research, Camden, NJ). Phytohaemagglutinin-stimulated human lymphocytes were used in the present investigation. The CBG banding technique for chimpanzee and human chromosomes was carried out following the standard protocol (Verma and Babu 1989).

The fluorescence in situ hybridization technique [FISH] of Pinkel et al. (1986) was used with minor modifications as suggested by the manufacturer (Oncor, Gaithersburg MD). To detect the telomeric DNA sequences of chimpanzee, a human telomeric probe (P5097–B.5) was used as follows. Briefly, after RNase treatment, chromosomal DNA was denatured in 70% formamide, 2 x SSC for 2.5 min at 70°C. Hybridization mixture containing telomeric probe (P5097–B.5) was also denatured at 70°C for 5 min and 40 μl of this hybridization mixture was then applied on each slide. Hybridization was carried out for 30 h in a humidified incubator. Posthybridization washing was carried out under appropriate stringency conditions for both species (50% formamide). Hybridization sites were detected fluorescein (FITC)-labelled avidin after signal amplification with biotin-linked anti-avidin antibody. Chromosomes were stained with propidium iodide which included antifade (30 μl per slide) and viewed under a Zeiss Axioskop fluorescence microscope with appropriate filter combinations. Photography was performed using Kodak Ektachrome (p800/1600) at 30–40 sec exposure.
Fig. 1. CBG-banded haploid karyotype of human (left) and chimpanzee (right)

Results and discussion

The differences in the distribution of various fractions of heterochromatin between these two species have been well documented by the CBG technique. Heterochromatin in humans is present in the pericentromeric regions of all chromosomes except the Y, while in chimpanzee chromosomes it is localized at pericentromeric, telomeric and intercalary positions (Fig. 1). Nonradioactive in situ hybridization with the human telomere-specific DNA probe P5097-B5 showed identical hybridization signals at the termini of both human and chimpanzee chromosomes (Fig. 2).

It is a known fact that centromeres as well as telomeres are important for chromosome stability (Moens and Pearlman 1990) while telomeres are necessary for the completion of DNA replication (Blackburn 1990; Sundquist and Klug 1989; Zakian 1989). The conservation of the tandemly duplicated telomeric DNA sequence (TTAGGG)n between human and chimpanzee chromosomes supports the evolutionary concept of "phylogenetic inertia" (Harvey and Purvis 1991). The existence of a functional subset of telomeric repeated DNA sequences associated with the telomeric heterochromatin of many chimpanzee chromosomes indicates the molecular heterogeneity of heterochromatic fraction. Constitutive heterochromatin in eukaryotes is a major site for various families of highly repetitive DNA sequences (Verma 1988, 1990).

The molecular nature of the repetitive DNA sequences of the telomeric heterochromatin adjacent to this ancestral telomeric repeat (TTAGGG)n is not known in chimpanzee chromosomes. However, the evolutionary conservation of human telomeric repeat sequences was traced back 400 million years in more than 100 species suggesting a common ancestor and a common function for this DNA family (Meyne et al. 1989). Human chromosome 2 is believed to be the product of fusion of chimpanzee chromosomes 12 and 13 (King et al. 1988; Luke and Verma 1992). Both chimpanzee chromosomes 12 and 13 have telomeric heterochromatin with (TTAGGG)n caps, yet human chromosome 2 does not show intercalary (TTAGGG)n repeats. However, in other species telomeric repeats have been found in both intercalary and telomeric positions (Scherthan 1990; Meyne et al. 1990; Kipling et al. 1991) which raises a perplexing enigma regarding the origin of human chromosome 2 and the evolution of heterochromatin during chromosome fusion. The evolution of new satellite DNA adjacent to the ancestral telomeric repeats may occur by a series of mutational changes or other amplification and homogenizing mechanisms. To the best of our knowledge, this is the first demonstration that even telomeric heterochromatin which is species specific needs caps to maintain the integrity of chromosomes. How these telomeres are conserved or reorganized during evolution will be the subject of detailed scrutiny in future investigations.