Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9051–9055, October 1991
Genetics

Origin of human chromosome 2: An ancestral telomere–telomere fusion

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Communicated by Alan Garen, July 8, 1991

ABSTRACT **We have identified two allelic genomic cosmids from human chromosome 2, c8.1 and c29B, each containing two inverted arrays of the vertebrate telomeric repeat in a head-to-head arrangement, 5′(TTAGGG)$_n$-(CCCTAA)$_m$3′. Sequences flanking this telomeric repeat are characteristic of present-day human pretelomeres. BAL-31 nuclease experiments with yeast artificial chromosome clones of human telomeres and fluorescence *in situ* hybridization reveal that sequences flanking these inverted repeats hybridize both to band 2q13 and to different, but overlapping, subsets of human chromosome ends. We conclude that the locus cloned in cosmids c8.1 and c29B is the relic of an ancient telomere–telomere fusion and marks the point at which two ancestral ape chromosomes fused to give rise to human chromosome 2.**

Similarities in chromosome banding patterns and hybridization homologies between ape and human chromosomes suggest that human chromosome 2 arose out of the fusion of two ancestral ape chromosomes (1–3). Molecular data show evidence that this event must have occurred only a few million years ago (refs. 4 and 5 and the references therein). Although the precise nature of this putative fusion is unknown, cytogenetic data point to either a centromeric or telomeric fusion in the vicinity of region 2q1 (1, 2, and 6). The observation that telomeric DNA is present in chromosomal band q13 suggests that telomeres, the extreme ends of chromosomes, may have been involved in this fusion (7, 8). Normally, telomeres form a dynamic buffer against loss of internal sequence and prevent chromosomes from fusing (for review, see ref. 9). By contrast, nontelomeric DNA ends are subject to degradation by nucleases and to fusion by ligation (10, 11).

The termini of human chromosomes consist of head-to-tail tandem arrays of TTAGGG, running 5′→3′ toward the end of the chromosome, with average lengths of 5–10 kilobases (kb) in somatic cells (7, 12, 13). The proximal ends of these arrays contain degenerate forms of this repeat, such as (TTGGGG)$_n$ and (TGAGGG)$_n$ (14). Sequences adjacent to these simple repeats have been characterized in a number of human chromosomes and shown to consist of repetitive elements, each shared by a subset of all chromosomes (13, 15–17). In addition, stretches of telomeric repeats are present at interstitial sites, usually in subtelomeric regions but also at a distinct internal site within band 2q13 (8). We describe here the architecture of the sequence at this internal locus at 2q13, which represents a relic of the fusion of two ancestral ape chromosomes in the evolution of human chromosome 2.‖

MATERIALS AND METHODS

Library Screening. Approximately 1.4 × 10^6 colonies from a human genomic cosmid library containing *Mbo* I partial digestion fragments of 35–41 kb in vector pWE15, propagated in host NM554 (Stratagene), were screened with pSC4, a human genomic 300-base-pair (bp) *Alu* I fragment present at the subtelomeric regions of five different chromosomes, as well as at a single nonterminal locus at 2q13, described elsewhere (8). Sixty positives were screened subsequently with a (TTAGGG)$_n$ probe (n varies between 10 and 1000). Fifteen cosmids hybridized to both pSC4 and (TTAGGG)$_n$.

Cosmid Mapping to Chromosome 2. Probe pSC4 detects five different size fragments on a *Pst* I digest of total human DNA representing the different chromosomal loci. Of these, a 2.5-kb *Pst* I fragment was assigned to chromosome 2 by means of hybridization of pSC4 to 34 somatic cell hybrid lines (obtained, in part, from BIOS, New Haven, CT). We identified two cosmids, c8.1 and c29B, containing both (TTAGGG)$_n$ and the same 2.5-kb fragment detected by pSC4 that maps consistently to chromosome 2 only and, therefore, these two cosmids must originate from chromosome 2 (data not shown).

Restriction Mapping of Genomic Cosmids. Restriction maps of genomic cosmids c8.1 and c29B were constructed by using partial digests of cosmids hybridized to kinase-labeled T3 and T7 primers, as well as cosmid double digests probed with subclone inserts.

DNA Sequencing. DNA sequencing was done by using the dideoxynucleotide chain-reaction procedure. Subclones with suitable insert sizes were generated from clone c8.1. Sequences were determined either by sequencing both DNA strands or by sequencing the same DNA strand twice.

Fluorescence *In Situ* Hybridization. Standard metaphase spreads were prepared from cultured phytohemagglutinin-stimulated peripheral blood lymphocytes. Six unrelated individuals were studied. Chromosome preparations were hybridized *in situ* with probes biotinylated by nick translation, under suppression conditions, essentially as described by Lichter *et al.* (18). The hybridization was done at 37°C in 2× standard saline citrate (SSC)/50% (vol/vol) formamide/10% (wt/vol) dextran sulfate/DNase I (1.5 mg/ml)-cut human genomic DNA (average size, 300–600 bp)/biotinylated probe at 3 μg/ml/sonicated salmon sperm DNA at 1 mg/ml. The probe was denatured in the hybridization mixture at 75°C for 10 min and annealed at 37°C for 20 min. Posthybridization washing was at 42°C in 2× SSC/50% formamide followed by three washes in 0.5× SSC at 60°C. Chromosome identification was based on *in situ* hybridization banding produced by adding heat-denatured digoxigenin-11-dUTP (Boehringer Mannheim)-labeled *Alu*-PCR products in the hybridization mixture (≈2 μg/ml after the reannealing step). This technique produces an R-banding pattern suitable for gene mapping studies (19). Biotin-labeled DNA was detected with fluorescein isothiocyanate-conjugated avidin DCS (5 μg/ml) (Vector Laboratories). Digoxigenin-labeled DNA was detected by

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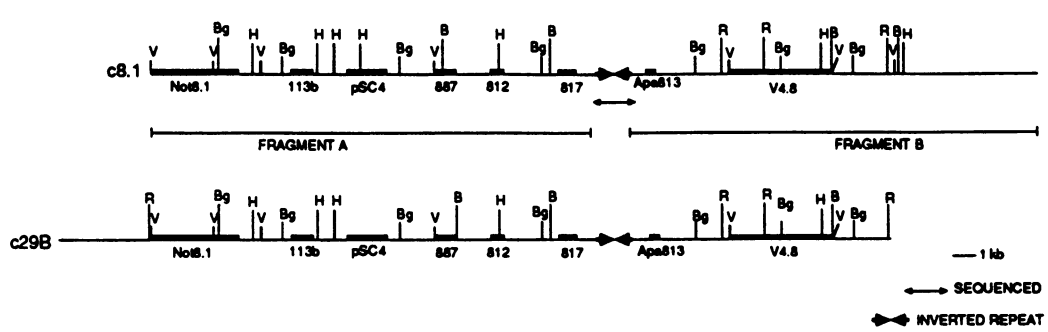
‖The DNA sequence reported in this paper has been deposited in the GenBank data base (accession no. M73018).

using a rhodamine-conjugated anti-digoxigenin antibody (Boehringer Mannheim). Ten metaphase spreads were analyzed per experiment.

RESULTS

Identification and Characterization of Genomic Cosmids Mapping to Band 2q13. To investigate the architecture of this putative fusion point, we isolated genomic cosmids containing the telomere-like repeats from band 2q13. A human genomic cosmid library was screened with a $(TTAGGG)_n$ probe and also with pSC4 (8). Cosmids containing both $(TTAGGG)_n$ and pSC4 were assigned to chromosomes by means of hybridization to 34 somatic cell hybrid lines (data not shown). We identified two cosmids, c8.1 and c29B, that map consistently to chromosome 2, according to the specific length of the pSC4-hybridizing fragment they contain, which is only shared with chromosome 2-containing human–rodent

A



B

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   1 GTGCCCCGGC GCCACGAGGG CGCTGGCGAC CACTGTAAGC AAGAGAGCCC TGCGCCTCTC TGCGCCTGCG CGGCCGGGCC
  81 GGCCGGCCGC CTTTGCGATG GCGGAGTTGC GTTCTCCTCA GCACAGACCC GGAGAGCACC GCAGGGCGAC CTGCGTTGTC
 161 TCTGCACAGA TTTCGGTGGT ACTGCGAAGG CGGACGAGAG TTCTCCTCAG GTCAGACCCG GGCCGGGCCG GCTAGGATAC
 241 CGCGAGGCGA GCTGCGTTCT GCTCAGCACA GACCTGGGGG TCACCGTAAA GATGGAGCAG CATTCCCCTA AGCACAGAGG
 321 TTGGGGCCAC TGCCTGGCTT TGTGACAACT CGGGGCGCAT CAACGGTGAA TAAAATCTTT CCCGGTTGCA GCCGTGAATA
 401 ATCAAGGTCA GAGACCAGTT AGAGCGGTTC AGTGCGGAAA ACGGGAAAGA AAAAGCCCCT CTGAATCCTG GGCAGCGAGA
 481 TTATCCCAAA GCAAGGCGAG GGGCTGCATT GCAGGG

 517 TGAGGG  TGAGGG  TGAGGG  TGAGGG  TTAGGG  TTTGGG  TTGGGG  TTGGGG  TTGGGG  TTGGGG
 577 TAGGG   TTGGGG  TTTGGG  TTGGGG  TTAGGG  TTAGGGG TAGGGG  TAGGG   TCAGGG  TCAGGG
 636 TCAGGG  TTAGGG  TTTTAGGG TTAGGG  TTAGGG  TTAAGG  TTTGGGG TTGGGG  TTGGGG  TTGGGG
 699 TTAGGGG TTAGGGG TTAGGGG TTAGGG  TTGGGG  TTGGGGG TTGGGG  TTGGGG  TTAGGGG TAGGGG
 764 TAGGGG  TAGGG   TTAGGG  TTAGGG  TTAGGG  TAAGGG  TTAAGGG TTGGGG  TTGGGG  TTGGGG
 824 TTAGGG  TTAGGGG TTAGGG  TTAG      CTAA  CCCTAA  CCCTAA  CCCCTAA CCCCTAA CCCCAA
 883 CCCAAA  CCCCAA  CCCCAA  CCCCAA  CCCTA   CCCCTA  CCCCTAA CCCCAA  CCCTTAA CCCTTAA
 945 CCCTTAA CCCTTA  CCCTAA  CCCTAA  CCCAAA  CCCTAA  CCCTAA  CCCTA   CCCTAA  CCCAA
1004 CCCTAA  CCCTAA  CCCTA   CCCTAA  GCCTAAAA CCCTAAAA CCGTGA  CCCTGA  CCTTGA  CCCTGA
1067 CCCTTAA CCCTTAA CCCTTAA CCCTAA  CCCTAA  CCATAA  CCCTAAA CCCTAA  CCCTAAA CCCTAA
1132 CCCTA   CCCTAA  CCCCAA  CCCCTAA CCCTAA  CCCCTATA CCCTAA  CCCTAA  CCCTA   CCCCTA
1193 CCCCTAA CCCCAA  CCCCAG  CCCCAA  CCCCAA  CCCTTA  CCCTAA  CCCTA   CCTAA   CCCTTAA
1253 CCCTAA  CCCCTAA CCCTAA  CCCCTAA CCCTA   CCCCAA  CCCCAAA CCCAA  CCCTAA  CCCAA
1313 CCCTAA  CCCAA   CCCTAA  CCCCTA  CCCTAA  CCCCTAA CCCTAA  CCCCTA  CCCTAA  CCCCTAA
1374 CCCTAA  CCCCTA  CCCTAA  CCCCTAA CCCTAG  CCCTAG  CCCTAA  CCCTAA  CCCTCA  CCCTAA
1435 CCCTCA  CCCTAA  CCCTCA  CCCTCA  CCCTCA  CCCTCA  CCCTAA  CCCAA

1482  CGTCTGTGC TGAGAAGAAT GCTCGTCCGC CTTTAAGGTG CCCCCCAGGT CTGTGCTGAA CAGAACGCAC GTCCGCCGTC
1561 GCAGTGCCCT CAGCCCGGGT CTGACCTGAG AAGAACTCTG CTCCGCCTTC GCAATAGCCC CGAAGTCTGT GCAGAGGAGA
1641 ACGCAGCTCC GCCCTCGCGA TGCTCTTCGG CTGTGTGCTA AAGAGAACGC AACTCCGCCC TCGCAAAGGC GGCGCCGCCG
1721 CGGAGGCCGG AGAGGCGCGG CGCCGCGGAG GCCGGAGAGG CGCGGCGCGC GGGAGGCCGG AGAGGCGCGG CGCCGCGGAG
1801 GCCGGAGAGG CGCGGCGCCG CGGAGGCCGG AGAGGCGCGG CGCCGCGGAG GCCGGAGAGG CGCGGCGCCG CGG
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FIG. 1. (*A*) Restriction maps of cosmids c8.1 and c29B indicate that they are allelic. Thick bars indicate positions of subclones that were used in hybridization studies to show that the two cosmids contain homologous sequences in the same order. B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; R, *Eco*RI; and V, *Eco*RV. (*B*) DNA sequence of region indicated in *A* shows degenerate head-to-head arrays of a tandem repeat having the consensus TTAGGG. The underlined flanking sequences show 80% identity over 269 bp (when inverted) and were 95% (left of telomere repeat) and 90% (right of telomere repeat) identical to sequences found adjacent to the telomeric tandem repeats in clones pTH14 and TelSau2.0, respectively (13, 17). The dashed line indicates sequence consisting of a 25-bp repeat unit with an 88% G + C content.

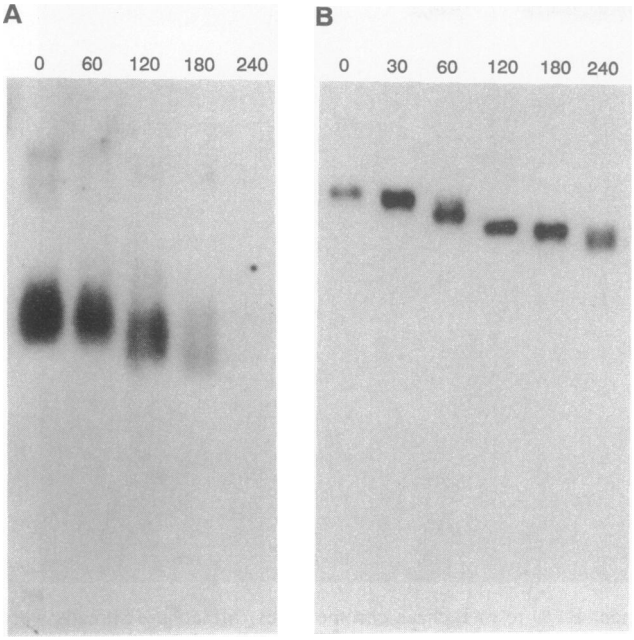

FIG. 2. Sensitivity of the sequences hybridizing to probe 817 in HTY243 (*A*) and to probe Apa813 in HTY275 (*B*) to digestion with BAL-31 nuclease. High-molecular-weight DNA isolated from HTY243 and HTY275 was treated with BAL-31 nuclease for the indicated time in min at 30°C. The DNA was then cleaved with *Pst* I (HTY243) or *Eco*RI (HTY275) and, after transfer to membrane, hybridized to probes 817 and Apa813, respectively. We have attributed the focusing of the Apa813-hybridizing fragments observed at 120 min to a decreased BAL-31 digestion rate when the enzyme reaches the C + G-rich area (21), marked with a dashed line in Fig. 1*B*.

hybrids. Fluorescence *in situ* hybridization experiments localized these cosmids to band 2q13. Restriction analysis and studies of eight subclones of these cosmids revealed that they are allelic (Fig. 1*A*). Both cosmids contain arrays of a telomere-like repeat. By sequence analysis, the telomere-hybridizing region in clone c8.1 was found to consist of two degenerate arrays of TTAGGG in an inverted (head-to-head) arrangement (Fig. 1*B*). The flanking sequences are also arranged in an inverted fashion and show 80% identity over 269 bp and are also 95% and 90% identical with sequences found adjacent to cloned "true" telomeres, pTH14 and TelSau2.0, respectively (13, 17).

The inverted arrangement of the TTAGGG array and the adjacent sequences, which are similar to sequences found at present-day human telomeres, is precisely that predicted for a head-to-head telomeric fusion of two chromosomes. Alternatively, a small duplication and inversion, which could have arisen by chance, might account for this structure. To distinguish between these possibilities we isolated subclones 817 and Apa813, which flank the telomeric repeat in clone c8.1 but lie outside the inverted-repeat region demonstrated by sequencing (Fig. 1*B*). These flanking subclones detect BAL-31-sensitive bands, respectively, in HTY243 and HTY275, two independently isolated yeast artificial chromosome clones that contain different human telomeres (Fig. 2) (20). These data provide strong evidence that the inverted repeats in c8.1 arose from the head-to-head fusion of ancestral telomeres.

Confirmation of the Subtelomeric Origin of the Sequences Flanking the Telomeric Array. Confirmation that sequence blocks from either side of the 2q13 telomere repeat are similar to sequences at human subtelomeres was obtained by fluorescence *in situ* hybridization experiments. Fragments A and B (Fig. 1*A*) from either side of the telomere repeat in c8.1, used as probes in fluorescence *in situ* hybridizations of metaphase chromosomes, showed a different, but overlapping, chromosomal distribution (Fig. 3 *A* and *B*). Both fragments hybridized to most telomeric bands of several chromosomes, as well as to the interstitial band 2q13 (Fig. 4 *A* and *B*). An additional interstitial hybridization signal was observed with fragment B at 3p14 in 5 of the 20 chromosomes analyzed. We also observed a wide range of signal intensity between different chromosomes and between homologous chromosomes, especially with fragment B. In some cases only one homologue was labeled in each of the 10 metaphases studied (i.e., the telomeric region on chromosome 6p with fragment A). The pattern of signal was consistent from cell to cell within an individual but not from individual to individual (e.g., in an unrelated individual, fragment A hybridized to only one copy of chromosome 20q) (data not shown). These data, along with the observations of others (17, 22), suggest that the terminal regions of human chromosomes are dynamic structures, from which stretches of sequence are gained and lost at a relatively high frequency.

DISCUSSION

We have isolated two allelic genomic cosmids that were localized to chromosome 2, each containing two arrays of telomeric repeat TTAGGG in an inverted arrangement.

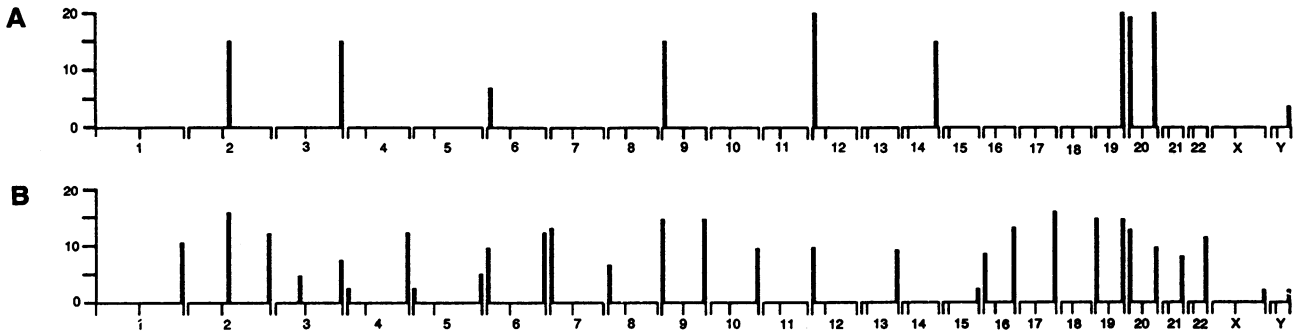

FIG. 3. Hybridization signals obtained with fragments A and B from cosmid c8.1. Ten banded metaphase spreads (20 chromosomes) were examined with each probe. The number of chromosomes hybridizing were summated for each point at which signal was observed. Both fragments hybridize to the most telomeric bands of several chromosomes (at 400-band resolution) as well as region 2q13 (confirmed at 800-band resolution) and show a different but overlapping chromosomal distribution. An additional interstitial hybridization was seen with fragment B at region 3p14 in 5 of 20 chromosomes. Note that, in some cases, only one telomere was labeled in each metaphase spread. For example, only one 6p terminus hybridized with fragment A *in this individual*; hybridization of both 6p telomeres was never observed (sensitivity of the technique is such that there was failure to detect hybridization above background in 3 of 10 spreads). Likewise, one homologue was labeled on 1q, 3q, 8p, 10p, 12p, 13q, and 16p with fragment B.

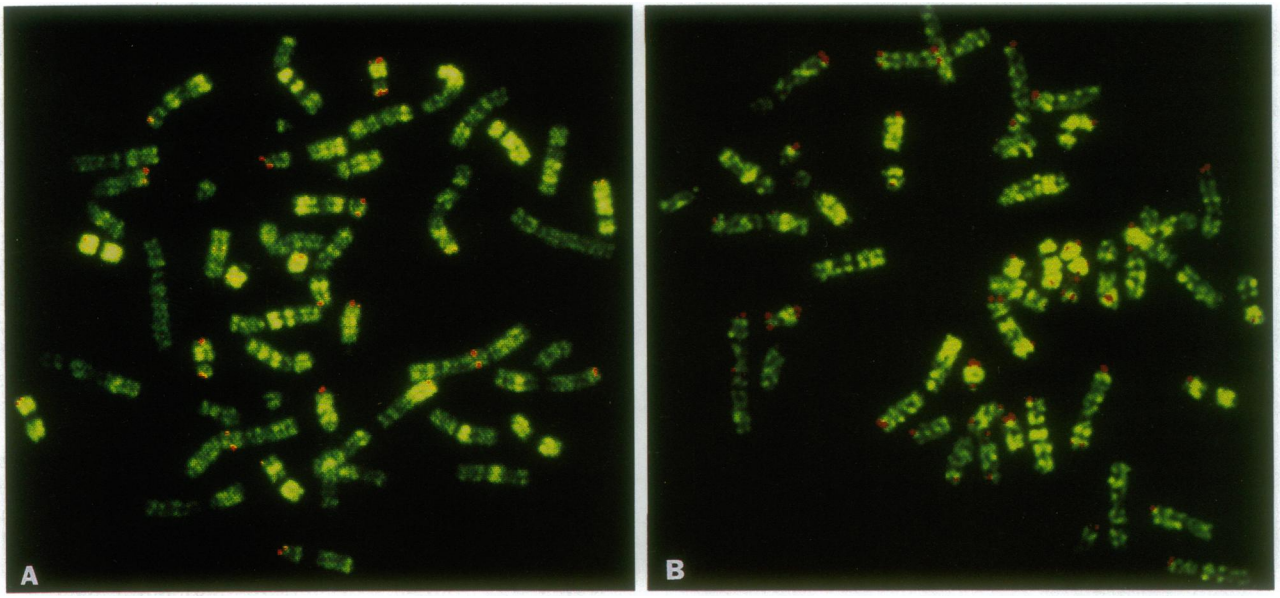

FIG. 4. Fluorescence *in situ* hybridization of fragment A (*a*) and fragment B (*b*) to metaphase chromosomes. Metaphase spreads were prepared from cultured lymphocytes and simultaneously hybridized with a biotinylated A or B probe (shown in red) and digoxigenin-labeled *Alu*-PCR products (shown in green) that generate an R-banding pattern, according to published protocols (19).

Flanking sequences are characteristic for the preterminal regions of human chromosomes. The data we present here demonstrate that a telomere-to-telomere fusion of ancestral chromosomes occurred, leaving a pathognomonic relic at band 2q13. This fusion accounts for the reduction of 24 pairs of chromosomes in the great apes (chimpanzee, orangutan, and gorilla) to 23 in modern human and must, therefore, have been a relatively recent event. Comparative cytogenetic studies in mammalian species indicate that Robertsonian changes have played a major role in karyotype evolution (23, 24). This study demonstrates that telomere–telomere fusion, rather than translocation after chromosome breakage, is responsible for the evolution of human chromosome 2 from ancestral ape chromosomes.

Fusion of telomeres is a rare occurrence in normal lymphoblasts and fibroblasts, although it has been observed in 20–30% of the cells of certain tumors, where it appears to be nonclonal (25–29). The telomere–telomere fusion at region 2q13 must have been accompanied or followed by inactivation or elimination of one of the ancestral centromeres, as well as by events that stabilize the fusion point. Hybridization studies suggest that there is a remnant of an ancestral centromere at band 2q21, which is consistent with the telomeric fusion proposed here (A.B., unpublished data).

The frequency with which telomere–telomere fusion has participated in chromosome evolution cannot readily be assessed. More ancient fusions than the one described here may not easily be detected because of subsequent mutation of the telomere-like repeats and their flanking regions. The observation of an additional weak interstitial hybridization signal at band 3p14 in 5 of 20 chromosomes with fragment B could be explained by the presence of a more degenerate subtelomeric remnant of another telomere–telomere fusion. Telomere-related sequences have also been found in subtelomeric regions of many human chromosomes (8). It has not, however, been determined whether these have arisen by telomere–telomere fusion or by another mechanism of illegitimate recombination. In the single example that has been studied in detail, an inverted telomere array was not present.

The likeliest explanation for the relatively short stretch of telomere-like repeat in cosmid clone c8.1, compared with the average length of human telomeres, is the instability of a long inverted tandem repeat sequence. An extensive inverted repeat, as described here, might be expected to facilitate the formation of secondary, cruciform structures. The ability of inverted-repeat sequences to form cruciform structures has been demonstrated *in vitro* (30, 31). Because of the formal analogy between cruciform structures and Holliday junctions, both are subject to site-specific cleavage and, hence, resolution by single-strand-specific nucleases (32, 33). We suggest such a phenomenon could have resulted in progressive shortening of this inverted sequence until relative stability was reached.

The cosmid clones described here will allow testing of the hypothesized association between the telomere-like sequence at region 2q13 and the rare folate-sensitive fragile site (FRA2B), which also maps to band 2q13 (7, 8, 34). Although the mechanism underlying chromosome fragility has not been determined, it is clear that rare fragile sites, including FRA2B, segregate as codominant traits, so that a heritable cis-acting difference must exist in the fragile chromosome. Hastie and Allshire (35) have cited several features of a putative telomere–telomere fusion that make it an attractive candidate for the fragile site in this band. In the sole example of a constitutional telomere–telomere fusion in human, a high rate of chromosome gaps and breaks was reported at the fusion point between chromosomes 6 and 19, although the presence of residual telomere repeats at the fusion point was not confirmed (36).

The sequences cloned in c8.1 and c29B promise to be extremely useful reagents for further study of chromosome evolution. Comparison of nucleotide sequence of regions flanking the inverted telomere repeats at band 2q13 with homologous sequences at human and ape telomeres should cast some light on the nature of telomere evolution, as the interstitial location of c8.1 will have sheltered the sequences therein from genomic turnover mechanisms peculiar to subtelomeric sequences.

We are grateful to M. Rocchi (University of Bari, Italy) for providing the somatic cell hybrid line RJ38791-CT8, U. Francke (Howard Hughes Medical Institute, Stanford University Medical Center, Stanford, CA) for use of a hybrid panel and H. C. Riethman (Wistar Institute, Philadelphia) for the half-yeast artificial chromosomes HTY243 and HTY275.

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