Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 8983–8988, August 1996
Cell Biology

Telomeric repeats $(TTAGGC)_n$ are sufficient for chromosome capping function in *Caenorhabditis elegans*

CHANTAL WICKY*†, ANNE M. VILLENEUVE‡, NATHALIE LAUPER*, LAURENCE CODOUREY*, HEINZ TOBLER*, AND FRITZ MÜLLER*§

*Institute of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg, Switzerland; and ‡Department of Developmental Biology, B300 Beckman Center, Stanford University School of Medicine, Stanford, CA 94305

Communicated by William B. Wood, University of Colorado, Boulder, CO, May 22, 1996 (received for review January 26, 1996)

ABSTRACT **Telomeres are specialized structures located at the ends of linear eukaryotic chromosomes that ensure their complete replication and protect them from fusion and degradation. We report here the characterization of the telomeres of the nematode *Caenorhabditis elegans*. We show that the chromosomes terminate in 4–9 kb of tandem repeats of the sequence TTAGGC. Furthermore, we have isolated clones corresponding to 11 of the 12 *C. elegans* telomeres. Their subtelomeric sequences are all different from each other, demonstrating that the terminal TTAGGC repeats are sufficient for general chromosomal capping functions. Finally, we demonstrate that the *me8* meiotic mutant, which is defective in X chromosome crossing over and segregation, bears a terminal deficiency that was healed by the addition of telomeric repeats, presumably by the activity of a telomerase enzyme. The 11 cloned telomeres represent an important advance for the completion of the physical map and for the determination of the entire sequence of the *C. elegans* genome.**

Telomeres are specialized structures that protect chromosome ends against degradation and fusion and ensure their complete replication. Cytological studies suggest that telomeres may also play a role in the spatial arrangement of the chromosome ends in the nucleus. In most species that have been investigated, telomeric DNA is marked by the presence of long stretches of conserved short tandem repeats that are synthesized by RNA-dependent DNA polymerases (telomerases) (1).

For a variety of reasons, we think that the nematode *Caenorhabditis elegans*, a well-characterized experimental organism that is amenable to a combination of genetic, cytological, and biochemical approaches, represents an excellent model system for a molecular and genetic analysis of telomeres. First, *C. elegans* has the best-developed physical map of any metazoan organism, with over 95% of the genome represented on contiguous stretches of overlapping yeast artificial chromosome (YAC) and cosmid clones (2–4) and over 35% of the genomic DNA sequenced (5–7). Nevertheless, the chromosome ends are notably missing from the map, accounting for most of the few remaining gaps. The reasons for this may be largely systematic, since the methods used to construct the map were biased against isolation and mapping of terminal clones. Cloning the *C. elegans* telomeres using methods specifically designed to isolate terminal clones will provide a key resource for filling in these terminal gaps, thereby allowing completion of the physical map and the genome sequence. Second, the end regions of the *C. elegans* chromosomes have been implicated in several important meiotic functions, including nuclear membrane attachment, pairing and synapsis of homologous chromosomes, and kinetic activity during the meiotic divisions (reviewed in refs. 8–10). Thus, completing the physical maps of the chromosome ends may facilitate the molecular dissection of chromosomal domains involved in meiosis.

Third, cloning of the *C. elegans* telomeres, coupled with the study of nematode telomerases, will provide an entry point for a genetic analysis of telomere function, regulation, and maintenance during development in a multicellular organism. Work is in progress to purify the telomerase enzyme from the parasitic nematode *Ascaris suum* (L. Magnenat, personal communication), which should provide an enriched source of telomerase activity because its embryos undergo a developmentally programmed process of chromatin diminution and new telomere formation during early cleavages (11). This will pave the way for molecular and genetic studies in *C. elegans*, where it may be possible to identify regulators of telomerase activity and other factors involved in the control of telomere length, a process which is not well understood in any organism.

In this paper, we report the first step in the molecular analysis of *C. elegans* telomeres. We establish that the *C. elegans* chromosomes terminate in the tandemly repeated hexamer TTAGGC, identical to that previously identified in *A. suum* (12). We have cloned and analyzed 11 of the 12 *C. elegans* telomeres, which represents an important advance for the completion of the physical map (2–4) and the sequencing of the *C. elegans* genome (5–7). Moreover, we show that the subtelomeric regions of these 11 telomeres share no sequences in common, implying that the telomeric repeats alone are sufficient for the general chromosome capping functions attributed to telomeres. Finally, we show that the *me8* meiotic mutant (13) bears a terminal chromosomal truncation, and that a new telomere was generated in this strain by a telomerase-mediated healing event.

MATERIALS AND METHODS

***Bal*31 Digestion and Southern Blotting.** *C. elegans* genomic DNA was extracted as described (14) and purified by CsCl gradient centrifugation (15). *Bal*31 digestions were performed at a DNA concentration of 100 ng/μl with 20 units/ml of nuclease at 30°C in 20 mM Tris·HCl (pH 8.0), 600 mM NaCl, 12.5 mM $MgCl_2$, 12.5 mM $CaCl_2$, 1 mM EDTA. The rate of *Bal*31 digestion was approximately 10 kb per 60 min. Reactions were terminated by the addition of EGTA to a final concentration of 20 mM and DNA was precipitated with ethanol. Restriction digestion, Southern blotting, and hybridization were carried out as described (12), as well as YAC DNA isolation (16). *Hin*dIII-cleaved λ DNA and *Bgl*II- or *Hin*fI-cleaved pBR328 DNA was used for size determinations.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: YAC, yeast artificial chromosome; wt, wild type.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. X97521–X97533, X98717).

†Present address: Department of Medical Genetics, University of British Columbia, 6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada.
§To whom reprint requests should be addressed.

Telomeric Libraries. To construct end libraries enriched for telomeric inserts, *C. elegans* genomic DNA was prepared by embedding wild-type (wt) or *me8* L1 larvae in 0.5% low gelling-temperature agarose in 0.125 M EDTA, 0.125 M Tris (pH 9) (ref. 17; R. H. Waterston, personal commmunication). Animals were lysed with 1% sarkosyl, 1 mg/ml proteinase K, and 7% mercaptoethanol for 48 hr at 50°C. After 24 hr, the lysis buffer was replaced by fresh solution. Agarose blocks were then washed several times with TE (10 mM Tris·HCl/1 mM EDTA) and treated with Klenow enzyme to generate blunt ends. The Klenow enzyme was removed by washing the agarose blocks with TE. DNA was then digested with *Xba*I, *Bam*HI, or *Sau*3A, and DNA fragments were isolated from the agarose by filtration through glass wool (18). To remove internal restriction fragments, DNA was self-ligated for 16 hr with T4 DNA ligase (25 Weiss units/ml) at a DNA concentration of 500 ng/ml at 14°C. The unligated telomeric fragments (which contained one blunt end and one sticky end) were cloned into λ Zap (*Xba*I/*Eco*RI treated with Klenow enzyme) or λ Zap Express (*Bam*HI/*Sma*I) (Stratagene). The unamplified libraries were screened with an *A. suum* telomeric $(TTAGGC)_{27}$ probe (12). Positive clones were isolated and Bluescript SK(−) or BKS–CMV plasmids containing the telomeric inserts were excised by following the Stratagene protocol. Plasmid DNA was prepared using standard procedures (15) and sequencing was carried out on both strands with the dideoxy chain termination method using Sequenase (United States Biochemical). To eliminate clones corresponding to already analyzed telomeres from subsequent screens of the library, probes derived from subtelomeric sequences were hybridized to the pool of telomeric clones.

Subtelomeric Probes. To generate specific probes for each telomere, subtelomeric DNA fragments were subcloned in Bluescript or pBS treated with the appropriate restriction enzymes. Subcloning experiments were performed using standard procedures (15). For the *Bal*31 experiments (see Fig. 3), the subcloned fragments indicated by hatched bars in Fig. 2 were used. The probe used in Fig. 4*A* is an *Xba*I-*Sac*I fragment from clone cTel7X (see Fig. 2), which was subcloned in pBS. The terminal *Dde*I fragment from cTel41B, subcloned in pBS, was used for the Southern blots shown in Fig. 4 *B* and *C*.

Genomic Library. The BP1 genomic clone was isolated from a *C. elegans* genomic library constructed by ligating *Bgl*II-cleaved genomic DNA to a *Bam*HI/calf intestinal phosphatase treated λ Zap vector from Stratagene following the manufacturer's recommendations, using the 0.37-kb *Dde*I subtelomeric fragment from cTel41B as a hybridization probe.

RESULTS

The Tandemly Repeated Sequence TTAGGC Is Located at the End of *C. elegans* Chromosomes. *C. elegans* genomic DNA was treated for various times with *Bal*31 exonuclease, followed by digestion with *Hin*fI, separation by agarose gel electrophoresis, and hybridization with an *A. suum*-derived probe containing 27 tandem copies of TTAGGC (12). This analysis revealed a broad smear of about 4–9 kb in length that is preferentially sensitive to shortening by *Bal*31, indicating that these TTAGGC sequences are located at the end of the *C. elegans* chromosomes (Fig. 1). In addition, the TTAGGC probe also detects many *Bal*31-insensitive shorter fragments, which correspond to internal blocks of TTAGGC repeats that were previously shown to be dispersed throughout the genome (19). If genomic DNA was digested with *Dde*I, which cuts once within every TTAGGC repeat, no hybridization signals were observed (data not shown), consistent with the probe hybridizing specifically to $(TTAGGC)_n$ sequences.

Cloning of the *C. elegans* Telomeres. To identify the DNA sequences adjacent to the telomeric repeats at chromosome ends, we used a cloning procedure that discriminated between

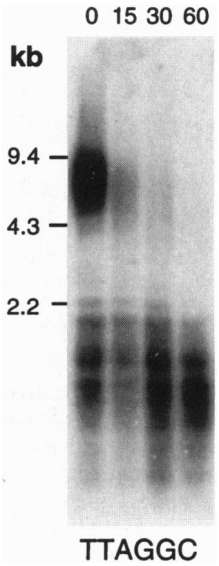
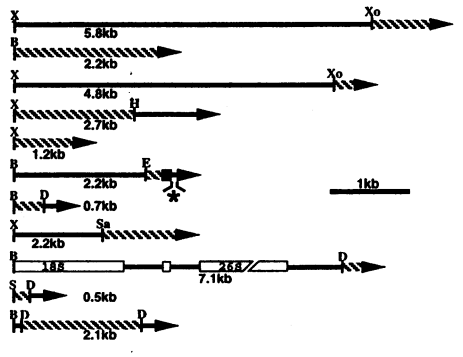


FIG. 1. TTAGGC repeats are located at the telomeres and at various internal sites of *C. elegans* chromosomes. Total *C. elegans* genomic DNA was digested with *Bal*31 for the times indicated in minutes followed by digestion with *Hin*fI, Southern blotting, and hybridization with a radiolabeled *A. suum* telomeric probe, containing 27 repeats of the hexamer TTAGGC (12).

interstitial and telomeric TTAGGC repeats to construct three different λ Zap end libraries. We treated high molecular weight DNA to create blunt ends at the natural telomeres and then digested to completion with restriction enzymes producing overhanging ends. After initial self-ligation to remove fragments bearing two sticky ends, the genomic DNA was ligated into a doubly cut vector with appropriate blunt and sticky end sites (see *Materials and Methods*). From the resulting libraries, we have isolated more than 200 independent TTAGGC containing clones, of which 108 turned out to be legitimate telomeric clones. By comparing the subtelomeric regions adjacent to the TTAGGC repeats, either by direct sequencing or by cross hybridization of subtelomeric probes, the clones could be grouped into 11 distinct classes of identical sequences. A representative clone of each class is shown in Fig. 2. Given that each of the 11 sequences was isolated several times from at least two of the three libraries, and that subtelomeric probes from each group hybridized to *Bal*31-sensitive restriction fragments (Fig. 3), we conclude that 11 out of the 12 native telomeres from *C. elegans* have been cloned. We did not identify a twelfth class of telomere clones in any of the three end libraries, despite the large number of clones that were isolated and analyzed. It is possible that the twelfth telomere is not represented in our collection due to lack of restriction sites, or to some other bias against cloning (possibly from repetitive sequences). Alternatively, the twelfth telomere may in fact be represented among our clones if two telomeres share homologous sequences adjacent to the telomeric repeats.

The restriction patterns of the cloned telomeres (Fig. 2) match those of the corresponding genomic DNA (data not shown), consistent with the interpretation that no appreciable loss or rearrangement of subtelomeric DNA sequences occurred during the cloning process; this holds true even for clones that contain short stretches of tandemly repeated sequences in their subtelomeric regions (cTel4X, cTel79B and cTel52S, Fig. 2). However, the number of TTAGGC sequences in the different clones varied from only 20 to 53 repeats, likely the result of deletion events during the cloning process in *E. coli*. (20). The G-rich strand of the telomeric repeats was oriented 5′ to 3′ toward the end of the insert, corresponding to the defined orientation of eukaryotic telomeric sequences

Telomeric Clones	Indep. Isolates		Subtelomeric Sequences
cTel3X	6	(3)	TGAATATAAACGATCAAAAATAATTTCCAAATGTT(TTAGGC)$_{53}$TTAGG
cTel17B	7	(3)	TGCGAAGGTACTGAACCACCTTTCTGGGGATTTGC(TTAGGC)$_{27}$TTAGG
cTel54X	9	(9)	TATAAATCCTTGAAATTTCATAGGATTTCGCGCTT(TTAGGC)$_{32}$TTAGG
cTel55X	11	(11)	TTGGGTTTACTATCGAATAATGAGAATTTTCAGGC(TTAGGC)$_{28}$TTAGG
cTel4X	2	(2)	CGTTTAAAAAATTGATTTTTCGGTTTGGTCTCTTC(TTAGGC)$_{24}$TTAGG
cTel33B	26	(6)	GAGTGTACTACCGTTTCTGTAGCTACCATATTCTT(TTAGGC)$_{20}$TTAGG
cTel5.3B	21	(5)	TCTGAAAAGCCATTAAAAGTGAATTTTTCTTTTTC(TTAGGC)$_{34}$TTAGG
cTel7X	5	(5)	ACAAGTCCGTCAGTTCGAAGGCTTTGGAGCACAGA(TTAGGC)$_{31}$TTAGG
cTel29B	11	(4)	ACTTTTGAGATACTAATGATGTTGCGGTATTGGTC(TTAGGC)$_{28}$TTAGG
cTel52S	6	(4)	AGTCACTATTTTAGAGAACTGCCAGAGTCACTATT(TTAGGC)$_{28}$TTAGG
cTel79B	6	(6)	ACTCTCTGGCTTCACCGTATTTTTTACTCTCTGGC(TTAGGC)$_{21}$TTAGG



*142bp insert: (cTel33B) (TTAGGC)$_n$CTAAACACAGGAGCATATAGGTTGGCAGGCAGGCAAAATTAGAGGTACCCGCCAAATATCTAGAAGCTTCA
CTAAAAAAAAAACGTTTTGATTTAGCATGAAAACAATTTTGAAAAAGTAAAATGTTTTCTTACTCAATTTT(TTAGGC)$_{20}$

FIG. 2. Nucleotide sequences of the subtelomeric and telomeric regions and partial restriction maps of a representative clone for each of the 11 classes of *C. elegans* telomeres. The letters behind each clone indicate the restriction enzymes used for cloning (B, *Bam*HI; S, *Sau*3A; X, *Xba*I). The second column indicates the number of independently isolated clones from the three end libraries and in brackets the number of partially sequenced clones. In the third column are shown the subtelomeric sequences from each telomere. The G-rich strand of the telomeric repeats (boldface letters) is oriented 5′ to 3′ toward the end of the insert. The subtelomeric regions of clones cTel4X, cTel52S, and cTel79B contain tandemly repeated sequences (first repeating units underlined). In cTel4X, a 20-bp sequence is repeated over a small region of less than 1 kb. cTel52S and cTel79B contain minisatellites with monomer lengths of 25 bp over 0.38 kb and 1.3 kb, respectively, which are located elsewhere in the genome, most likely as large blocks of repeated sequences (see Fig. 3). Ambiguous nucleotides in the telomeric junction region of cTel29B, cTel52S, and cTel79B are boxed (see text). A partial restriction map for each of the telomeric clones is shown in the fourth column (B, *Bam*HI; D, *Dde*I; E, *Eco*RI; H, *Hin*dIII; S, *Sau*3A; Sa, *Sac*I; X, *Xba*I; Xo, *Xho*I). The arrowheads in each clone and the black box in cTel33B represent tandem repeats of the sequence TTAGGC. Subtelomeric probes used for the *Bal*31 experiments shown in Fig. 3 are depicted by hatched bars (see *Materials and Methods*). The telomeric repeats TTAGGC in cTel33B are interrupted by a 142-bp insertion (*), the sequence of which is shown below the figure.

(for review see refs. 1 and 21). Most surprisingly, the telomere-vector junctions of all clones were identical, always ending with the sequence 5′-TTAGG-3′ (Fig. 2). This may reflect a conserved structural feature of the native ends of the *C. elegans* chromosomes. The fact that different clones of the same telomeres were identical in sequence suggests that there are no microheterogeneities among individuals in the position of the junction between telomeric repeats and subtelomeric sequences, as might be expected for a clonal inbred population.

Absence of Similarity Between Subtelomeric Sequences. Except for the TTAGGC repeats, no other sequences are shared by the different classes of telomeric clones, and their subtelomeric sequences do not cross-hybridize with each other (data not shown). The subtelomeric regions of the clones cTel3X, cTel17B, cTel54X, and cTel55X are single copy and thus specific for their telomeres (Fig. 3). Subtelomeric probes derived from the other clones, however, also hybridize with *Bal*31-insensitive genomic DNA fragments, which must be located at internal genomic sites (Fig. 3). The subtelomeric regions of cTel4X, cTel52S, and cTel79B contain short stretches of satellite-like repeats, that is, tandem repeats of 20 bp and 25 bp length (see Fig. 2). The minisatellite in cTel4X is located at a single additional genomic site on chromosome IV (cosmid T05C7), whereas the satellites of cTel79B and cTel52S hybridize to many genomic fragments (see Fig. 3). The telomere identified by the clone cTel33B has a particularly unusual structure. The cTel33B subtelomeric sequence shares similarity with the *C. elegans* transposon-related sequence Tc6.4 (22), and also has an insertion of 142 bp embedded within the telomeric repeats (Fig. 2). On Southern blots with total genomic DNA, this 142-bp sequence hybridizes to many additional *Bal*31-insensitive fragments (data not shown), suggesting that it is present at multiple internal chromosomal sites within the genome of *C. elegans*.

An rDNA Unit Is Immediately Adjacent to the Telomeric Repeats. The telomeric clone cTel29B contains a 7.1-kb insert, which encodes an almost complete *C. elegans* rDNA repeating unit (23). The insert starts at the *Bam*HI site located 113 bp after the beginning of the 18S rRNA coding region and extends to the NTS/ETS region, where it abruptly changes into the tandem telomeric repeats. The 0.27-kb *Dde*I fragment immediately adjacent to the TTAGGC sequences of cTel29B was subcloned and used as a hybridization probe to genomic DNA digested with *Dde*I (Fig. 3, panel cTel29B). The probe detects the cTel29B-specific *Bal*31 sensitive *Dde*I fragment at 0.27 kb with the intensity of single copy DNA, as well as a strong band at 0.33 kb, which corresponds to the same region in the uninterrupted rDNA repeating units (Fig. 3, panel cTel29B), indicating that cTel29B represents a true *C. elegans* telomere. Genetic and physical mapping data consistently map the *C. elegans* ribosomal genes to a single cluster at the right end of chromosome I (24). We therefore propose that cTel29B corresponds to the right end of chromosome I, which consequently represents the first completely mapped chromosomal end of *C. elegans*.

Terminal Deletion and New Telomere Formation in *C. elegans*. Recently, a *C. elegans* mutation was identified that disrupts the function of a cis-acting locus important for normal X chromosome crossing over and segregation during meiosis (13). Since this mutation, *me8*, maps to the extreme left of the X chromosome (13) and since the leftmost known DNA from the X chromosome was deleted from the *me8* chromosome (data not shown), we probed total genomic DNA of *me8* homozygotes with the complete set of our telomeric clones and found that the telomeric restriction fragment corresponding to cTel7X was absent (Fig. 4*A*). Our data demonstrate that the *me8* X chromosome harbors a terminal chromosomal truncation and allows us to assign the cTel7X telomere to the left end of the X chromosome. This chromosomal assignment has been confirmed using a second independently derived deletion of the left end of X (ref. 13; data not shown).

Because the endogenous left X telomere was deleted from the *me8* chromosome, we investigated whether the chromosome end had been healed by acquisition of a new telomere.

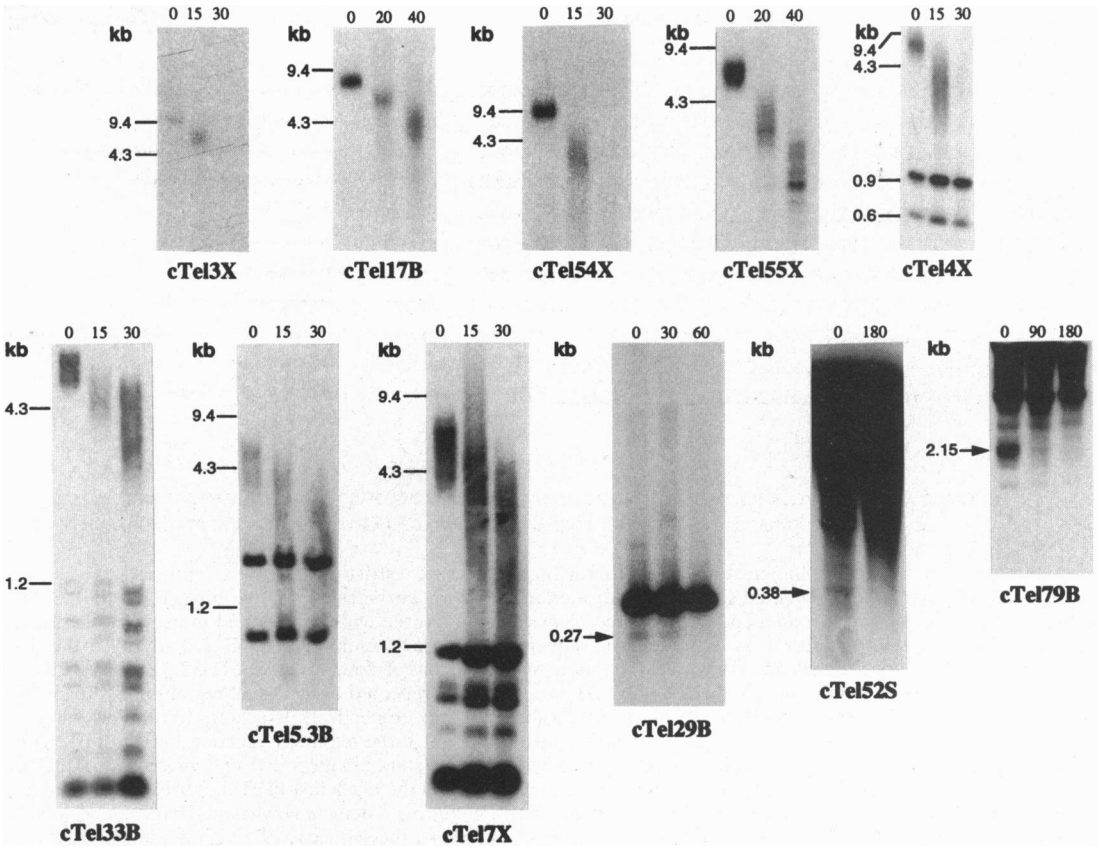


FIG. 3. Telomere-adjacent sequences hybridized to *Bal*31-sensitive restriction fragments. Total *C. elegans* genomic DNA was treated for the times indicated in minutes with *Bal*31 and digested with the following restriction enzymes: cTel3X, *Xba*I; cTel17B, *Bam*HI; cTel54X, *Bam*HI; cTel55X, *Xba*I; cTel4X, *Hin*fI; cTel33B, *Taq*I; cTel5.3B, *Hin*dIII; cTel7X, *Hin*fI. Subtelomeric probes derived from these clones hybridized to *Bal*31-sensitive telomeric smears. For the clones cTel29B, cTel52S, and cTel79B, genomic DNA was digested with *Dde*I, which cuts once within every TTAGGC repeating unit and thus leaves the ends of the chromosomes free of telomeric repeats. The bands corresponding to the subtelomeric *Dde*I fragments of the telomeric clones (marked with an arrow) are *Bal*31 sensitive. The ratio of single copy to repetitive DNA in cTel29B and cTel52S makes difficult a definitive statement concerning *Bal*31 sensitivity. As a control, the filters were stripped and reprobed with a radiolabeled fragment of *C. elegans* rDNA (data not shown).

Indeed, from an end library constructed from *me8* genomic DNA (see *Materials and Methods*), we isolated a new telomeric clone, cTel41B. In Southern blotting experiments, the 0.37-kb *Dde*I fragment immediately adjacent to the TTAGGC repeats in cTel41B was present in *me8* DNA, but absent from wt DNA (Fig. 4*C*). Furthermore, this probe detected a broad band of approximately 9.4 kb present in *Bcl*I digests of *me8* DNA, but not of wt DNA (Fig. 4*B*); this *me8*-specific band was *Bal*31-sensitive, indicating that it corresponds to a new telomere present in the *me8* strain. Genetic mapping experiments confirm that this telomere indeed represents the new left end of the X chromosome (data not shown).

To determine the nature of the healing event responsible for new telomere formation in the *me8* strain, we cloned the wt DNA spanning the region from which the telomere was derived. This wt genomic clone, BP1, overlaps the 0.7-kb *Dde*I fragment (and the 5-kb *Bcl*I fragment) detected by the cTel41B subtelomeric probe in both wt and *me8* genomic DNA (Fig. 4 *B* and *C*). Unexpectedly, we found that this DNA maps to the YAC clone Y51B4 located in the middle of chromosome V (Fig. 4*C*). Thus, it is likely that the original *me8* mutation resulted from a complex mutational event involving fusion of the terminally deleted X chromosome with a segment of chromosome V, accompanied by breakage within the chromosome V segment and addition of a new telomere. Comparison of the cTel41B and BP1 sequences revealed that the new telomere had been generated by *de novo* telomere addition at a site that lacked preexisting telomeric TTAGGC repeats (Fig. 4*D*).

DISCUSSION

We have cloned and analyzed 11 of the 12 telomeres of the free-living nematode *C. elegans*. The chromosome ends of this organism consist of 4–9-kb long tandem arrays of the simple invariant hexamer TTAGGC, where the G-rich strand is oriented 5′ to 3′ toward the end of the chromosome. This sequence is related to the telomeric repeats of otherwise widely divergent eukaryotic organisms, most notably the TTAGGG repeats found at vertebrate and trypanosome telomeres (21). TTAGGC may be a general feature of nematode telomeres, since it is also present at the telomeres of *A. suum* (12) and hybridizes to the genomes of other free-living nematodes (8, 19).

Telomeric TTAGGC repeats are not only present at the ends of the *C. elegans* chromosomes, however, but are also found at many different internal sites (see Fig. 1). Sequence analysis of randomly cloned fragments (unpublished data) and data from the genome sequencing project revealed that these interstitial telomeric repeats are in most cases highly degenerated, suggesting a rapid evolution. Interstitial telomeric repeats are also present in a wide variety of organisms, such as plants, ciliates, and vertebrates (25), but their origin and possible function(s) are still unclear. It has been suggested that telomeric repeats could act as hotspots of recombination in *Paramecium* (26) and in rodents (27, 28). In *C. elegans*, this idea is supported by the observation that the interstitial telomeric repeats are concentrated within the 30% proterminal portion of the chromosomes (19), where recombination frequency is

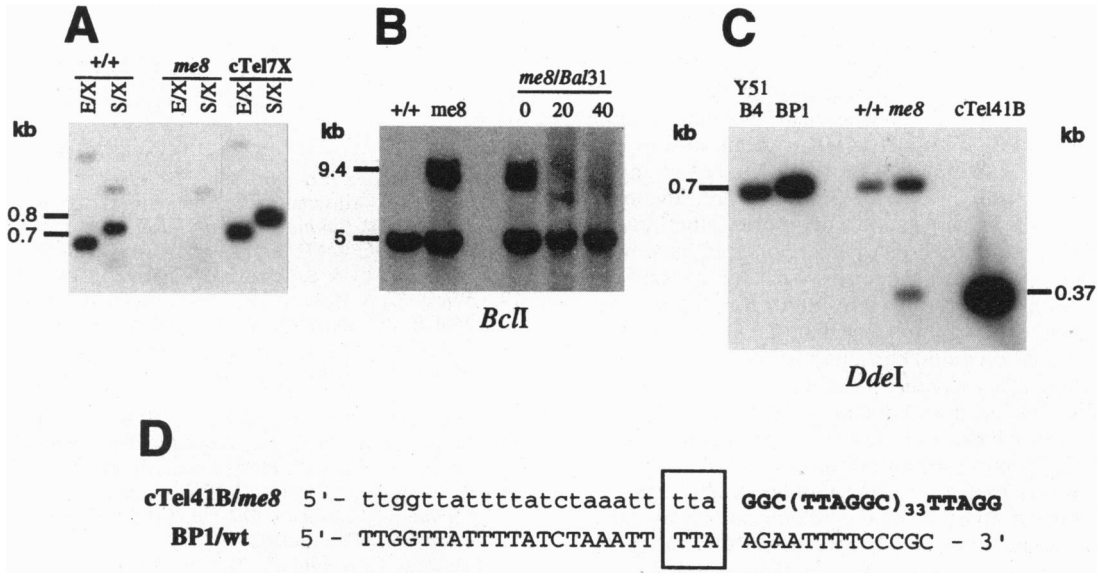


FIG. 4. Terminal deletion and new telomere formation in the *me8* mutant. (*A*) The telomere corresponding to cTel7X is absent from the *me8* genome. Wild-type and *me8* genomic DNA and cTel7X plasmid DNA were digested with *Sac*I (S), *Xba*I (X), or *Eco*RI (E) and hybridized with an *Xba*I–*Sac*I fragment derived from cTel7X (see Fig. 2). The 0.8- and 0.6-kb cTel7X-specific fragments present in the wt DNA are absent from the *me8* genome. Additional weak bands in both DNAs represent repetitive sequences located elsewhere in the *C. elegans* genome. (*B* and *C*) The clone cTel41B corresponds to the healed chromosome end in *me8* animals. (*B*) Wild-type and *me8* genomic DNAs, as well as *Bal*31 digested *me8* DNA, were cleaved with *Bcl*I and hybridized with the 0.37-kb *Dde*I fragment immediately adjacent to the telomeric repeats in cTel41B, which was isolated from a *me8* end library (see text). The probe hybridized with a broad band of 9.4 kb present in the *me8* DNA but not in wt DNA. (*C*) The same subtelomeric probe was hybridized with a Southern blot containing wt and *me8* genomic DNAs, and DNA from the YAC clone Y51B4 and plasmids BP1 and cTel41B, digested with *Dde*I. The *me8*-specific *Dde*I fragment is identical in size to the subtelomeric fragment from the cTel41B telomeric clone, confirming that cTel41B corresponds to the new telomere in the *me8* strain. Similarly, the 0.7-kb fragment present in both wt and *me8* DNA (the ancestral fragment from which the new *me8* telomere was derived) corresponds to a fragment present in the clone BP1 and Y51B4. (*D*) *De novo* addition of telomeric repeats. DNA sequence across the junction between telomeric repeats and subtelomeric DNA at the new *me8* telomere (from cTel41B) compared with the wt ancestral sequence from which the new telomere was derived (from plasmid BP1). Nucleotides that are identical between the two sequences are indicated in lowercase letters in the sequence of cTel41B. The framed trinucleotide TTA represents an ambiguity with respect to the exact position of the telomere addition site, because it is present in both the ancestral sequence and in the TTAGGC repeat.

enhanced compared with that observed in the central gene cluster regions (29). In the parasitic nematode *A. suum*, interstitial telomeric repeats are present in the germ-line DNA, but are eliminated from the somatic genome during the process of chromatin diminution (ref. 12 and unpublished data). It is tempting to speculate that the germ-line-specific location could reflect a germ-line-specific function of these sequences, such as, promoting recombination during meiosis in *A. suum*.

A key finding of the current work is that proximal to the simple TTAGGC repeats, the *C. elegans* telomeres do not share sequence similarity with each other. This is in contrast to the subtelomeric sequences identified from six cloned *Tetrahymena* micronuclear telomeres, which are 55–87% identical to each other (20), and to the subtelomeric regions of *Saccharomyces cerevisiae* chromosomes, which often contain two types of subtelomeric repeats, X and Y′ (30). The subtelomeric repeated sequences observed on subsets of chromosomes in these and other systems (including humans) do not exhibit any phylogenetic conservation, and numerous attempts have failed to find an essential function for these sequences in *S. cerevisiae*. Nevertheless, the prevalence of such sequences has led some workers to hypothesize that they may play an important role in metazoan biology (reviewed in ref. 31). *C. elegans* is the first metazoan reported in which the subtelomeric regions lack repeated sequences that are shared among at least a subset of the different telomeres. The absence of shared sequences makes it very unlikely that subtelomeric sequences contribute to telomere function in this organism.

Whereas the subtelomeric regions of the telomeric clones cTel3X, cTel54X, cTel55B, and cTel17X contain single-copy DNA sequences, the sequences adjacent to the TTAGGC repeats in cTel4X, cTel5.3B, cTel7X, cTel33B, and cTel29B are repeated once to several times at nontelomeric sites. The telomeres corresponding to the clones cTel4X, cTel79B, and cTel52S have tandemly repeated sequences in their subtelomeric regions (see Fig. 2). Tandemly repeated subtelomeric sequences have been found in the genomes of several organisms, where they appear to rearrange frequently to produce a high degree of polymorphism at the chromosomal ends (31). It has been suggested that the function of the subtelomeric regions is to allow frequent recombination or to act as a buffer zone between the chromosome ends and the most distal genes. In the clone cTel29B, however, telomeric repeats are added directly to an almost complete rDNA repeating unit. Either no subtelomeric sequences are required, or the subtelomeric functions are carried out by the most distal rDNA repeats themselves. In the algae *Chlorarachniophyte sp.*, the rDNA cluster of the chloroplast nucleus is also localized adjacent to the telomeric repeats (32), and abrupt transition from the rDNA sequence to telomeric repeats has been found in the protozoan *Giardia lamblia* (33, 34). Perhaps the terminal chromosomal position of rDNA in these organisms permits the frequent recombination thought to be necessary to maintain a high degree of homogeneity between the tandem copies of ribosomal RNA genes (35).

The new telomere present in the *me8* strain was generated by the addition of TTAGGC repeats to a chromosomal breakage site that lacked preexisting telomeric repeats. It is likely that these telomeric repeats were added by the activity of a telomerase. This interpretation is supported by the presence of three bases homologous to the *C. elegans* telomeric repeats within the genomic DNA at the *me8* breakpoint that may have provided limited pairing with the RNA template of

telomerase (36–38), thereby allowing priming of telomere addition. Furthermore, telomere addition at the *me8* breakpoint appears to be directly analogous to the developmentally regulated telomere addition occurring during the process of chromatin diminution in *A. suum*, where chromosomal healing is mediated by the addition of TTAGGC repeats to sites that are also marked by ambiguous bases (refs. 11, 12, 39 and S. Jentsch, personal communication). Furthermore, the existence of ambiguous bases at the telomeric junctions of cTel29B, cTel52S, and cTel79B, where telomeres have been added to tandemly repeated sequences (see Fig. 2), suggests that these telomeres may also have resulted from telomerase-mediated healing events, implying that *de novo* telomere formation by telomerase might be an important mechanism for genome evolution in *C. elegans*. Telomerase-mediated chromosomal healing has been well documented during macronucleus formation in ciliates (40–45) and is thought to occur spontaneously in human (38) and mice (46).

The observations that *C. elegans* telomeres share no sequences in common apart from the telomeric repeats, that telomeric repeats are directly adjacent to rDNA coding sequences and that a broken chromosome end was healed in the *me8* strain by the addition of telomeric sequences, suggest that the terminal TTAGGC repeats are sufficient for general chromosome capping functions in *C. elegans*. In the case of the *me8* chromosome, the addition of TTAGGC repeats did not rescue the meiotic mutant phenotype, however, suggesting that the telomeric repeats *per se* are not sufficient to ensure normal pairing and recombination between homologs. Further experiments will be required to determine whether the meiotic defect is caused by the deletion of key pairing center sequences located internal to the telomeric repeats, or to the juxtaposition of a new chromosome end adjacent to the pairing center.

The cloning of the telomeres and their adjacent sequences represents a significant advance toward the completion of the physical map of the *C. elegans* chromosome ends. In this study, we were able to assign two classes of clones to their chromosome ends of origin (chromosome I right, chromosome X left), and work is in progress to determine the chromosomal locations of the remaining clones. We attempted to map clones by hybridizing probes derived from their subtelomeric regions with filters containing ordered arrays of cosmids or YACs of the physical map, but the only hybridization signals obtained in these experiments corresponded to internal locations of repeated sequences (data not shown). Other approaches for mapping the clones include PCR methodology to identify telomeres that are missing in embryos homozygous for suspected terminal deficiencies, or in segmental aneuploid embryos arising among the segregants of translocation heterozygotes; cTel3X has been assigned to the left end of chromosome V using the latter approach (J. Gawne and A. M. Rose, personal communication). Once assigned to their respective chromosome ends, the telomere clones will serve as stepping-off points for bridging the terminal gaps and completing the physical map of *C. elegans*.

We thank A. Coulson for various *C. elegans* genomic YAC and cosmid clones, H. Gachoud for technical assistance, and colleagues from our laboratory for helpful discussions. This work was supported by the Swiss National Science Foundation and in part by a senior fellowship from the American Cancer Society (California Division) to A.M.V.

1. Kipling, D. (1995) *The Telomere* (Oxford Univ. Press, Oxford).
2. Coulson, A. R., Sulston, J. E., Brenner, S. & Karn, J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7821–7825.
3. Coulson, A. R., Waterston, R., Kliff, J., Sulston, J. E. & Kohara, Y. (1988) *Nature (London)* **335,** 184–186.
4. Coulson, A., Kozono, Y., Lutterbach, B., Shownkeen, R., Sulston, J. & Waterston, R. (1991) *BioEssays* **13,** 413–417.
5. Waterston, R. & Sulston, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10836–10840.
6. Sulston, J., Du, Z., Thomas, K., Wilson, R., Hillier, L., *et al.* (1992) *Nature (London)* **356,** 37–41.
7. Wilson, R. Ainscough, R., Anderson, K., Baynes, C., Berks, M., *et al.* (1994) *Nature (London)* **368,** 32–38.
8. Zetka, M.-C. & Müller, F. (1996) *Semin. Cell Dev. Biol.* **7,** 59–64.
9. Zetka, M.-C. & Rose, A. (1995) *Trends Genet.* **11,** 27–31.
10. Wicky, C. & Rose, A. M. (1996) *BioEssays* **18,** 447–452.
11. Müller, F., Bernard, V. & Tobler, H. (1996) *BioEssays* **18,** 133–138.
12. Müller, F., Wicky, C., Spicher, A. & Tobler, H. (1991) *Cell* **67,** 815–822.
13. Villeneuve, A. M. (1994) *Genetics* **136,** 887–902.
14. Wood, W. B. (1998) *The Nematode Caenorhabditis elegans* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
16. Guthrie, C. & Fink, G. R. (1991) *Guide to Yeast Genetics and Molecular Biology* (Academic, San Diego).
17. Schwarz, D. C. & Cantor, C. R. (1984) *Cell* **37,** 67–75.
18. Heery, D. M., Gannon, F. & Powell, R. (1990) *Trends Genet.* **6,** 173.
19. Cangiano, G. & La Volpe, A. (1993) *Nucleic Acids Res.* **21,** 1133–1139.
20. Kirk, K. E. & Blackburn, E. H. (1995) *Genes Dev.* **9,** 59–71.
21. Zakian, V. A. (1989) *Annu. Rev. Genet.* **23,** 579–604.
22. Dreyfus, D. H. & Emmons, S. W. (1991) *Nucleic Acids Res.* **19,** 1871–1877.
23. Ellis, R. E., Sulston, J. E. & Coulson, A. R. (1986) *Nucleic Acids Res.* **14,** 2345–2364.
24. Albertson, D. G. (1984) *EMBO J.* **3,** 1227–1234.
25. Biessmann, H. & Mason, J. M. (1994) *Chromosoma* **103,** 154–161.
26. Katinka, M. D. & Bourgain, F. M. (1992) *EMBO J.* **11,** 725–732.
27. Ashley, T. & Ward, D. C. (1993) *Cytogenet. Cell Genet.* **62,** 169–171.
28. Ashley, T., Cacheiro, N. L., Russell, L. B. & Ward, D. C. (1993) *Chromosoma* **102,** 112–120.
29. Barnes, T. M., Kohara, Y., Coulson, A. & Hekimi, S. (1995) *Genetics* **141,** 159–179.
30. Louis, E. J., Naumova, E. S., Lee, A., Naumov, G. & Haber, J. E. (1994) *Genetics* **136,** 789–802.
31. Biessmann, H. & Mason, J. M. (1992) *Adv. Genet.* **30,** 185–249.
32. Gilson, P. & McFadden, G. I. (1995) *Chromosoma* **103,** 635–641.
33. Adam, R. D., Nash, T. E. & Wellems, T. E. (1991) *Mol. Cell. Biol.* **11,** 3326–3330.
34. Blancq, S. M., Reggie, S. K. & Van der Ploeg, L. H. T. (1991) *Nucleic Acids Res.* **19,** 5790.
35. Petes, T. D. (1980) *Cell* **19,** 765–774.
36. Blackburn, E. H. (1992) *Annu. Rev. Biochem.* **61,** 113–129.
37. Wilkie, A. O. M., Lamb, J., Harris, P. C., Finney, R. D. & Higgs, D. R. (1990) *Nature (London)* **346,** 868–871.
38. Morin, G. B. (1991) *Nature (London)* **353,** 454–456.
39. Tobler, H., Etter, A. & Müller, F. (1992) *Trends Genet.* **8,** 427–432.
40. Greider, C. W. & Blackburn, E. H. (1989) *Nature (London)* **337,** 331–337.
41. Greider, C. W. & Blackburn, E. H. (1985) *Cell* **43,** 405–413.
42. Greider, C. W. & Blackburn, E. H. (1987) *Cell* **51,** 887–898.
43. Zahler, A. M. & Prescott, D. M. (1988) *Nucleic Acids Res.* **16,** 6953–6985.
44. Shippen-Lentz, D. & Blackburn, E. H. (1989) *Mol. Cell. Biol.* **9,** 2761–2764.
45. Yu, G. L. & Blackburn, E. H. (1991) *Cell* **67,** 823–832.
46. Prowse, K. R., Avilion, A. A. & Greider, C. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1493–1497.