Chlamydomonas telomere sequences are A+T-rich but contain three consecutive G·C base pairs

(telomere-associated sequences/chromosome structure)

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ABSTRACT   We have isolated telomeric DNA and telomere-associated sequences from *Chlamydomonas reinhardtii*. The terminal telomere sequences of the green alga *Chlamydomonas* are composed of (TTTTAGGG)$_n$ repeats that are similar, but not identical, to those of the higher plant *Arabidopsis thaliana*. We demonstrate that these repeats are telomeric by their preferential sensitivity to nuclease Bal-31 digestion, their similarity to *A. thaliana* telomeres, their orientation relative to the end of the chromosome, and the methods used for their isolation. Five independent telomere clones were isolated, and three of these clones include closely related telomere-associated sequences. One of these telomere-associated sequences hybridizes to a number of genomic fragments sensitive to digestion with the exonuclease Bal-31. Like telomere sequences from other organisms, the *C. reinhardtii* telomeres display a bias for guanine and thymine nucleotides on the 3'-end strand. However, the sequence of *Chlamydomonas* telomeres is more A+T-rich than any other known telomere sequence. We propose that the common feature of all known telomeres is the frequent occurrence of tracts of three or more adjacent guanine residues.

Eukaryotic chromosomes terminate in specific structures, the telomeres, which are composed of highly repetitive DNA sequences and nonnucleosomal proteins (for review, see refs. 1–3). The terminal telomere sequences (telomere repeats) include some variation of the consensus sequence d[T$_{0-4}$ (W)G$_{1-8}$, where W = A or T], with this "G-strand" oriented 5' to 3' toward the chromosome end. The telomere repeats known to date are listed in Table 1. Telomere sequences fall into two general classes: those with simple primary repeats that rarely or never diverge (e.g., *Tetrahymena* (6) and *Arabidopsis* (17) and those with a variable primary telomere sequence pattern [e.g., *Saccharomyces cerevisiae* (7, 8), *Schizosaccharomyces pombe* (19), and *Dictyostelium* (4)]. It is assumed that all chromosome ends in a given organism have the same telomere repeat motif. This conservation may be due to the fact that telomerase, a ribonucleoprotein enzyme that has been isolated from ciliates (20–22) and human cells (23), can elongate G-strand telomere repeats by using a portion of its RNA as a template (24).

Telomere repeats from organisms as distantly related as *Tetrahymena* (25), *Oxytricha* (26), and *Homo sapiens* (27–29) can be recognized and used as telomeres on linear plasmids in *Sa. cerevisiae* cells. *Sc. pombe* can recognize and maintain linear plasmids with telomere repeats from *Tetrahymena* and *Sa. cerevisiae* (19, 30, 31). Both yeasts add their own telomere repeat motif distal to the heterologous sequences on linear plasmids (8, 19). Furthermore, single-stranded (G-strand) telomere repeats from a broad range of organisms can function as primers in *in vitro* telomerase assays (21–23, 32) and can fold

into unusual structures that have altered mobilities on nondenaturing gels (33). Because telomere repeats from such divergent organisms have similar properties *in vivo* and *in vitro*, it is assumed that telomeres in all eukaryotes are replicated, maintained, and recognized by similar molecular mechanisms.

Telomere-associated sequences (TASs) are defined as the sequences just proximal to the telomere repeats (1). In higher organisms, moderately repetitive TASs have been identified by their cytogenetic localization to telomeres (34–36). Some of these repeat sequences are also found in other heterochromatic loci—e.g., the pericentric regions (35). Human TAS clones include sequences shared with other telomeres (37), as well as a region of *Alu* repeats (29). In *Sa. cerevisiae*, two classes of TASs, Y' and X, are located proximal to the telomere repeats. These TASs recombine at very high rates, and the Y' TASs may be propagated via a circular intermediate (38, 39). Both of the yeast TASs (40) and a region of *Tetrahymena* telomere-flanking sequence (41) contain sequences that enable the autonomous replication of plasmids in *Sa. cerevisiae*. However, neither the Y' nor the X classes of TASs are essential for general telomere function in *Sa. cerevisiae* (42, 43).

Chlamydomonas reinhardtii is a unicellular green alga that is amenable to genetic, biochemical, and molecular analysis. It can be transformed with exogenous DNA by simple inexpensive methods (44–48). An extensive genetic map with numerous phenotypic and molecular markers is available. While 18 of the linkage groups have linear genetic maps, the 19th, or *uni* linkage group, which includes a large number of markers important for flagellar and basal-body function, has been reported to be genetically circular (49, 50). It has recently been reported (51) that, at least during some portion of the cell cycle, the *uni* linkage group may be physically located in the basal bodies rather than in the nucleus, although this finding has been disputed (68). Cloned *Chlamydomonas* telomeres and TASs would provide useful molecular markers for the *Chlamydomonas* linkage map and would facilitate analysis of the structure and inheritance of the *uni* linkage group.

In this paper, we describe the cloning and sequencing of a number of *Chlamydomonas* telomeres. The *Ch. reinhardtii* telomere repeat, (TTTTAGGG)$_n$, is similar to, but more A+T-rich than, other known telomere sequences. We have isolated and sequenced a number of *Chlamydomonas* clones that contain this telomere repeat motif and adjacent telomere-associated sequences. One class of related TASs is present on some, but not all, of the *Chlamydomonas* chromosomes.

MATERIALS AND METHODS

Strains. *Ch. reinhardtii* NO⁺ and field isolate S1-D2 (52) were maintained as described (53). These two strains were crossed, sporulated, and dissected by standard methods (54).

Abbreviation: TAS, telomere-associated sequences.
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Table 1. Comparison of %G+C content in telomere repeats and genomic DNA

Organism	Telomere repeat (ref.)	G+C in telomere,* %	G+C in genome, % (ref.)
Dictyostelium	AG_{1-8} (4)	83	22 (5)
Tetrahymena	TTGGGG (6)	67	25 (5)
Sa. cerevisiae	$(TG)_{1-3}[TGG(G)]$ (7, 8)	62	39 (5)
Plasmodium berghei	TTYAGGG (9)	52	41 (5)
Homo sapiens	TTAGGG (10)	50	40 (11)
Physarum	TTAGGG (12)	50	42 (5)
Oxytricha nova	TTTTGGGG (13)	50	42 (14)
Trypanosoma	TTAGGG (15)	50	50 (5)
Neurospora	TTAGGG (16)	50	54 (5)
A. thaliana	TTTAGRG (17)	42	41 (18)
Lysopersicum esculentum	TTWAGGG†	42	37‡
Ch. reinhardtii	TTTTAGGG	37.5	64 (5)

Y, thymine or cytosine; R, guanine or adenine; W, thymine or adenine.
*When sequences varied, G+C content was calculated from the available published telomere sequences.
†M. Ganal, personal communication.
‡R. Messeguer, personal communication.

Escherichia coli strains MC1061 [*araD139*, Δ*(ara–leu)7697*, Δ*(lac)X74*, *galU⁻*, *galK⁻*, *hsr⁻*, *hsm⁺*, *strA*] (55) and XL1-blue {*recA1*, *endA1*, *gyrA96*, *thi*, *hsdR17*, *supEff*, *relA1*, *lambda⁻(lac)* [F', *proAB*, *lacIq*, *ZΔM15*, *Tn10 (tetR)*]} (Stratagene) were used.

Enzymes. Restriction enzymes, T4 DNA ligase, and T4 polynucleotide kinase were purchased from New England Biolabs. Nuclease Bal-31 was from Life Technologies, Inc. (Gaithersburg, MD), and DNA polymerase I large fragment (Klenow fragment) was purchased from both New England Biolabs and Pharmacia. All enzymes were used according to the manufacturer's instructions.

Cloning *Chlamydomonas* Telomeres. Primary end-libraries were constructed essentially as described by Richards and Ausubel (17). *Chlamydomonas* genomic DNA was digested for 30–60 sec at room temperature with Bal-31 and then ligated to plasmid pSDC12 (56) or pJK-kmf(−) (57) digested with *Bam*HI and *Hinc*II. The genomic DNA/vector hybrids were then digested with *Bam*HI and *Mbo* I, circularized with T4 DNA ligase, and transformed into *E. coli* strain MC1061 by electroporation [BTX, San Diego, CA (58)]. Transformants were screened by colony hybridization (59) on nitrocellulose (Schleicher & Schuell) by using as probes either the *A. thaliana* telomere insert from pAtT4 (17) labeled with [α-³²P]dATP by random priming (60) or CT1 oligonucleotide (5'-AAAACCCTAAAACCCTAAAACCCT-3') end-labeled with [γ-³²P]ATP (59).

The first end-library, constructed in pSDC12, was screened by hybridization with gel-purified insert fragment from pAtT4. Hybridizations were carried out in 5× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/1% SDS/herring sperm DNA at 100 μg/ml/1× Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin) at 42°C. Washes were performed in 2× SSC/1% SDS at 65°C. From ≈6000 transformants, we identified two hybridizing clones, pC2 and pC9. These were subcloned into the *Eco*RI/*Hin*dIII sites of pBluescript-KS-minus (Stratagene) to form pCT20 and pCT17, respectively. pCT17TAS was constructed by digestion of pCT17 with *Mnl* I, gel purification of the telomere-flanking fragment, and ligation of the fragment to pBluescript-SK-plus (Stratagene), which had been digested with *Sma* I.

The second end-library was constructed directly in sequencing vector pJK-kmf(−). Genomic DNA for this library was prepared from the F₁ progeny of a cross between *Ch. reinhardtii* strains NO⁺ and S1-D2. The library was screened by hybridization with oligonucleotide CT1 in 6× SSC/0.1%

SDS/5× Denhardt's solution/herring sperm DNA at 100 μg/ml/0.05% sodium pyrophosphate at 42°C. Washes were performed at 57°C in 6× SSC/0.1% SDS/0.05% sodium pyrophosphate.

Bal-31 Digestion Experiments. *Chlamydomonas* genomic DNA was digested with nuclease Bal-31 (Life Technologies, Inc., Gaithersburg, MD) in 200 mM NaCl/20 mM Tris·HCl (pH 8.1)/12 mM MgCl₂/12 mM CaCl₂/1 mM EDTA at 30°C for time periods as indicated in the figure legends. The enzyme was inactivated with 33 mM EGTA, and the DNA was then digested with either *Bam*HI or *Pst* I and fractionated by electrophoresis on 0.8% agarose gels. The DNA was transferred to a nylon membrane (Nytran, Schleicher & Schuell, or Biotrans, ICN) as described by the manufacturer. Hybridization and wash conditions for CT1 oligonucleotide were as described above. Plasmid DNA hybridizations of pAtT4 (17), pCT17, pCT17TAS, pcf6-100 (61), and pMN24 (45) were in 6× SSPE (1× SSPE is 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA)/0.3% SDS/50% (vol/vol) formamide/herring sperm DNA at 100 μg/ml, and 2× Denhardt's solution. Washes were performed as described for colony screening with pAtT4.

DNA Sequencing. Double-stranded plasmid DNA was prepared by the boiling miniprep method (Stratagene) and denatured by using NaOH (62). Sequenase 2.0 (United States Biochemical) was used for dideoxynucleotide sequencing according to manufacturer's instructions. DNA sequences were analyzed using Intelligenetics programs (University of Minnesota Molecular Biology Computing Facility).

RESULTS

***Ch. reinhardtii* Telomere Repeats Cross-Hybridize with Telomere Repeats from *A. thaliana*.** Telomere repeats from a broad range of organisms can cross-hybridize with telomere repeats from unrelated organisms (17, 63). In initial experiments we found that an *A. thaliana* telomere repeat probe hybridizes to sequences from *Ch. reinhardtii*. To determine whether the *Ch. reinhardtii* sequences were telomeric, we performed nuclease Bal-31 digestion experiments. Bal-31 degrades both the 5' and 3' strands of duplex DNA. Because of its position at the chromosomal termini, telomeric DNA is much more sensitive to exonuclease digestion than are internal DNA sequences. When intact genomic DNA is digested with Bal-31 for increased amounts of time and subsequently digested with restriction endonucleases, telomeric DNA sequences rapidly decrease in size and eventually are lost from the preparation. When *Chlamydomonas* genomic DNA is digested with Bal-31 for increased lengths of time (Fig. 1,

lanes 2–5), most of the DNA fragments that hybridize to pAtT4 get smaller and eventually disappear, indicating that they are telomeric. At least some of these hybridizing fragments appear heterodisperse, a feature common to the telomeres of many, but not all, organisms (for review, see ref. 1). As a control, a molecular probe that maps near the centromere on linkage group III was shown to be insensitive to Bal-31 digestion (Fig. 1C).

Cloning *Chlamydomonas* Telomeres in *E. coli*. We used clone pAtT4 as a probe to identify putative clones of *Ch. reinhardtii* telomeres. Because telomere DNA is rarely represented in conventional DNA libraries, a primary genomic-end-library of *Ch. reinhardtii* was prepared (17). Genomic DNA was treated briefly with Bal-31 to generate blunt ends at the chromosomal termini and to remove DNA distal to any nicks or gaps (see *Materials and Methods*). In the initial cloning scheme, two independent clones were isolated, their inserts were subcloned, and both strands were sequenced. The most obvious feature of both clones was the presence of a long tract (272 bp in pCT17 and 320 bp in pCT20) of simple repetitive sequence (Fig. 2A). The repeats in both clones were composed of the simple repetitive sequence (TTT-TAGGG)$_n$. Subclone pCT17 (Fig. 2A) contained a tract of (TTTTAGGG)$_{34}$ sequence adjacent to the blunt-end cloning site. The orientation of the repeats was consistent with the TTTTAGGG strand forming the 3' end at this site.

In a number of organisms, telomere repeats and TAS repeats are also found at centromeric or pericentric regions (10, 35; E. Richards, personal communication). We wanted to determine whether the Bal-31-resistant restriction fragments that hybridize to pCT17 (Fig. 1B) are due to hybridization to telomere repeats or to TASs. To determine the location of the simple repeats, we used a synthetic oligonucleotide, CT1 [5'-(AAAACCCT)$_3$-3'], to probe a blot of Bal-31-digested genomic DNA. Oligonucleotide CT1 hybridized to a number of *Ch. reinhardtii* bands, many of which were heterodisperse and all of which were sensitive to Bal-31 digestion (Fig. 2B). Therefore, we conclude that most or all of the detectable (TTTTAGGG)$_n$ sequences in the *Ch. reinhardtii* genome are located at, or very near, the physical ends of the chromosomes and thus can be considered to be telomere repeat sequences. This telomere repeat is 1 bp longer than the *A. thaliana* telomere repeat and is only 37.5% G+C.

FIG. 2. Bal-31-sensitivity of *Ch. reinhardtii* telomere repeats and telomere-associated sequences. (A) Primary DNA sequence of the telomere repeats in pCT17. Dideoxynucleotide chain-termination sequencing reactions were performed as described. (B–D) *Ch. reinhardtii* genomic DNA was incubated with 1.2 units of Bal-31 at 30°C for 0, 1, 3, and 10 min (lanes 1–5, respectively) as described for Fig. 1. DNA was subsequently digested with *Pst* I, size-fractionated by agarose gel electrophoresis, and transferred to a Nytran membrane. The same blot was probed with oligonucleotide CT1 [(AAAACC-CT)$_3$] (B), pCT17TAS (265 bp of TAS only) (C), or pMN24 (the *Ch. reinhardtii nit1* gene) (D).

Cloning of a *Ch. reinhardtii* Telomere-Associated Sequence. We subcloned the region of pCT17 adjacent to the TTT-TAGGG repeats (pCT17TAS) and determined whether genomic sequences that hybridize to this presumed TAS subclone were sensitive to nuclease Bal-31 digestion. The results in Fig. 2C demonstrate that pCT17TAS hybridizes to a number of *Pst* I restriction fragments in the *Ch. reinhardtii* genome. Some of these fragments are heterodisperse and very sensitive to Bal-31 digestion, disappearing within 3 min of incubation with Bal-31 (Fig. 2C). Two hybridizing fragments appear Bal-31-resistant: they persist after 10 min of Bal-31 digestion. Furthermore, these Bal-31-resistant bands appear less heterodisperse than the Bal-31-sensitive bands. Therefore, two fragments that hybridize to pCT17TAS are resistant to Bal-31 digestion and are presumably found at internal chromosomal loci. However, in preliminary experiments with more extensive Bal-31 digestion times, these TAS-hybridizing fragments appear more sensitive to Bal-31 digestion than are the pcf6-100-hybridizing fragments (M.E.P., unpublished results). In human cells, TASs have also been shown to cross-hybridize to both Bal-31-resistant genomic DNA fragments and to Bal-31-sensitive fragments (37). Because clone pCT17 contains telomere repeats adjacent to the TAS sequence, we conclude that clone pCT17 was isolated from a chromosomal terminus.

Four additional telomeric clones were isolated from a secondary end-library by their hybridization to oligonucleotide CT1. Two of the isolated clones had identical sequences for their entire length, including the number of TTTTAGGG repeats, suggesting that they represent duplicate isolates of the same cloned fragment, rather than independent telomere clones. We treated these two isolates as one clone in our subsequent analyses. The five independently isolated clones contained telomere repeat tracts of 211–320 bp. This length represents a minimum size for the *Ch. reinhardtii* telomere sequence because the genomic DNA was treated with Bal-31 before cloning. Cloned terminal restriction fragments obtained by Bal-31 digestion can be ≈50–500 bp shorter than the genomic restriction fragment (2). Restriction analysis of *Ch.*

FIG. 1. *Ch. reinhardtii* genomic DNA that hybridizes to *A. thaliana* telomere sequence is sensitive to nuclease Bal-31 digestion. *Ch. reinhardtii* genomic DNA was digested with 1.2 units of Bal-31 at 30°C for 0, 1, 3, 7, and 10 min (lanes 1–5, respectively), as described. The DNA was then digested with *Bam*HI, size-fractionated by agarose gel electrophoresis, and transferred to a Biotrans membrane. The same blot was probed with the *A. thaliana* telomere sequence from pAtT4 (A), clone pCT17 [34 telomere repeats plus 271 base pairs (bp) of TAS] (B), or a centromere-proximal *Ch. reinhardtii* probe pcf6-100 (C).

Genetics: Petracek *et al.*

Proc. Natl. Acad. Sci. USA 87 (1990)   8225

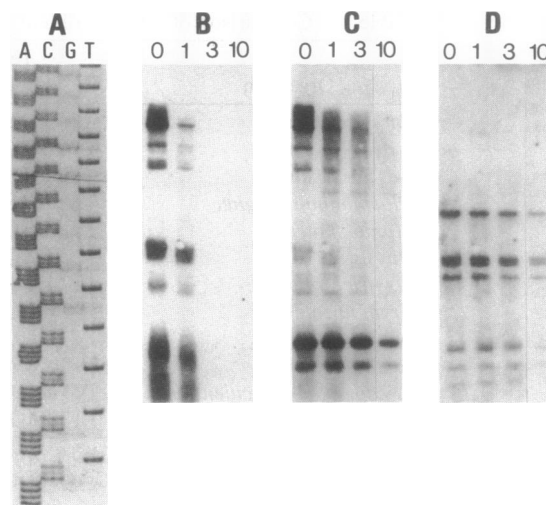*reinhardtii* genomic DNA suggests that the genomic telomere repeats are at least 300 bp in length (M.E.P., unpublished results).

Sequence Analysis of *Chlamydomonas* Telomere Clones. The *Chlamydomonas* telomere repeat is composed of regular, simple primary-sequence repeats. Of 1280 bp of cloned telomere repeat (160 repeat units) in five independent clones, we found only one aberrant repeat of the form TTT-TAAGGG. This insertion, of one extra adenine residue to the repeat, may have been present in the original *Chlamydomonas* DNA or it may have arisen during replication of the plasmid in *E. coli*.

In three of the independent clones, the DNA flanking the telomere repeats (and oriented to the 5' side of the G-strand) has a high degree of sequence similarity. When these sequences are aligned at the proximal end of the telomere repeats, the three clones are identical at ≥78% of the residues (Fig. 3). The sequence similarities within the distal TAS region appear organized into a number of regions. At the TAS–telomere repeat border are one or two aberrant telomere repeats of the form GTTTAGGG, GTTTAGGGTTTA, or TTAGGATTTTT. Imperfect repeats have also been found at the TAS–telomere repeat border of human telomeres (37). Proximal to these repeats is a 25- to 30-bp region of related repeats reminiscent of, but not identical to, the complementary 5'-CCCTAAAA strand. Approximately 35 bp proximal to these C-strand repeats is a 15-bp conserved sequence that includes a 10-bp inverted repeat: TGACATGTCA. The remainder of the TAS region retains high levels of sequence similarity between the three analyzed clones and continues to be 38% G+C, much lower than the average G+C content of the *Ch. reinhardtii* genome (64%) (5). Because we have only analyzed the distal most 97–274 bp of these TAS sequences, there could be additional long-range repeats in the TAS region.

DISCUSSION

We have isolated and sequenced a number of genomic clones that derive from *Ch. reinhardtii* telomeres. They share the properties seen for telomeric DNA from other organisms: they are sensitive to Bal-31 digestion, they are composed of simple, invariant repeats similar to other known telomere repeats, they are oriented with the G–T strand toward the 3' end of the chromosome, and they can be cloned by brief Bal-31 treatment of intact genomic DNA before ligation to a blunt cloning site. The *Ch. reinhardtii* telomeres have an average length of 300–350 bp, similar to the average telomere lengths seen for *Sa. cerevisiae*. From the heterodisperse appearance of the fragments that hybridize to CT1 we infer that the number of telomere repeats at individual *Chlamydomonas* chromosome ends varies. This is consistent with the dynamic nature of telomere length documented in other organisms (for review, see ref. 2). Like the human telomere repeats (10), the *Ch. reinhardtii* telomere repeats are found primarily at the chromosome ends.

The *Ch. reinhardtii* telomere repeat TTTTAGGG is similar but not identical to the *A. thaliana* telomere repeat: it includes a single additional thymine residue. This 8-bp repeat is also reminiscent of the hypotrichous ciliate telomere repeat TTTTGGGG: the adenine in *Ch. reinhardtii* is a guanine in *Oxytricha* and related species.

The first telomere repeats characterized were usually G+C rich (1, 64). However, many telomere repeats are ≤50% G+C (Table 1). Because many of the diverse telomere repeats can be recognized as telomeres in yeast, a high G+C content clearly is not essential for telomere function. Interestingly, many organisms appear to have an inverse relationship between their genomic G+C content and the G+C content of their telomere repeat motif (Table 1).

Studies of telomere structure have focused on the G-rich *Tetrahymena* telomere sequence. A number of independent groups have proposed that telomeric G-strand 3' ends assume specific folded structures based upon G·G base-pairing and stacking interactions (33). These G-quartet (65) or guanine-tetrad (66) structures appear to require a number of adjacent guanine residues. The only feature that all of the known telomere sequences share is that the 3' end strand includes multiple adjacent guanine residues (≥3 at frequent intervals) and few, if any, cytosine residues (Table 1). The presence of at least 3 guanine residues at frequent intervals may be required for telomere function, consistent with the G-strand folding models proposed for *Oxytricha* and *Tetrahymena* telomeric G-strand structure (65, 66). In preliminary studies, we find that a G-strand *Ch. reinhardtii* telomere repeat (TTTTAGGG)$_4$ migrates with an altered mobility in nondenaturing acrylamide gels but does not appear to form the same types of mobility shifts as those seen with *Oxytricha* and *Tetrahymena* telomere-repeat oligonucleotides (33, 65, M.E.P., unpublished data). Because the *Chlamydomonas* telomere repeat contains fewer potential G·G bp and longer intervening A+T loops than the other studied telomere repeats, further analysis of its structure should reveal specific features of telomere structure that are important for its function.

The cloned TASs from *Sc. pombe* (19) and humans (29, 37) are members of a moderately repetitive class of DNA present on some, but not all, of the chromosome arms. In *Sa. cerevisiae*, the Y' class of TASs are present on some, but not all, of the chromosome ends in up to four tandem repeats (40, 67), and the TAS strand contiguous with the G-strand telomere repeats contains many guanine residues and very few cytosine residues. In *Sc. pombe*, the TAS strand contiguous with the G-strand telomere repeat also contains four times more guanine residues than cytosine residues. Three of our *Chlamydomonas* telomere clones include sequences that are related to one another. Pulse-field separation of *Chlamydomonas* chromosomes suggests that the pCT17TAS is present on a subset (<50%) of the chromosomes in the *Chlamydomonas* genome (J.B., unpublished data). The pCT17 class of TASs

pCT17 tatgctaattatacggtctatacaatttctgacatgtcacacatttcaaacctttcaaaaccccctcaaaacccccttagggttttagggtttt(agggtttt)$_{34}$
RxG33 ||||||t||atagittcg||c|tc|||||||||||||||||ac|t|||||||a|c|g|||||||||||tc||||||||*|||t||||||||||||(||||||||)$_{34}$
RxG1 ||||||at|tat|||gt|c|a|||||||||||||||||||ca|||c||||c||c||||||||||||a|||||||||a*******|||||||*|||(||||||||)$_{26}$

consensus
sequence tatgctaattatgctgtgta-atcatttctgacatgtcacacttttcaaaccttgcaaaacccctaaaacccccT-tagg-ttttagggtttt(agggtttt)$_n$

FIG. 3. *Chlamydomonas* telomeres and telomere-associated sequences are highly conserved in three independent clones. The conserved telomere repeats and adjacent TASs of three independent clones are shown. The physical end of the chromosome is to the far right. |, Nucleotide is identical to the above nucleotide; boldface letters, nucleotides different from the other two clones; ★, missing nucleotide. In the consensus sequence, a - indicates no conservation of sequence at that position. The diagram at top illustrates the different features of the TAS and telomere repeats. Stippled area, conserved TAS; arrows, 10-bp palindrome; wavy vertical lines, sequence reminiscent of the C-strand telomere repeat; wavy horizontal lines, G-strand repeats that diverge slightly from the telomere-repeat motif. Straight horizontal lines represent the perfect telomere repeats.

maintains the A+T-rich character of the *Chlamydomonas* telomere repeat (38% G+C), but there is no apparent strand bias of guanine vs. cytosine residues in the TAS region. The pCT17 class of TASs needs further investigation, and additional, unrelated *Ch. reinhardtii* TAS sequences should be isolated to study their organization and distribution. In addition, the TASs and specific DNA sequences that we find adjacent to them will be useful tools for the *Ch. reinhardtii* restriction fragment length polymorphism mapping effort (53). With the recent development of efficient transformation procedures for *Chlamydomonas*, it should be possible to analyze telomere function *in vivo*.

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