Identification of a telomere-binding activity from yeast

(DNA binding protein/poly(CA) runs/agarose gel binding assay)

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Communicated by Leon A. Heppel, January 31, 1986

ABSTRACT In yeast, the ends of the chromosomes (telomeres) terminate in repeated poly(C13 A) sequences. We have identified a yeast activity that binds specifically to these poly(C13 A) repeats. An agarose gel binding assay was used to detect and characterize this activity in cell extracts using both cloned telomere DNA and yeast genomic DNA as substrates. The activity appears to bind specifically to poly(C13 A) sequences, despite their different primary sequences, yet does not bind specifically to telomeric repeats, such as poly(CA3 A2), poly(CA3 A4), and poly (C13 A-T) from other lower eukaryotes.

Linear chromosomes terminate in specific structures, the telomeres, which must be replicated and stably maintained during the cell division cycle. While the ends of broken chromosomes or naked double-stranded DNA are recombinogenic and susceptible to nuclease degradation (1-4), intact telomeres seem to be protected from these activities. In cytological studies, frequent association of telomeres with one another and with the nuclear membrane have been observed in meiotic and mitotic cells (5, 6), leading to the suggestion that telomeric interactions may be important for the proper pairing and segregation of chromosomes. In addition, telomeric DNA must be replicated by a special mechanism that ensures complete synthesis of both daughter DNA strands. As Watson (7) first noted, if telomeres were replicated by the prototypic semiconservative mechanism requiring RNA primers (8), excision of the 5’-terminal primer would leave one of the daughter DNA strands shorter than its parental strand. Linear viruses overcome this obstacle by either concatenemerization of genomes (7) or by the use of a protein primer at both genomic termini (9, 10). Telomeric DNA probably interacts with specific enzymes and structural proteins to facilitate the replication, maintenance, and proper segregation of chromosomes.

The DNA structure at the ends of eukaryotic chromosomes and linear plasmids has been studied extensively [reviewed by Blackburn and Szostak (11)]. In every case examined, telomeric DNA contains repetitive DNA sequences. Fig. 1 illustrates the general organization of yeast telomeric DNA. Two types of long repeated sequences, Y' and X, are associated with telomeres. Both of these “telomere-associated sequences” contain autonomous replication sequences that are presumed to be replication origins. The Y' repeat is present in one to four tandemly repeated copies on the ends of most, but not all, chromosomes. The X repeat is believed to be present on every yeast telomere (12).

The macronuclear chromosomes of a number of lower eukaryotes terminate in multiple simple repeats, or “telomere sequences,” that are characteristic of the organism. For example, Tetrahymena RNA telomeres terminate in 300–500 base pairs (bp) of tandemly repeated C13 A sequence; hypotrichous ciliates such as Stylonichia and Oxytricha have macronuclear telomeres that terminate in tandem repeats of C13 A (13). Non-nucleosomal telomeric complexes have been identified on the extrachromosomal ribosomal RNA genes of Tetrahymena (14). In Oxytricha, the terminal ∼100 bp of the macronuclear DNAs are involved in a DNA-protein complex (15).

Yeast chromosomes terminate in several hundred base pairs of tandemly repeated units of C13 A. The length of the terminal poly(C13 A) run, or T sequence, of a particular telomere is heterogeneous in a cell population (observed as a heterodisperse DNA band on Southern blots), and this length distribution also varies among different yeast strains (16). Shorter poly(C13 A) runs (25–150 bp) are also located at the junctions between Y' and X repeats and between tandem Y' repeats (Fig. 1) (17, 18).

Linear plasmid DNA containing yeast autonomous replication sequences and Tetrahymena telomere sequence at the ends can replicate autonomously in yeast cells (19). These minichromosomes are maintained as linear molecules and acquire poly(C13 A) tracts distal to the Tetrahymena poly(C13 A) sequence (20). In at least one case (17, 20), the identical poly(C13 A) primary sequence was found within a natural Y' junction sequence and within a terminal sequence acquired on a linear minichromosome, leading to the suggestion that there may be interactions between the terminal and internal poly(C13 A) sequences. Alternatively, the addition of terminal poly(C13 A) runs (termed the addition reaction) may proceed via a type of terminal deoxynucleotidyl transferase activity in a template-independent manner (20, 21).

In this paper we report the identification of an activity in yeast that binds specifically to telomeric poly(C13 A) runs. An agarose gel binding assay was used to detect and characterize this activity in cell extracts. Cloned plasmid DNA, as well as total genomic DNA, were used as substrates in these studies. The activity appears to bind exclusively to poly(C13 A) sequences: it does not bind to telomeric repeats from Tetrahymena, Oxytricha, or Dictostyllum.

MATERIALS AND METHODS

Strains and Plasmids. The Escherichia coli strain HB101 (thr leuB pro hsr hsm recA) was used as the host for plasmid propagation. The Saccharomyces cerevisiae strain S288C (MAT a mll-1) was used as a standard wild type for the genomic Southern blot in Fig. 4. Plasmids YRp120, YRp131S, and YRp131 have been described (12).

Preparation of Yeast Cell Extracts. Yeast cells (S288C unless noted otherwise) were grown to late logarithmic phase in YEPL (yeast extract/peptone/dextrose), harvested by centrifugation, washed once in cold distilled water, resuspended 1:1 (wt/vol) in buffer A [50 mM KCl, 50 mM Hepes (pH 7.5), 5 mM EDTA, 5%(vol/vol) glycerol], frozen in liquid nitrogen, and stored at −70°C. Thawed cells were brought to 2 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol, mixed with an equal weight of acid-washed glass beads (0.45–0.5 mm, B. Braun Instruments), and...
disrupted by Vortex mixing (for small scale extractions) or by mixing in a Bead Beater (Biospec). Disruption was for 1-min periods interspersed with 1-min incubations of the cell lysate on ice, repeated 5—7 times. The cell debris and unlysed cells were removed by centrifugation at 8000 × g for 20 min. The remaining supernatant is referred to as the crude cell extract or fraction 1. Fraction 2 was prepared by precipitation of fraction 1 with 50% saturated ammonium sulfate. The pellet was resuspended in buffer A containing 1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol and dialyzed against the same solution. Fraction 2 was loaded on a DE-52 (Whatman) column, washed with 100 mM KCl, 50 mM Hepes (pH 7.5), 5 mM EDTA, and 5% (vol/vol) glycerol, and eluted with 250 mM KCl in the same buffer. Active fractions were pooled and dialyzed against buffer A to yield fraction 3. Protein determinations were by the method of Bradford (22) using Bio-Rad dye reagent concentrate and the micro assay conditions suggested by the supplier.

**Agarose Gel Binding Assays.** Restriction endonuclease-digested plasmids were end-labeled with an [α-32P]dNTP and reverse transcriptase (Molecular Genetic Resources) as described (23). In some cases, plasmids were subsequently cut with other restriction enzymes. Reactions (20 μl) consisted of 2–10 ng of labeled plasmid DNA, a 100-fold excess (0.2–1.0 μg) of *E. coli* DNA (sonicated to 1-kb average size), 15 mM MgCl₂, 50 mM KCl, 10 mM Hepes (pH 7.5), 12.5% (vol/vol) glycerol, and protein extracts as indicated in figures. For the genomic Southern blot, 2 μg of DNA from strain S288C was used per reaction mixture, and no *E. coli* DNA was added. Reaction mixtures were incubated for 5–15 min at 22°C and then electrophoresed on agarose gels (0.7, 1.0, or 1.5% agarose (Bethesda Research Laboratories), 40 mM Tris-maleate base, 12.5 mM NaOAc, 3.3 mM EDTA, brought to pH 7.9 with HCl) at up to 100 V. Time and length of electrophoresis were varied to optimize visualization of the band of interest. The voltage, amperage, and timing of electrophoresis did not appear to influence the activity observed. Gels were dried and exposed to Kodak XAR-5 film for 5–48 hr with a single intensifying screen.

**DNA Preparation.** Plasmid DNA was amplified in *E. coli* cultured in Luria broth (24) supplemented with chloramphenicol at 150 μg/ml. All DNA preparations (25, 26) were treated with RNase A (Worthington) and small molecular weight molecules were removed by precipitation with polyethylene glycol (27). Total yeast DNA was isolated essentially as described by Cryer et al. (28).

**Construction of pCA120-390 and Its Deletion Derivatives.** Plasmid p120-390 was constructed by inserting the 390-bp *Spel* 1/His1 fragment from plasmid YRP120 into plasmid pBR322 cut with Spel and PvuII. Plasmid pCA120-390 and its deletion derivatives were constructed by first cutting plasmid p120-390 with Spel I and then removing the 3′ protruding ends with the Klenow fragment of *E. coli* DNA polymerase I. To generate deletion derivatives, the plasmid was then treated with exonuclease III and S1 nuclease. EcoRI linkers (Collaborative Research, Waltham, MA) were ligated onto the plasmid at the EcoIII/S1 nuclease-treated ends (for deletion derivatives) or at the blunted Spel I site (for pCA120-390). The ligation mixtures were cut with EcoRI, religated with T4 ligase, and then cut with Hinfl (to linearize any religated p120-390). The sizes of pCA120-390 and the deletion derivatives (see Fig. 3) were determined by electrophoresis of end-labeled fragments next to size standards and sequence ladders on sequencing gels.

**Other Procedures.** Restriction enzymes, T4 ligase, *E. coli* DNA polymerase I, exonuclease III, S1 nuclease, and DNase I were purchased from New England Biolabs and Bethesda Research Laboratories and were used according to suppliers’ instructions.

*E. coli* transformation was carried out as described (29). Preparation of nick-translated 32P-labeled DNA probes was as described (30). Poly(d(GT));poly(d(CA)) (Boehringer Mannheim) was nick-translated under the same conditions but DNase I was omitted from the reactions. Treatment of agarose gels for the transfer of DNA to Bioyte (Pall Ultrasafine Filtration) was as recommended by the supplier. The conditions for DNA hybridization were as described (31).

**RESULTS**

**Agarose Gel Binding Assays.** An agarose gel binding assay was used for the detection of telomere-specific binding activities in yeast cell extracts. Initially, substrates for specific binding were prepared from plasmids containing large fragments of cloned telomere sequences (X or Y′). These plasmids were cut with a restriction enzyme, end-labeled with a single [32P]dNTP using reverse transcriptase, and then, in some cases, cut with additional restriction endonucleases. The resulting substrate contained approximately stoichiometric amounts of at least two end-labeled DNA fragments: one containing the telomere sequence specifically bound by a protein in the extract and the other(s) containing vector DNA sequences that provide an internal, negative control for nonspecific DNA binding activities in the extract. Excess unlabeled DNA was also added to minimize the detection of nonspecific DNA binding activities in the crude extracts. The fragments were then separated on horizontal agarose gels and visualized by autoradiography. DNA binding activity was detected as a decrease in the electrophoretic mobility of a specific DNA fragment when increasing amounts of cell extract were added.

Using the agarose gel assay, we could demonstrate the specific and preferential binding of telomeric X- and Y′-containing DNA fragments by yeast cell extracts. Plasmids YRP120 and YRP131S were used as substrates for the binding assay (Fig. 2). In each case, the mobility of the fragment containing the poly(C₃₋₃A) sequence was retarded in agarose gels after incubation with crude cell extract. New bands, presumably corresponding to DNA-protein complexes appeared as increasing amounts of cell extract were added. Experiments using unlabeled plasmids containing cloned telomeric DNA indicated that plasmid YRP131S, which includes 62 bp of poly(C₃₋₃A), could compete for the binding of a cell extract component to the telomeric X fragment from plasmid YRP120 (Fig. 2d). Plasmid YRP131, which includes only 25 bp of poly(C₃₋₃A), could also compete for binding to plasmid YRP120 (data not shown).

The binding activity appears to be due to one or more proteins. Activity is lost upon treatment of the extract with proteinase K, 0.1% NaDodSO₄, phenol, or high temperatures (data not shown).

**The Protein Binds Specifically to poly(C₃₋₃A) Sequences.** The 390-bp *Spel* 1/His1 fragment from plasmid YRP120 contains the telomeric sequence specifically bound by protein(s) in the extract. This fragment contains a portion of a telomeric X-Y′ junction. This *Spel* 1/His1 fragment was subcloned in plasmid p120-390. Deletions from the *Spel* I site were generated with exonuclease III and S1 nuclease and...
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Fig. 2. Gel-binding assays. (A) Binding to telomeric X sequence (YRp120). Binding reactions mixtures including 8 ng of labeled YRp120 plasmid DNA (see C) and 1 µg of unlabeled E. coli DNA were electrophoresed on a 1% agarose gel. Lane 1, no extract added. Fraction of 1 cell extract was added to the reactions in lanes 2-5 as follows: lane 2, 0.1 µg; lane 3, 3.0 µg; lane 4, 3 µg; lane 5, 8.0 µg. A-E refer to the fragments indicated in C. Arrows indicate bands that presumably represent DNA-protein complexes.

(B) Binding to telomeric Y' sequence (YRp131S). Binding reaction mixtures including 10 ng of labeled YRp131S plasmid DNA (see C) and 1 µg of unlabeled E. coli DNA were electrophoresed on a 1.5% agarose gel. Lane 1, no extract added. Fraction 3 extract was added to the reactions in lanes 2-4: lane 2, 0.52 µg; lane 3, 1.3 µg; lane 4, 2.6 µg. A and B refer to the fragments indicated in C. Arrows indicate bands that presumably represent DNA-protein complexes. (C) Plasmids.

Plasmid YRp120 (12) contains a 4-kb Sal I fragment of X sequence inserted into plasmid pSZ57 (44). It was digested with Nco I, end-labeled with [α-32P]dATP, and cut with Hpa I to yield six labeled DNA fragments: a 4.4-kb fragment, A; a 3.2-kb fragment, B; a 1.7-kb fragment, C, containing the autonomous replication sequence 120; a 0.65-kb fragment, D, containing the 139-bp poly(C13A) sequence; and a 0.15-kb fragment, E. Plasmid YRp131S (12) contains a 5.2-kb Sal I fragment of Y' sequence inserted into plasmid pSZ57. It was digested with Xho I, end-labeled with [α-32P]dATP, and cut with Sph I to yield two major labeled fragments: a 1.7-kb fragment, A, and an 0.8-kb fragment, B, that contains the Y'-Y' junction region including the autonomous replication sequence 131 and 62 bp of poly(C13A) sequence. (D) Competition for binding. The substrate in all binding reactions was plasmid pCA120-390 (see Fig. 3) digested with EcoRI, end-labeled with [α-32P]dATP, and cut with Fok I to yield two labeled bands, the 380-bp fragment B from pBR322 and the 181-bp fragment B from YRp120 including 139 bp of poly(C13A) sequence. Each reaction contained 3.3 ng of pCA120-390, 300 ng of unlabeled E. coli DNA, and 0.65 µg of fraction 3 extract. In lane 1, no additional DNA was added. Additional, unlabeled plasmid DNA was added in lanes 2-10. Lanes 2-4, pSZ57 (vector); lanes 5-7, YRp131S; lanes 8-10, YRp120. A 5-fold excess (16.5 ng) of the unlabeled plasmid DNA was added in lanes 2, 5, and 8; a 10-fold excess (33 ng) of competing DNA was added in lanes 3, 6, and 9; and a 15-fold excess (49.5 ng) of competing DNA was added in lanes 4, 7, and 10. Mixtures were electrophoresed on 1.5% agarose gels. Arrows indicate bands that are presumed to represent DNA-protein complexes.

Fig. 3. Deletion analysis of the binding substrate. Plasmid pCA120-390 and its deletion derivatives pCA120-Δ50, -Δ80, and -Δ150 were digested with EcoRI, end-labeled with [α-32P]dATP, and cut with Fok I to yield two labeled fragments, the larger fragment A contains 580 bp of pBR322 DNA and the smaller insert fragments B-E of approximately 180, 130, 100, and 30 bp, containing 139, 109, 79, and 7 bp of poly(C13A) sequence, respectively, for each of the above plasmids. All samples contain 6 ng of labeled plasmid DNA and 600 ng of unlabeled E. coli DNA. In each experiment, lane 1 represents the reaction mixture without added extract. Fraction 3 extract was added to the reactions in lanes 2-4. Lane 2, 0.32 µg; lane 3, 0.65 µg; and lane 4, 3.25 µg. The mixtures were electrophoresed on a 1.5% agarose gel.

linkers were inserted at the deletion endpoints. Plasmid pCA120-390 contains the intact 390-bp fragment. The 139-bp poly(C13A) sequence begins 20 bp from the EcoRI site and terminates 22 bp before the first Fok I site (Fig. 3). The 181-bp EcoRI/Fok I fragment (Fig. 3, band B) is required for specific binding by the extract. At least three different discrete bands, representing different DNA-protein complexes, are seen in this gel (Fig. 3).

Although it is conserved in a few different telomere clones, the 20-bp sequence on the 5' side of the poly(C13A) tract is not required for the specific binding observed: the deletion of 50 bp (pCA120-Δ50) and 80 bp (pCA120-Δ80) from the Sph I site does not abolish binding to the remaining EcoRI/Fok I fragment (Fig. 3). These fragments contain approximately 110 and 80 bp of poly(C13A), respectively. When the fragment contains 7 bp of poly(C13A) sequence (pCA120-Δ150), no specific binding is observed (Fig. 3). Also, there is no specific binding to an EcoRI/Acc I fragment from pCA120-Δ190 in which the entire poly(C13A) sequence has been deleted (data not shown).

Binding to Intact Telomeres. There is some evidence suggesting that telomeric DNA from a number of lower eukaryotes contains unusual structures such as single-stranded gaps and foldback termini (19, 32). While there is no evidence that conclusively supports or refutes the existence of such structures within yeast telomeric DNA, we wanted to know whether the yeast protein(s) that bind poly(C13A)
tracts in cloned telomeric junction fragments would also bind to the genomic T sequences (see Fig. 1). Genomic yeast DNA was digested with Xho I and incubated with various amounts of fraction 3 extract. The mixtures were then electrophoresed on agarose gels. The DNA was transferred to Biodyne filters and hybridized with 32P-labeled poly[d(GT)]•poly[d(CA)] (Fig. 4). The poly[d(GT)]•poly[d(CA)] probe enables visualization of the 1.3- to 1.5-kb Y' Y' terminal fragment present at the ends of most chromosomes (12, 33). This fragment usually appears as a heterodisperse band (19), presumably due to the variable lengths of the terminal poly(C13A) runs (16). The mobility of this heterodisperse terminal fragment decreases upon the addition of increasing amounts of extract (Fig. 4). A number of other genomic DNA fragments (e.g., the 6.7-kb Y' Y' Y' Y' Y' Y' lacZ/A3 A3 A3 A3 A3 A3 A3 fragment) that hybridize to poly[d(GT)]•poly[d(CA)] are also bound by the activity in the extract. Yet the mobility of some poly[d(GT)]•poly[d(CA)]-hybridizing fragments remains unaffected upon incubation with the extract. These unbound DNA fragments probably contain the repeating poly(CA) sequences found at nontelomeric loci (17, 33). As a control, the mobility of a fragment containing the A3 A3 A3 A3 A3 A3 A3 A3 gene was also examined and found to be unaltered by the addition of extract to the reactions (data not shown).

Other Telomeric Sequences. Since repetitive telomeric sequences from a number of organisms can be used as substrates for the addition reaction in yeast cells, we wanted to know whether these sequences could also be bound by the telomere-binding protein(s) in the yeast cell extracts. Plasmids containing telomere sequences from the macronuclear DNA of Tetrahymena [poly(C13A2)] and Oxytricha [poly(C13A2)], as well as telomere sequences from the extrachromosomal rDNA of Dicyostelium [poly(C13A2)], were tested. We did not detect any specific binding of proteins to these repeats (Fig. 5) even when as much as 10 μg of fraction 3 extract was used (data not shown). In addition, the synthetic oligonucleotide poly[d(GT)]•poly[d(CA)] that hybridizes to poly(C13A) runs (33) was not a specific substrate in filter binding assays nor could it compete for binding to poly(C13A) sequences in agarose gel assays (data not shown).

**DISCUSSION**

We have shown that yeast extracts contain a protein(s) that specifically binds to telomeric DNA. An agarose gel assay was used to detect the binding activity and to demonstrate binding specificity for DNA substrates containing poly(C13A) sequence. Similar assays have been used to study the binding of purified proteins to small DNA fragments (34–40).

**FIG. 4.** The protein binds to genomic termini. Yeast genomic DNA (2 μg per reaction) was digested with Xho I and used in binding reactions with fraction 3 extract before electrophoresis on an agarose gel (0.7%). DNA in the gel was denatured, transferred to biodyne membrane, and hybridized to nick-translated poly[d(GT)•poly[d(CA)]]. Lane 1, no extract; lane 2, 0.16 μg; lane 3, 0.32 μg; lane 4, 0.65 μg; and lane 5, 1.6 μg of extract. Bracket denotes the 1.3-kb heterodisperse Y'-terminal Xho I fragment. The long arrow points to the 6.7-kb Y'-Y' internal fragment. The short arrow points to a band that does not display a shifted mobility upon the addition of extract.

**FIG. 5.** Binding to other telomeric DNA sequences. Plasmid pDel5 (obtained from A. Murray and J. Szostak) contains 300 bp of C13A2 repeats from Tetrahymena thermophila rDNA telomeres inserted as a Xho I/BamHI fragment in plasmid pBR322 between the Cla I and BamHI sites. Plasmid pDel5 was digested with BamHI, end-labeled with [α-32P]dATP, and cut with Xho I to yield two labeled fragments. Fragment A, pBR322 (4 kb) and fragment B, the C13A2 insert (300 bp). Plasmid pLOT1A-4 (obtained from D. Gottschling) contains 11 or 12 repeats of C13A from Oxytricha nova macronuclear telomeres inserted into the Sma I site of plasmid pUC19. Plasmid pLOT1A-4 was digested with EcoRI and HindIII and end-labeled with [α-32P]dATP to yield two labeled bands. Fragment C, a 2.7-kb vector sequence and fragment D, a 0.14-kb insert containing about 90 bp of tandemly repeated C13A. Plasmid pCT14 was obtained from Emery and Weiner (45). It contains an approximately 1-kb EcoRI/BamHI fragment that includes 135 bp of C13A-T satellite sequence from the terminus of Dicyostelium discoideum DNA inserted into the EcoRI and BamHI sites of plasmid pBR322. Plasmid pCT15 was digested with BamHI, end-labeled with [α-32P]dATP, and cut with EcoRI to yield two labeled bands. Fragment E, pBR322 (~4 kb) and fragment F, a 1-kb telomere fragment from D. discoideum including the tandemly repeating C13A-T sequence. For plasmids pDel5 and pLOT1A-4, 2 ng of labeled plasmid and 200 ng of E. coli DNA were used per reaction. For plasmid pCT15, 2.5 ng of labeled plasmid DNA and 250 ng of unlabeled E. coli DNA were used per reaction. In each experiment, lane 1 contained no extract. In lane 2, 0.65 μg and in lane 3, 1.3 μg of fraction 3 extract were incubated with the DNA. Plasmid pDel5 was electrophoresed on a 1.0% agarose gel, the pLOT1A-4 and pCT15 plasmids were electrophoresed on a 1.5% agarose gel.

Strauss and Varshavsky (41) showed that the polycarylamide gel system could be used as an assay to detect and purify a DNA-binding protein from crude nuclear extracts. Here, the use of agarose gels for the binding studies permitted us to screen DNA fragments as large as 7 kb for sequences that act as specific binding substrates. The binding sequence could then be carefully delimited by restriction enzyme and deletion analysis.

The poly(C13A) binding protein forms complexes specifically with DNA fragments that include poly(C13A) tracts. We have not yet determined the minimum size of a poly(C13A) tract required for the specific binding, but it appears to be between 7 and 25 bp. We do know that plasmid YRP131, which contains a 25-bp tract of poly(C13A) repeats (C. Chan, personal communication) can compete efficiently for specific binding, while neither the vector sequence (pS257) nor plasmid pCA120-D190, which lacks poly(C13A) sequence, can compete for poly(C13A) binding. However, our experiments do not rule out the possible contribution of the 3'-flanking sequence (which is different in each of the telomeric clones) to the binding of poly(C13A) sequences. Further deletion analyses as well as nuclease-protection experiments should clarify these points.

A number of discrete bands (presumed DNA–protein complexes) are observed when extract is added to an appropriate substrate. The deletion studies show that the number of complexes is somewhat proportional to the length of poly(C13A) sequence present on the substrate. Furthermore,
plasmids that have shorter poly(C1.3-A) runs (YRp131, YRp131S, and pCA120-Δ30), do not compete for binding as efficiently as does pCA120-389. This, in conjunction with substrate mixing studies (J.B., unpublished results), suggests that the bands represent complexes with different amounts of protein bound to a single substrate molecule.

The actual structure of the poly(C1.3-A) sequences at the chromosomal termini has not been determined. The genomic DNA binding experiment in Fig. 4 shows that the terminal Y’ fragment as well as other fragments, including the 6.7-kb internal Y’-Y’ junction fragment, are bound by the extract. The mobility of other fragments remains unchanged in the presence of the extract. These are presumably the nontelomeric poly(CA) repeats (17). The fact that the protein can bind to genomic T sequences as well as cloned poly(C1.3-A) sequences, suggests that the two sequences are structurally and that the binding is not specific to structures unique to telomeres such as hairpins and single-stranded gaps proposed for *Tetrahymena* telomeres (42).

Since the primary sequences of poly(C1.3-A) runs differ from one another, perhaps runs of poly(C1.3-A) exhibit a unique conformation that is recognized by the protein(s).

No specific binding of the extract to the simple telomere repeats from *Tetrahymena* and *Oxytricha* was observed, despite the fact that these runs can function as substrates for the addition reaction in *vivo*. Furthermore, the protein(s) did not bind specifically to poly(C1.8-T) or to poly(CA). Therefore, the protein(s) binds to poly(C1.3-A) sequences does not bind specifically to CA-type repeats or to telomeric sequences from other organisms. It is possible that the binding affinity of the protein for these substrates is much lower than the binding affinity for its natural poly(C1.3-A) substrate and, therefore, that its binding to these substrates is not visualized in the agarose gel assay.

Greider and Blackburn (21) reported the identification of a terminal transferase-type activity in *Tetrahymena* crude extracts. This activity is capable of adding repeats of G4T2 to telomere sequences from yeast or *Tetrahymena*. This species-nonspecificity of the *Tetrahymena* addition activity for telomeric primers is in sharp contrast to the specificity of the yeast poly(C1.8-A)-binding protein(s). It is possible that the poly(C1.3-A)-binding protein is not directly involved in the addition reaction but plays a structural role in the telomeric function. The protein could serve to protect telomeres from nuclease digestion by binding to poly(C1.3-A) sequences, or it could be important as a mediator of inter- and/or intrachromosomal interactions between poly(C1.3-A) runs (17, 43). Alternatively, the poly(C1.3-A)-binding protein could be instrumental in the associations between telomeres and the nuclear membrane. Further experiments will be required to elucidate the function of this DNA binding protein.

We thank Dr. Shlomo Eisenberg for his invaluable advice and Dr. Clarence Chan for providing unpublished data on the nucleotide sequence of poly(C1.3-A) tracts from telomeric DNA. This work was supported in part by Grant MV-233 from the American Cancer Society and in part by Grant GM34190 from the National Institutes of Health. J.B. is a Chain Weizmann Postdoctoral Fellow.