Microinjected *Tetrahymena* rDNA Ends Are Not Recognized as Telomeres in *Xenopus* Eggs

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**Abbreviation used in this paper: TtrDNA, *Tetrahymena* rDNA.

Abstract. Telomeres are essential structures that stabilize the ends of eukaryotic chromosomes and allow complete replication of linear DNA molecules. We examined the structure and replication of telomeres by observing the fate of the linear extrachromosomal rDNA of *Tetrahymena* after injection into unfertilized *Xenopus* eggs. The rDNA replicated efficiently as a linear extrachromosomal molecule, increasing in mass 30-50-fold by 15-20 h after injection. In addition, the molecules increased in length by addition of up to several kilobases of DNA to their termini. Sequence analysis demonstrated that the added DNA bore no resemblance to known telomeres. The junction between the rDNA and added DNA was apparently random, indicating that the addition reaction did not involve a site-specific recombination or integration event. Surprisingly, Southern blot analysis showed that the added DNA did not derive from *Xenopus* DNA, but rather from co-purifying and therefore co-injected *Tetrahymena* DNA. The nonspecific ligation of random DNA fragments to the rDNA termini suggests that microinjected *Tetrahymena* rDNA ends are not recognized as telomeres in *Xenopus* eggs.

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Figure 1. Tetrahymena thermophila rDNA is a 20-kb palindrome. The origins of replication are marked at the center. The large arrows represent coding regions for 17-, 5.8-, and 26-S rRNAs. The molecules are heterogeneous in length due to varying numbers of CaA repeats at the termini. A sequence that functions as a weak arc (autonomously replicating sequence) in yeast is present 200 bp from the CaA repeats.

 efficiently in a semiconservative manner after injection into Xenopus eggs. This replication follows the regulated pattern maintained by Xenopus chromosomal DNA during development (Hara et al., 1980; Newport and Kirschner, 1982). The general ability of injected DNA to replicate in frog eggs has been verified in several later reports (Bendig, 1981; Rusconi and Schaffner, 1981; Hines and Benbow, 1982; Mechali and Kearsley, 1984).

We describe here the efficient replication of TrrDNA after injection into unfertilized Xenopus eggs. During this process, sequences are added to the rDNA termini. We examine here the nature and origin of these newly acquired sequences.

Materials and Methods

DNA

DNA from Tetrahymena thermophila strain B7 was the generous gift of R. Craig Findly (University of Georgia, Athens, GA), Fritz Mueller (Universität Freiburg, Freiburg, Switzerland), or Karen Vavra (Eastman Kodak Co., Rochester, NY). Total Tetrahymena DNA was a gift of R. Craig Findly, and micronuclear-specific Tetrahymena DNA was very kindly provided by Meng-Chao Yao (Washington University, St. Louis, MO). pGY39, a clone containing the terminal HindIII fragment of TrrDNA (Kiss et al., 1981) was a gift from Ron Pearlman (York University, Downsview, Ontario). Bill Taylor (Vanderbilt University, Nashville, TN) provided SP6-TFIIIA clones containing cDNA for Xenopus laevis 5 S RNA transcription factor IIIA. X laevis DNA was prepared from blood cells or liver tissue according to Kavenoff and Zimm (1973). Synthetic oligomers were produced by Michael Sepanski (Carnegie Institution of Washington, Baltimore, MD) on DNA synthesizer (model 380 A; Applied Biosystems, Inc., Foster City, CA).

Microinjections

Unfertilized eggs were obtained as described by Gurdon (1967). Females were injected with 500 IU human chorionic gonadotropin (CG-2; Sigma Chemical Co., St. Louis, MO) several times over a 1-2-d period before oviposition. Eggs were stripped into a dry petri dish and their jelly coats were removed by repeated washes with 2% cysteine (pH 7.8). The dejellied eggs were rinsed several times with modified Barths' saline containing 88 mM NaCl and 10 mM Tris-HCl (pH 7.6), 2.4 mM NaHCO3, and 10 μg/ml each penicillin and streptomycin), and left in modified Barth's saline during and after injection. The eggs were not irradiated with UV light to inactivate the female pronucleus. DNA at a concentration of 60 μg/ml was diluted into a buffer containing 88 mM NaCl and 10 mM Tris-HCl (pH 7.6) to a final concentration of 30 or 10 μg/ml. Approximately 10 μl of this solution was injected into a region near the animal pole of each egg. Eggs were then incubated at room temperature for various times up to 24 h.

Extraction of Injected DNA

Eggs were homogenized briefly in 300 mM NaCl, 100 mM Tris-HCl (pH 7.5) 10 mM EDTA, and 2% SDS. Proteasease K (Boehringer Mannheim Diagnostics, Inc., Houston, TX) was added to a final concentration of 1 mg/ml and the homogenates incubated at 37°C for 2-12 h. Samples were extracted two to three times with 1:1 phenol:chloroform, and four times with ether. Total nucleic acids were precipitated with EtOH. The pellets were rinsed once with 70% EtOH, briefly dried, and resuspended in TE (10 mM Tris-HCl and 1 mM EDTA (pH 8.0)).

Cloning the Added DNA

Xenopus eggs were injected with 250 pg TrrDNA and incubated for 20 h in modified Barths' saline. Total nucleic acids from 202 pooled eggs were isolated as described above, then further purified by CsCl gradient centrifugation. The DNA was lightly digested with BAL-31 under conditions that removed 0-4 bp/end over the 10-min period used (Stefano, J., and C. Berg, unpublished observations). The ends of the molecules were repaired with the Klenow fragment and ligated to 8-bp EcoRI linkers (Boehringer Mannheim Diagnostics, Inc.). The DNA was cleaved with HindIII and EcoRI, ligated into HindIII/EcoRI-cut pBR322, and used to transform Escherichia coli HB101. The library was screened as described by Grunstein and Hogness (1975) using a nick-translated, gel-purified fragment from pGY39 containing the terminal HindIII fragment of TrrDNA (Kiss et al., 1981).

Sequencing

Portions of the added DNA regions from four clones (pTX043, 220, 529, and II7) were sequenced according to Maxam and Gilbert (1980). These four inserts and eleven others were transfected into the single-strand phage M13 mpl (Messing and Vieira, 1982) and sequenced by the dideoxyn method of Sanger et al. (1977). Sequence comparisons were carried out using the Staden or Conrad and Mount programs for sequence analysis (Staden, 1977; Conrad and Mount, 1982).

Restriction Digestion and Southern Blot Analysis

Restriction digests were performed as recommended by the supplier (New England Biolabs, Beverly, MA; Bethesda Research Laboratories, Gaithersburg, MD; or International Biotechnologies Inc., New Haven, CT). Digests of Xenopus DNA were phenol-extracted and EtOH-precipitated before loading on the gel. Fragments were separated on either 0.7 or 1.0% agarose gels and transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, NH) essentially according to Southern (1975), with pretreatment of the gels in 0.24 N HCl before denaturation and neutralization. Hybridization and washing conditions varied depending on the type of probe. The conditions used for nick-translated or SP6 RNA probes were as follows: filters were prehybridized at 42°C for 1-12 h in 50% formamide, 5X SSPE (IX SSPE = 180 mM NaCl, 10 mM Na3PO4 (pH 7.0), 1 mM EDTA), 3X Denhardt's solution (IX Denhardt's solution = 0.02% BSA, 0.02% polyvinylpyrrolidone, and 0.02% Ficoll) 0.1% SDS, 0.1% Na3PO4, and 100 μg/ml each yeast RNA and denatured, sonicated salmon sperm DNA. Hybridizations were at 42°C in the same buffer for 12-16 h, using ~105 cpm/ml probe. All genomic blots also used 10% dextran sulfate in the prehybridization and hybridization buffers. Filters were washed once for 30 min at 42°C in 50% formamide, 5X SSPE, and 0.1% SDS, once for 30 min at 65°C in 1X SSPE and 0.1% SDS, and four times for 15 min at 65°C in 0.1X SSPE and 0.1% SDS. Filters that had been hybridized with RNA probes were also treated with 20 μg/ml pancreatic RNase A in 2X SSC at 37°C for 30 min.

The following conditions were used for oligomer probes: filters were prehybridized in 4X SSPE, 10X Denhardt's solution, and 0.1% SDS overnight at 25°C below the apparent melting temperature. The melting temperature was calculated by assigning 4°C for each G or C and 2°C for each A or T in the oligomer (Suggs et al., 1981). Hybridizations were carried out in 4X SSPE, 5X Denhardt's solution, and 0.1% SDS for 20-24 h at a melting temperature of ~25°C. Washes were three times for 10 min in 4X SSPE, 1X Denhardt's solution, and 0.1% SDS at a melting temperature of ~12°C.

Probes were made in several ways. Nick-translation of gel-purified fragments or whole plasmids was carried out according to Rigby et al. (1977). SP6 RNA probes were made as described by Green et al. (1983). Synthetic oligomers were kinased according to Maxam and Gilbert (1980) and purified on 20% acrylamide, 8-M urea gels. The labeled fragments were excised from the gel, placed in a polyurethane bag with the filter and hybridization buffer, and crushed.
Figure 2. (A) 100 pg of TrtDNA was injected into unfertilized Xenopus eggs. The eggs were incubated at room temperature 0, 2, 6, or 18 h, then total nucleic acids were isolated from pooled eggs. The DNA was left uncut (−), or digested with restriction enzymes (PstI, HindIII, BamHI). The equivalent of one egg’s DNA was loaded into each lane and electrophoresed through a 1% agarose gel, which was then stained with ethidium bromide. The blank lanes were loaded with 100 pg of TrtDNA, too little to see by staining. The band visible in all the egg samples (m) is the 15.8-kb circular mitochondrial DNA of Xenopus, present at 3.8 ng/egg. A single PstI site within the DNA linearizes the molecule. (B) The DNA in the gel shown in A was transferred onto nitrocellulose and probed with a nick-translated fragment containing numerous C4A2 repeats. The terminal rDNA fragments appear as a broad smear extending from the expected size of the terminal fragment to the limit of mobility of DNA in the gel (Fig. 2 B). In addition, discrete bands could be seen within the smear, one of which had the size of a dimer of the terminal fragment (arrow).

The apparent lack of replication between 0 and 6 h suggests that not all the injected DNA replicated. If unfertilized eggs maintain a cell cycle similar to that of fertilized eggs (Newport and Kirschner, 1982), one would expect the rDNA to replicate once by 2 h postinjection and an additional 5–6 times by 6 h postinjection. However, if only a portion of the injected rDNA replicates, the expected exponential replication pattern would be masked at early time points by hybridization to the 100 or 250 pg of injected material. In addition, as the injected material becomes heterogeneous in length, it becomes more diffuse on the gel and therefore more difficult to visualize. An exponential pattern of incorporation is observed when TrtDNA replication is examined after co-injection with radioactive nucleotide precursors (data not shown).

To characterize the structure of the replicated rDNA molecules, we examined total DNA from injected eggs by electron microscopy (data not shown). In addition to the expected mitochondrial DNA circles (16 kb), we saw primarily linear molecules over 40 kb long. No large circles or unusual terminal branching structures were observed. We therefore hypothesized that the ends of the rDNA molecules were being extended in some unknown manner by the linear addition of nucleic acid.

Results

Replication of TrtDNA and Discovery of Terminal Addition

TrtDNA was injected into unfertilized eggs of the toad Xenopus. Approximately 100 or 250 pg of DNA was injected per egg. The DNA was allowed to replicate for varying lengths of time up to 20 h, and then total nucleic acids were isolated from batches of eggs. The recovered DNA was deproteinized with proteinase-K and phenol before examination by restriction digestion, gel electrophoresis, and Southern blot analysis.

By 15–20 h after injection, eggs contained an average of 5 ng of rDNA. This represents a 30–50-fold mass increase. 5 ng of DNA can be readily visualized by ethidium bromide staining on an agarose gel (arrow, Fig. 2 A). Restriction analysis of the replicated DNA showed all expected internal fragments as sharp bands (arrowheads). The terminal fragment, which normally appears slightly diffuse on gels due to varying numbers of C4A2 repeats, could not be seen by ethidium bromide staining. However, hybridization of a C4A2 probe to a Southern blot of injected DNA revealed a broad smear extending from the expected size of the terminal fragment to the limit of mobility of DNA in the gel (Fig. 2 B). In addition, discrete bands could be seen within the smear, one of which had the size of a dimer of the terminal fragment (arrow).
C4A2 Repeats Are Not Extended

During macronuclear development in ciliates, the genomic DNA is fragmented and newly synthesized telomeric sequences are added to the ends of these chromosomal pieces (Roth and Prescott, 1985; Greider and Blackburn, 1985). We considered that such an addition reaction might occur on injection of TtrDNA into *Xenopus* eggs. We used three techniques to test this hypothesis: Southern blot analysis using a C4A2 probe (Southern, 1975), G+C content determination and nearest neighbor analysis of the terminal fragments (Rae, 1973). Using these methods, we found no support for the hypothesis that the rDNA was specifically elongated by the addition of extra C4A2 repeats. Because we later describe the added sequences, these data are not shown.

Cloning the Added DNA

To determine the nature of the elongation reaction, we cloned sequences added to the rDNA termini. Replicated rDNA molecules were lightly digested with BAL-31 to remove any hairpin loop at the termini (Blackburn and Gall, 1978). After addition of EcoRI linkers, the DNA was cut with HindIII and EcoRI and inserted into the vector pBR322. 35 positive clones were selected by hybridization to a probe containing the terminal HindIII fragment of the rDNA. Because there were no EcoRI sites distal to the HindIII site in the rDNA, the cloned fragments extended from the HindIII site in the rDNA to either an EcoRI site in the "added DNA", or to the EcoRI site of the linker. Since all known telomeres consist of simple repeats, we did not expect EcoRI sites in the added DNA.

We examined 15 of the 35 positive clones (Fig. 3). The largest insert was only 2.5 kb, much smaller than the 10- or 12-kb lengths predicted by Southern blot analysis. Since linkers contain a specific sequence of DNA longer than the 6 base pair (bp) EcoRI site, it was possible to demonstrate by sequence analysis that most of the inserts extended from the HindIII site in the rDNA to an EcoRI site in the added DNA. Five of the 15 examined clones contained the linker-specific sequences, and might therefore contain all of the added DNA. Thus, although all the clones contained the junction between the rDNA and the added DNA, most did not possess the true ends of the extended molecules.

Sequence Analysis

We transferred the inserts from the 15 selected clones into M13 for sequence analysis (Fig. 3). Unlike other known telomeric sequences, the added DNA did not contain multiple repeats of a simple C(A/T)/G(T/A) sequence (Blackburn, 1984). The only distinguishing feature of these sequences was a general A+T richness. With the exception of pTX213, whose base composition was 46% A+T, the clones bore added DNAs whose A+T content averaged 65-70%.

We obtained two different pairs of identical clones. We sequenced each member of the first pair, pTX722 and pTX117, on one strand for ~300 nucleotides. They matched each other along this stretch. The second pair, pTX446 and pTX420, matched each other along their entire lengths on both strands. In addition, the junctions between the rDNA and the added DNAs in pTX446 and pTX420 were identical. Both sets of clones most likely resulted from duplication during cloning.

**Figure 3.** Sequence analysis of added DNA. Inserts from 15 selected clones are shown. To the left lies the HindIII site in the rDNA, followed by 400 bp of A+T-rich region, then a thick block of C4A2. Although the clones all have varying numbers of C4A2 repeats, they are depicted here as being identical. The added DNA lies to the right of the C4A2. The sequenced region is shown by a dashed line. At the top of the figure is pGY39, a clone containing the original TtrDNA end. pFX446 has an EcoRI* site at its right end. pTX 213, 529, 722, 1117, and 108 have EcoRI sites consistent with the linker. The other clones have EcoRI sites in the added DNA.

**Figure 4.** rDNA/added DNA junction. Sequence analysis of the rDNA/added DNA junction was complicated by an artifact of dideoxy sequencing, shown in A. The arrow in A shows the junction between the vector and the rDNA in the clone pGY39 (the original TtrDNA end). DNA polymerase falls off the template every sixth base when synthesizing C4A2 (also, E. Blackburn, personal communication). The enzyme appears to fall off at the second A in the repeat. However, the polymerase readily synthesizes G1T2. In B, the arrow points out the junction between the rDNA and added DNA in the clone pTX446. The junction probably occurs after the first T in G1T2. Since the single-strand gaps in the rDNA occur at the first C in C4A2, this junction did not form by intercalation or ligation of added DNA into the rDNA at the single-strand gaps.
We examined the junction between the rDNA and the added sequences in a total of four clones (Fig. 4). The added sequences immediately adjacent to the rDNA were different in all the examined cases (except the pair 420 and 446). In addition, the transition occurred within the CaA₂ repeats at either A or C. This kind of junction suggests that the addition reaction did not involve a site-specific integration or recombination event, nor did it require a particular sequence of added DNA.

Finally, we examined two clones for long repeating units, since numerous species have satellite-like sequences near their termini (Blackburn and Szostak, 1984). In particular, Jamrich et al. (1983) showed by in situ hybridization that 77-bp-long sequences are highly repeated at the telomeres of Xenopus chromosomes. We therefore sequenced the two longest added DNAs, pTX1043 and pTX920, along one strand for ~1400–1500 nucleotides. However, we found no long internal homologies.

**Origin of the Added DNA**

DNA might be added to the TtrDNA termini in Xenopus eggs by one of several mechanisms. These include the rearrangement of the rDNA molecule, homologous or nonhomologous recombination with other DNA species present in the egg, transposition of DNA into the CaA₂ repeats, de novo synthesis using Xenopus DNA as a template, or de novo synthesis without a template (a terminal transferase mechanism). To some extent, these processes can be distinguished by determining the source of the added DNA.

A Xenopus egg contains 3.8 ng of mitochondrial DNA (Dawid, 1966), ~25 pg of extrachromosomal rDNA (Gall, 1968; Brown and Dawid, 1968), and 6 pg of genomic DNA (Thiebaut and Fishberg, 1977). These DNAs could have provided material or a template for elongation of the TtrDNA ends. In addition, the 100–250 pg of injected DNA could have been a source of added DNA, either by rearrangement of the rDNA, or by ligation of contaminating Tetrahymena genomic DNA. We tested these possibilities by Southern blot analysis.

We probed Southern blots of HindIII-cut TtrDNA with several nick-translated pTX clones: 108, 114, 213, 311, 420/446, 529, 722/1117, and 1043 (data not shown). Because all of the pTX clones contained the original end of the rDNA, they hybridized to the terminal fragment. One clone, 108, also hybridized to a 2-kb HindIII fragment containing the 5' flanking sequences and the 5' coding region of the pre-17-S rRNA. The other clones were negative in this test, indicating that the rDNA had not rearranged to produce these terminal extensions.

Uninjected egg DNA was purified as a source of Xenopus mitochondrial DNA. pTX probes (420/446, 722/1117, 920, and 1043) failed to hybridize to this DNA, although the mitochondrial DNA could be easily visualized by ethidium bromide staining (data not shown). Because all of the pTX clones contained the original end of the rDNA, they hybridized to the terminal fragment. One clone, 108, also hybridized to a 2-kb HindIII fragment containing the 5' flanking sequences and the 5' coding region of the pre-17-S rRNA. The other clones were negative in this test, indicating that the rDNA had not rearranged to produce these terminal extensions.

We isolated Xenopus DNA according to Kavenoff and Zimm (1973). Although nick-translated pTX probes hybridized to a series of bands in each of the Xenopus lanes, these same bands were detected when the terminal fragment of the rDNA alone was used as a probe, or, when a kinased, synthetic, 20-nucleotide oligomer of CaA₂ was used (data not shown). Among the numerous bands hybridizing in each lane, no additional bands were distinguished with added DNA probes.

To verify that the added DNA had no homology to Xenopus sequences, we used BAL-31 to delete the rDNA from three clones: pTX446 (one of the pairs), pTX 920, and pTX1043 (the two longest added sequences). These deletions were subcloned into the vectors SP64 and SP65 (Green et al., 1983) to permit production of probes containing only added DNA. These deleted clones are called SP4c7 (446), SP9c1 (920), and SP10c6 (1043).

Using strand-specific RNA probes generated by SP6 polymerase, we detected no hybridization of added DNA sequences to Xenopus DNA (Fig. 5 A). Plasmid standards showed that these probes were sensitive enough to detect in 2–4 d the equivalent of one-tenth of a single copy genomic sequence. An SP6 RNA probe made from the TFI11A gene of Xenopus easily detected in an overnight exposure the few genomic copies of this gene (Fig. 5 B). From these experiments we concluded that Xenopus DNA did not serve as the source for the added sequences found at the telomeres of TtrDNA after replication in frog eggs.

Because the methods for preparing TtrDNA do not allow purification of the linear molecules to homogeneity (Cech and Rio, 1979), we tested the possibility that co-purifying (and therefore co-injected) Tetrahymena genomic DNA had served as a source for the added sequences. We probed Southern blots of total Tetrahymena DNA with SP6 RNAs made from the three rDNA-deleted clones. Because all of the original pTX clones contain the terminal HindIII fragment of the rDNA, they would hybridize extremely well to the very abundant rDNA present in the preparation, as well as to the CaA₂ repeats present throughout the rest of the genome. Therefore, we tested only the three rDNA-deleted clones. Two of the three clones (SP4c7 and SP9c1) hybridized to single copy sequences in the Tetrahymena DNA (Fig. 5 C). Digestion of the plasmid and Tetrahymena DNAs with a series of enzymes verified that the added DNAs corresponded exactly to DNA segments present in the Tetrahymena genome (data not shown).

The third clone (SP10c7) did not hybridize to total Tetrahymena DNA. It was possible that the 10c7 sequence originated from a micronuclear-specific DNA, and was therefore greatly underrepresented in the total Tetrahymena DNA preparation. However, hybridization of the SP10c7 probe to micronuclear-specific DNA again failed to detect any homologies (data not shown). We have not been able to determine the origin of this added DNA.

We tested the remaining added DNA sequences for homology with Xenopus and/or Tetrahymena DNA by using synthetic oligomers (25mers) generated from sequence data for each of the clones. In this way we avoided formation of hybrids between C + A rich sequences present in either of the two genomes and CaA₂ repeats in the clones. Five oligomers (I14, 220, 311, 343, and 1027) hybridized to single copy sequences in the Tetrahymena genome (Fig. 5 D). The remaining oligomers (213, 529, and 722/1117) cross-hybridized with TtrDNA even though previous analysis with full length probes had suggested that these sequences were not derived from the rDNA. Construction of rDNA-deleted subclones would not be necessary to further characterize the origin of these added DNAs.
Discussion

We have observed the efficient replication of TtrDNA after injection into unfertilized Xenopus eggs. This replication is characterized by the elongation of the rDNA termini through addition of heterogeneous fragments of co-purifying and therefore co-injected Tetrahymena genomic DNA. We believe that all of the added DNA sequences are derived by ligation to other DNA molecules present in the eggs. On a molar ratio of ends, co-purifying Tetrahymena genomic DNA is most abundant and therefore appears most frequently in our added DNA clones. This is a potential problem in examining the replication of any linear DNA molecule which cannot be purified to homogeneity.

Terminal Addition Reaction

The mechanism by which extra sequences are added to the rDNA ends is unclear, but the sequencing data suggest a nonspecific ligation reaction. Our reasoning is outlined below.

We examined the junction between the rDNA and the added DNA to determine if a specific splice at the single-strand gaps had occurred (Fig. 4). Within the rDNA telomeres, single nucleotide discontinuities occur at the first C of a C4A2 repeat on average every three repeats (Blackburn and Gall, 1978). However, the rDNA/added DNA junction occurred at either A or C in the C4A2 sequence, indicating that a specific splice at the gap had not occurred.

It is unlikely that homologous recombination between C4A2 repeats in the added DNA and C4A2 repeats in the rDNA produced the terminal elongation. Greater than 90% of all C4A2 sequences present in the macronuclear genome lie at the ends of the chromosomes (Yao and Yao, 1981). Since all C4A2 repeats run 5' to 3' from the telomere inward (Blackburn, 1984), any recombination between C4A2 repeats at the rDNA termini and C4A2 repeats at other macro-
nuclear DNA termini would result only in the exchange of CaA sequences and could not have generated added DNA.

We suggest that the added DNA was ligated to the extreme end of the rDNA. Recent work by Greider and Blackburn (1985) suggests that TrtDNA ends consist of hairpins formed by self-annealing G4T2 repeats. The rDNA/added DNA junction in our clones is consistent with their model for the terminal rDNA structure. However, we cannot rule out the possibility that the ends of the rDNA were degraded before ligation. Since the rDNA molecules normally contain a variable number of CaA repeats, we cannot test this possibility.

**Telomere Recognition**

One feature of this ligation reaction stands out. The addition of random DNA fragments to the ends of TrtDNA in *Xenopus* eggs demonstrates that TrtDNA behaves like other linear DNA molecules, which undergo ligation to form circular or concatemeric molecules shortly after injection (Harland and Laskey, 1980; Bendig, 1981; Rusconi and Schaffner, 1981; Bromley, S. K. Vavra, and C. Berg, unpublished observations). We suggest therefore that under these conditions, TrtDNA ends are specifically not recognized as telomeres in *Xenopus* eggs. Our observations contrast with those made by Szostak and Blackburn (1982), who described the ability of TrtDNA ends to provide at least some telomere functions in a heterologous system by stabilizing linear plasmids in yeast.

There are several reasons that TrtDNA ends might not function as telomeres in *Xenopus* eggs. It is possible that the injected rDNA molecules are in some way damaged and therefore cannot be recognized as telomeres by the cellular repair machinery (McClintock, 1941; Muller and Herskowitz, 1975). This seems unlikely, since the procedures used to isolate TrtDNA for *Xenopus* egg injections are essentially the same as those used to prepare functional TrtDNA ends for yeast transformations or *Tetrahymena* microinjections (Szostak and Blackburn, 1982; Tongrav and Yao, 1986).

A second possibility is that telomere-specific proteins in the *Xenopus* egg fail to bind rapidly enough to the TrtDNA ends to prevent ligation. Alternatively, these putative telomere-specific proteins might bind so inefficiently that the ligation machinery fails to recognize the ends as telomeres. Similarly, the quantity of telomere-specific proteins might be limiting relative to the large number of injected TrtDNA ends. The ends are then either ligated or exposed to nucleolytic degradation.

Non-nucleosomal telomeric protein complexes have been described for TrtDNA (Blackburn and Chiolu, 1981), *Physarum* rDNA (Cheung et al., 1981), and *Oxytricha* macronuclear DNA (Gottschling and Cech, 1984). Although no studies have been published that specifically examine rates of telomeric complex formation in *Xenopus*, Wylie et al. (1978) describe assembly of nucleosomes onto injected SV40 DNA. In vitro studies by Laskey and his colleagues (1978) indicate that this process requires several hours to go to completion. It is possible that telomeric protein complex formation in *Xenopus* is not rapid or efficient enough to prevent ligation or degradation of the rDNA ends.

A final possibility is that the mechanism by which *Xenopus* recognizes and maintains telomeres may be too different from that used by lower eukaryotes to allow TrtDNA ends to function properly in the egg.

In summary, we have observed the efficient replication of TrtDNA injected into *Xenopus* eggs. This replication is characterized by the addition of heterogeneous fragments of *Tetrahymena* DNA to the ends of the rDNA, suggesting that microinjected TrtDNA ends are not recognized as telomeres in *Xenopus* eggs.

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