Telomeres inhibit end to end fusion and enhance maintenance of linear DNA molecules injected into the *Paramecium primaurelia* macronucleus

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ABSTRACT

Direct Injection into the macronucleus of *Paramecium tetraurelia* of DNA molecules coding for the A-antigen leads to expression of the gene and autonomous replication (1). When injected into *Paramecium primaurelia* DNA from probably any origin, procaryote or eucaryote, can replicate as linear telomerized molecules and the number of copies maintained can be very high (up to 200000 copies). We present here evidence that if the injected linear DNA molecules harbour preexisting telomeres at both extremities they are protected from degradation, the number of DNA molecules maintained being 15- to 30-fold higher than if the molecules are injected without telomeres. Some of the injected molecules replicate as multimers, but, only when the fused ends are devoid of preexisting telomeric repeats.

INTRODUCTION

The *Paramecium* cell like all ciliates exhibits the interesting feature of nuclear dimorphism: two differentiated types of nuclei coexist in the same cell. The diploid micronucleus is transcriptionally inactive, divides mitotically and undergoes meiosis during the sexual phases of the cellular cycle, conjugation or autogamy, to form the zygote. It is believed to be the germinal nucleus. The somatic macronucleus is transcriptionally active and highly polyploid (800—1000n). It divides amitotically during the vegetative phase of the cell cycle and is degraded during the sexual phases. A new macronucleus is then formed for each caryonide by complex differenciation of the zygote. The term caryonide refers to clonal cells issued from a single macronuclear differenciation event. For a review see Blackburn and Karrer in reference 2.

When foreign DNA is introduced into the ciliated protozoan *Paramecium tetraurelia* by direct microinjection into the macronucleus it can be readily replicated, and in the case of *Paramecium* DNA, expressed (1,3,4). Thus, plasmids harbouring the cloned *P. tetraurelia* surface antigen A-gene can be detected when injected into cells deleted for this gene after screening for A-gene expressing transformants (1). When DNA is injected in a superhelical form, the DNA molecules are first linearized at random, and then, telomeric repeats are added (3). These modifications suffice to allow formation of replicons or pseudochromosomes that are stably maintained throughout the vegetative cell cycle. When autogamy occurs, all the injected DNA disappears along with the macronuclear chromosomes (3).

We are interested in studying expression of homologous and heterologous genes in *Paramecium primaurelia*. To maintain DNA as autonomously replicating molecule, it is necessary to unravel the structural features that permit efficient maintenance as macronuclear linear, telomerized chromosomes. We describe here the fate of a number of DNA molecules from different biological origins directly microinjected into the macronucleus of the *Paramecium primaurelia* 168 strain. We find that these molecules are maintained in the paramecium macronucleus throughout vegetative growth and present quantitative data showing that the presence of telomeric sequences at the ends of the DNA molecules prevents degradation and, more surprisingly, concatenation of the injected DNA and lead to maintenance of the injected molecules at 15- to 30-fold higher copy number than if the DNA molecules were injected without telomeres. Nonetheless, a portion of all the injected molecules, even those bearing telomeres, were integrated into the macronuclear chromosomes. Our results will be discussed in relation to the possible structural features and mechanisms of macronuclear DNA replication.

MATERIALS AND METHODS

*Paramecia*

*Paramecium primaurelia* wild-type strain 168 used in the present study was isolated at Sendai in Japan and was described by G. Beale (5). Cells were grown prior to microinjection at 18°C in a grass infusion infected with *Klebsiella pneumoniae* and supplemented with 0.8 μg/ml of beta-sitosterol (Merck; Darmstadt FRG). At 18°C the 168G gene is preferentially expressed, and this favored the coexpression of the cloned 156G gene used as the reporter DNA throughout this study. After microinjection cells were transferred to 24°C.

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Injected DNA
Plasmid UXi3 was a pUC19 inserted derivate of the plasmid described previously (6,7), harbouring the entire 156G surface antigen gene. The plasmid carrying the wild-type A2 polyoma virus genome DNA was previously described (8). Plasmid YA-C3 was donated by Bernard Dujon and was a variant of pYAC2 (9). In pYAC3 the unique Sma I cloning site was replaced by a Sna BI site. Restriction maps are given, when helpful in the results section.

Microinjection
The technique used was essentially that described by Tondrav and Yao (10). Plasmidides used for microinjection were alkali-extracted from E. coli bacteria and purified twice on CsCl density gradients. DNA was eventually digested with the appropriate restriction endonucleases and fragments were purified twice by electrophoresis on low melting point agarose gels (BRL; Bethesda USA). DNA containing agarose was equilibrated in 100 mM NaCl and 5 mM EDTA (pH 7.5), melted for 10 min. at 68°C and then solubilized by agarase (Calbiochem; San Diego USA) at 40°C for at least 5 h (1 unit of agarase per 20 µl of Agarose), followed by phenol extraction and ethanol precipitation. Prior to microinjection DNA was resuspended in water and filtered through a 0.2 µ DynaGard-ME filtration unit. In all of the experiments described, DNA was injected at an overall concentration of 5 mg/ml.

DNA extraction and electrophoresis
One volume of 10 mM Tris (pH 7.2) washed, pelleted paramecia was lysed at 55°C with two volumes of 0.5 M EDTA (pH 9.0), 0.5% N-laurylsarcosine, 0.5% NaDodSO4 and 0.5 mg/ml proteinase K (Merck) for at least 5h. The lysate was then gently extracted twice with phenol and dialysed twice against 25% ethanol containing 10 mM Tris-HCl and 1 mM EDTA (pH 8.0) and once against Tris-EDTA. To prepare agarose inserts of intact macronuclear DNA in low melting agarose one volume of washed pelleted cells was mixed with two volumes of 1 % low melting agarose in 125 mM EDTA (pH 9.0) at 40°C. After solidifying at 4°C the agarose inserts were incubated for at least 20 h at 55°C in the lysis solution described above. The inserts were then washed three times in 0.5 M EDTA at 55°C. Pulsed-field electrophoresis in a contour-clamped homogenous electric field (CHEF) homemade apparatus was carried out according to the method described by Chu et al. (11) in 0.25 TBE (TBE is 89 mM Tris, 89 mM borate and 2.5 mM EDTA) at 6V/cm, with cooling to 12°C and a commutation period of either 10 or 20 seconds. For endonuclease restriction digestions and electrophoresis, standard methods were used (12).

Southern hybridization and density scanning
DNA was transferred from agarose gels to hybondN+ membranes (Amersham; Amersham UK) in 0.4 M NaOH after depurination in 0.25 M HCl. Hybridization was carried out according to Church (13) in 7% NaDodSO4, 0.5 M sodium phosphate, 1% bovine serum albumin and 1 mM EDTA (pH 7.2) at 64°C for 15 to 20 h. Membranes were washed at high stringency (temperatures between 55°C and 65°C depending on the sequence) in 2 SSC (SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.5% NaDodSO4 for 30 min and once in 0.1-SSC and 0.5% NaDodSO4 for 60 min prior to autoradiography. Probes were purified by the same techniques used to purify DNA for microinjection, and were labelled using a random priming kit (Boehringer; Mannheim FRG) and (a-32P)dATP (Amersham, reference PB10204) up to a specific activity of 3 X 109 cpm/µg. Densitometry scans of autoradiograms were carried out on a Hoefer (San Francisco USA) GS300 densitometer driven by the GS370 electrophoresis data system. Radiograms to be scanned were always exposed in the absence of an intensifying screen.

RESULTS
Injection of the 156G gene DNA into the macronucleus
It has been shown that DNA injected into P. tetraurelia is maintained in the cell when injected into the macronucleus (1, 3). Eric Meyer in our laboratory (14 and manuscript in preparation) has shown that when the circular cloned P. primaurelia 156G surface protein gene (6,15) is injected (fig 1a) into the macronucleus of the 168 allelic strain, the 156G gene is expressed. This can be monitored by an immobilization test using a rabbit anti-156G serum that does not recognize the endogeneous allelic 168G protein (16). Moreover, the injected DNA is maintained throughout vegetative growth. We injected

Figure 1. Maintenance of the injected 156G gene. The scheme of the 16 kb 156G gene (pUXi3) is presented in a. The dark box indicates the coding region for the gene and the 3' black box the telomeric repeats. Eco RI sites are given by the vertical separations. The HphI, Sca I and Bam HI sites used in generating the gene and the 3' black box the telomeric repeats. Eco RI sites are given by the vertical separations. The HphI, Sca I and Bam HI sites used in generating the gene and the 3' black box the telomeric repeats. Eco RI sites are given by the vertical separations. The HphI, Sca I and Bam HI sites used in generating the gene and the 3' black box the telomeric repeats. Eco RI sites are given by the vertical separations. The HphI, Sca I and Bam HI sites used in generating the gene and the 3' black box the telomeric repeats. Eco RI sites are given by the vertical separations.
the linear 156G gene after excision from its pUC19 vector by Bam HI restriction. Either, the 16-kb insert plus vector was injected, or the insert was purified prior to injection. In both cases results were similar, and the percentage of transformants obtained in several injection experiments varied between 50 and 80% of the cells injected. Undigested total cellular DNA from clones expressing the 156G gene was electrophoresed on 0.6% agarose gels, transferred to a nylon membrane and hybridized with the 156G specific Pvl probe (Pvl is a 222 base-pair probe cloned from the 156G gene central repeats (7) and is specific for that gene; fig. 1a). DNA extracted about 20 generations following injection with the purified 16-kb gene showed that the number of copies per cell, varied from 10000 to 200000 copies (fig. 1b, lc and results not shown). In a typical experiment, shown in figure 1b, two DNA molecular species, one of 16-kb (the size of the 156G gene) and another of 32-kb, could be detected with the Pvl probe. In cells where a high copy number was observed, the 16-kb molecules represented 50 to 70% of the total 156G molecules and the 32-kb molecules 30 to 50%. To verify that the 32-kb species reflected the presence in the cells of a dimer of the injected 16-kb molecules we cut the total cellular DNA with Bam HI showing that most of the 32-kb molecules were resolved into 16-kb sized molecules. Results were similar when the restricted 156G DNA plus pUC19 was injected but two supplementary molecular species of 19-kb and 22-kb were observed. These molecular species arose from concatenation of the 156G molecule with one or two copies of the co-injected pUC19 vector, and disappeared after Bam HI digestion. The pUC19 vector as a monomer or multimerized could be likewise detected (results not shown).

To understand the orientation in which the 32-kb tandem molecules were concatenated, we cut cellular DNA with Hph I or Sca I in the presence or absence of Bam HI (restriction sites are given in fig. 1a). These sites offer the advantage of cutting the 16-kb molecule near both the 5' and the 3' ends of the linear DNA molecule. As shown in the scheme of dimers in figure 2a (presented are digestions by Hph I and Bam HI only), this allows the determination of the orientation in the dimers. When cut with Hph I or Sca I and probed with Hpl, Hp2 (figure 2b and 2c) or Sc1 and Sc2 (not shown), densitometry analysis of the autoradiograms presented in figure 2b revealed that about 50% of the dimers were in a head to tail (HT) orientation (Head is defined as the 5' end of the gene, and Tail the telomeric 3' end) and 50% in a head to head (HH) orientation. No tail to tail (TT) orientation, in which telomeric ends are joined, could be detected. When Bam HI restriction was included (figure 2b and 2c) all the HT dimers, but only about half of the HH dimers, could be

Figure 2. Configuration of the 156G gene dimers. The three theoretical configurations of the 32-kb dimers are shown in a: HT (head to tail), HH or TT. The terminal 0.9-kb Hpl and the 2-kb Hp2 fragments used as probes, and the internal 2.9, 1.8 and 4-kb fragments, are shown. The effective percentages of the three forms are given. Panels b and c show the results obtained with 0.2 μg of clone 6 of figure 1b digested with the corresponding enzymes and electrophoresed on a 1% agarose gel, transferred to nylon membranes and hybridized with the Hpl probe in panel b and with the Hp2 probe in panel c. B, Bam HI and H, Hph I. The faint bands in the control lanes (c) in the Hpl hybridization experiment are due to cross-hybridization with the endogenous 168 alleles in the injected cells. Background in the Hp2 experiment is due to cross-hybridization with cellular DNA telomeric repeats. Autoradiograms shown were exposed for 6 h.

Figure 3. De novo addition of telomeric repeats. The membrane was prepared as in figure 2b and hybridization was carried out with pure Paramecium telomeric repeats as a probe. The arrows at 0.9-kb and 1.1-kb indicate the telomeres added de novo to the 5' end of the 16-kb 156G linear molecule. Autoradiography was performed for 19 h.
resolved into monomers. These results suggest that the intramacronuclear concatenation is a primary event following injection, preceding telomerization and probably replication, since it proceeds without destruction of the Bam HI site. The incomplete resolution of the HH species might be explained by the instability of the palindrome generated which might lead to deletions. The 3' terminal Hp2 and Sc2 probes contain the cloned 156G telomeres and the background smear on the autoradiogram presented in 2c (Hp2 probe), arises from hybridization with telomeres of the 168 strain cellular DNA.

When digested by Hph I (fig. 2b), or Sca I, the 5' or 3' terminal fragments of the detected 156G molecules were 0.1 to 0.15-kb longer then expected, and did not appear as a sharp band, but as an heterogeneous smear on the autoradiogram. In order to assess the nature of the complementary sequence, the membranes of 2b were hybridized with a telomeric containing pure C4A2 repeats. The 5' Hph I and Sca I terminal fragments were hybridized with the telomeric probe (fig. 3) and as expected, so did the 3' fragments harbouring the prexistant telomeres of 2-kb and 0.9-kb, but not the internal 1.8-kb fragment. This shows that telomeres were added de novo to the extremities of the injected DNA, and that the size increase in the terminal fragments is undoubtedly due to addition of about 20 to 30 G1T2 or G1T2 telomeric repeats (17) over the 20 generations following microinjection. The heterogeneous nature of bands on an autoradiogram is characteristic of the presence of telomeric ends in Paramecium, the number of telomeric repeats added being variable during vegetative growth (1, 17).

Eight clones transformed by the 156G gene were allowed to undergo autogamy, and their DNA was extracted 20 generations later, submitted to electrophoresis and blotted. Hybridization with the Pvl probe did not reveal any persistance of the injected 156G molecules in any clone. This shows that injected DNA has the same fate as macronuclear chromosomes during autogamy.

Maintenance of injected polyoma virus cloned DNA

In order to assess whether maintenance of injected DNA through replication was dependent on particular Paramecium sequences, we examined the maintenance of a totally different DNA, cloned wild-type polyoma (Py) A2 strain DNA. This DNA is not only different in sequence but also in base composition (31% guanine+cytosine in 156G 16-kb fragment versus 50% in Py). Py DNA was linearized prior to injection at the unique Bgl I site (fig. 4a). This DNA was co-injected with an equal amount of linearized 156G DNA (roughly two Py molecules for one 156G molecule). The 156G DNA was added to monitor transformants and to compare maintenance efficiencies. The rate of transformed 168 cells was 50 to 60%, reminiscent of the transformation rate observed when 156G DNA alone was injected. Moreover, Py DNA is not toxic to Paramecium cells as the growth rate of the Py injected 168 cells was identical at 24°C to that of un.injected 168 cells.

As shown in figure 4, when total native DNA from transformed cells was blotted and probed with either the Pvl probe (fig. 4b), or with the Py probe (fig. 4c) both DNAs were present in the cell. Moreover, for a given clone, the relative ratio between the two DNAs was the same in the total cellular DNA extracted from different injected cells (figures 4b and 4c). Thus, in a cell with a high copy number of 156G molecules, the copy number of Py DNA was also high, and vice versa. However, comparing on the same blot the hybridization signal with either the Py or the Pvl probes, the Py/ Pvl ratio should have been 5. As shown in figure 4 this was not the case, the hybridization signal being three fold stronger with the Pvl than with the Py probe. Thus, Py is maintained in the Paramecium macronucleus 15 fold less efficiently than the 156G molecules.

Like the 156G DNA, the Py DNA formed multimers following injection (fig. 4b). The Py probe revealed 3 molecular species of 9-kb (60%), 18-kb (25%), and 27-kb (15%) in size (fig. 4c). Thus, at least up to trimers of the injected linear Py molecules could be formed. Two of the transformed clones shown in figure 4b (clones 1 and 2) were digested with Bgl I, as shown in fig. 5. These molecules could not be resolved into monomers, which suggests that the Bgl I site was lost in the multimerization process. In the Bgl I site of Py (5' GCCTTGG/AGGC 3') the 3'- protruding cohesive ends TGG (designated as the H extremity) and CCA (designated as the T extremity) are complementary in the Head to Tail (HT) orientation only. Thus, the HT orientation should have been largely favored. Indeed, when digested with Bam HI and probed with Py DNA (fig. 5), more than 80% of
the molecules were shown to be in the HT orientation and less than 20% were in the TT orientation. None were in the HH configuration. The 3'-TGG cohesive ends are reminiscent of half a telomeric repeat and when only one pseudotelomeric sequence is present, dimerization could be detected, but in all cases the Bgl I site was lost, showing that the cell destroys the TGG and/or the CAA 3'-protruding cohesive ends prior to ligation.

Telomere repeats and replication

Both 156G and Py injected DNA possess at their extremities respectively, one telomeric or pseudo-telomeric sequence. To test whether telomeres play a role in the maintenance of injected DNA molecules, we tested a number of DNA fragments derived from the *Saccharomyces cerevisiae* - *E. coli* shuttle vector pYAC3 (fig.6a). The use of this DNA offers several advantages. First, as shown in fig.6b, restriction by either Bam HI or by *Xho* I, yields a 9.8-kb or an 8.4-kb fragment (designated as BB and XB below), with or without telomeric ends, respectively. Secondly, a Bam HI digestion of pYAC3 generates a pure yeast *His* 3 gene fragment of 1.7-kb (designated BS; fig. 7). And thirdly, the *Xho* I digestion leads to a 3.1-kb fragment (designated XS; fig 7) with reversed telomeres (the 3' strand carrying the cytosine-rich sequence). Finally, since the telomeric repeats of the YAC molecule originate from *Tetrahymena*, they represent one of the two natural telomeric repeats in *Paramecium* (17). Thus, when injecting BB and XB we were able to compare the maintenance of two sequences identical in every respect, but for the presence of telomeric repeats at their extremities. Each one of these fragments was injected in the presence of circular *pUXi3* plasmid (roughly two BB or XB molecules for one *pUXi3* molecule).

As shown in figure 6c, when probed with the *Pst* I fragment of 3.8-kb (Ps1) comprising YAC sequences between *ARS1* and the beginning of *URA3* (fig 6a), BB molecules clearly have an advantage over the XB molecules. The results were normalized using the coinserted circular *pUXi3* detected with the *Pv1* probe (figure 6c). Circular *pUXi3* was linearized at random (results not shown). If one takes into account the number of injected *pUXi3* and BB molecules and the relative size of the Ps1 and *Pv1* probes utilized at the same specific activity, the BB/ *pUXi3* ratio should be of about 5—7 on the scanned autoradiogram, both molecules were maintained at the same rate. This was indeed the case since the ratios obtained for a number of injected clones were ranged from 4.5 to 6.8. Thus, circular DNA seems to be as stable as linear DNA injected with telomeres. One explanation is that the
Figure 7. Replication and reversed telomeres. The injected 1.7-kb HIS3 fragment (BS) and the 3.1-kb HIS3 fragment with the reversed telomeres (XS), both excised from pYAC3 (fig. 6a) by Bam HI or Xho I digestion, respectively, are shown in the upper panel. DNA from 2 clones injected with BS and 2 clones injected with XS was electrophoresed on 1% agarose either uncut (U), or digested by Eco RI (E), Bam HI (B) or Xho I (X). After blotting, DNA was hybridized with the BS fragment as a probe. Exposure time was 42 h. The small triangles indicate the different multimers obtained after injection of the BS fragment. The monomer is hardly visible and the heptamers were visible after longer exposure times. The large arrows indicate molecules integrated into macronuclear chromosomes.

cellular machinery that linearizes pUXi3 likewise protects it against cellular nucleases. On the contrary, the XB/pUXi3 ratio was at best 0.2. Thus, intact XB molecules are some 30-fold less numerous then pUXi3 or BB molecules. The XB fragments were not only in lower numbers but their size heterogeneity reflected degradation of the replicated DNA (fig. 6c). On the other hand, although the hybridization signal was weak, we could detect the presence of dimers in the XB injected clones, but none at all in the BB injected clones in spite of the higher hybridization signal.

BB and XB have Bam HI and Xho I cohesive ends, respectively. In order to exclude any influence of the different cohesive ends in the relative stabilities of the injected molecules, we have constructed a plasmid (pYBl) identical to XB when linearized, but containing Bam HI extremities (by the addition of Bam HI linkers). In a parallel experiment, either XB or Bam HI linearized pYB1 were injected in the presence of circular pUXi3 into 168 cells (results not shown). The calculated XB/pUXi3 ratio compared to the pYB1/pUXi3 ratio (0.1 and 0.2) testified that replicative maintenance of both DNA molecules in the injected cells was basically the same. Thus, telomeres themselves suffice to explain the stabilisation of the BB as compared to the XB molecules.

Figure 8. Integrated and free species of injected molecules. Agarose inserts containing DNA from paramecia coinjected with 156G and Py DNA (clone 1 in fig. 4) were submitted to pulsed-field electrophoresis for 20 to 24 h at 6 V/cm, at 12°C, according to the CHEF technique with commutation times of either 10 or 20 sec. The gels were blotted and hybridized with either the Py or the Pv1 probes. The 10 sec. commutated blot was exposed for 4 h with an intensifying screen and the 20 sec. ones for 18 h without a screen. The whole triangles represent the integrated 156G or Py molecules and the hollow triangles the free molecules.

Injected 1.7-kb BS molecules, as shown in figure 7, were readily replicated but, almost no monomers subsisted (about 3% of the total) and massive multimerization was detected up to heptamers (35% dimers and 23% trimers). The injected 3.1-kb XS fragment harbouring the reversed telomeric repeats scored no better in maintenance (fig. 7, XS clone), but almost no concatenation could be observed (only 8% of the molecules were dimers). Thus, if both BS and XS molecules were replicated in the cell, the rate was some 20 to 30-fold lower then that of the coinjected 156G DNA (results not shown). The difference in replicative maintenance was similar to that observed between the XB and the 156G molecules. The molecules harboring reverse telomeric repeats were not protected from degradation, but were somewhat leakily protected against multimerization. One should remember, however, that in these DNA molecules the telomeres are not only reversed, but are distant by 0.4-kb of Tetrahymena sub-telomeric sequences from the ends.

Integration of injected molecules into macronuclear chromosomes

In the experiments described above we observed that when intact DNA from injected cells was submitted to electrophoresis, the material migrating at a high molecular weight position hybridized with all the probes specific to the injected DNA (e.g. figures 4c and 7). No hybridization could be detected in DNA from uninjected cells. The integrated material was variable and seemed not to depend on the DNA molecule injected (not shown). When cellular DNA was cut with different restriction enzymes the hybridized material migrated as a large homogeneous smear of lower molecular weight (e.g. figure 7, BS clone 2 and XS clone 3). DNA agarose inserts from Py injected cells were
extremities of linear DNA molecules in eucaryotes (26, 18, 20). Paramecium macronucleus as well. Our protection against exonuclease degradation via proteins that molecules during successive replication cycles and, to offer Their two major roles are: to prevent shortening of linear chromosomes of the macronuclear chromosomes is now in any DNA molecule introduced into Paramecium. telomeres and the telomerase (19) machinery. An attractive however, sequence dependent, it could be in close relationship control of the number of chromosomes or pseudo-chromosomes needed to initiate replication in the polyploid macronucleus, but telomerized linear molecules of probably any foreign sequence a solution would be that telomeres themselves are origins of replication arresting. Paramecium cells on the other hand are vegetatively growing cells that actively replicate their DNA. Nevertheless, they allow replication in their macronucleus, as telomerized linear molecules of probably any foreign sequence whether introduced in a circular or a linear form. This suggests that no specific Paramecium sequences other than telomeres are need to initiate replication in the polyploid macronucleus, but control of the number of chromosomes or pseudo-chromosomes is nevertheless fairly stringent. If copy number control is, however, sequence dependent, it could be in close relationship to telomeres and the telomerase (19) machinery. An attractive solution would be that telomeres themselves are origins of replication in Paramecium. Any DNA molecule introduced into the macronucleus once it is telomerized acquires the origin of replication. Work attempting to map the initiation site and sense of replication of the macronuclear chromosomes is now in progress.

Telomeres are structures present at practically all the extremities of linear DNA molecules in eucaryotes (26, 18, 20). Their two major roles are: to prevent shortening of linear molecules during successive replication cycles and, to offer protection against exonuclease degradation via proteins that interact with the telomeres (26, 27, 28). These molecular tasks probably apply to the Paramecium macronucleus as well. Our results show that two telomeric ends of injected molecules do not fuse, but one telomeric and one non-telomeric end do. The biological interest of such inhibition of concatenation in the protection of chromosomes against multimerization, thus maintaining the normal number, size and function of chromosomes, seems obvious. Such a role was originally proposed by McClintock (15, 29). The fusion of telomere to non-telomeric ends might be explained by a directed process in which telomeric ends function as an initiator or a target in fusion, but not both. Telomere to telomere fusions might not occur at all, or if they do, go undetected, being rapidly resolved as suggested by the work of Murray et al. (30). In their experiments, head to telomere structures introduced into yeast chromosomes were resolved at a frequency of $10^{-2}$ per generation.

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