Characterization of telomeric regions from *Ustilago maydis*

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The isolation of telomeres from the phytopathogenic fungus *Ustilago maydis* is described. The telomeric repeat from the plant *Arabidopsis thaliana*, TTAGGG, cross-hybridizes to *Bal*31-sensitive fragments of *U. maydis* DNA and detects many or all of the *U. maydis* chromosomes separated by pulsed-field gel electrophoresis (PFGE). This telomeric repeat was used to screen a library enriched for chromosome ends. Three clones were isolated which contained the tandemly repeated sequence TTAGGG. This sequence is identical to some known telomere repeats found in humans and other vertebrates as well as in some protozoa and moulds. In addition, the three telomeric clones had an almost identical 376 bp segment of middle-repeated telomere-associated sequences adjacent to the telomeric repeat. This segment hybridized to many or all *U. maydis* chromosomes separated by PFGE and showed a hybridization pattern in genomic digestions similar to that of the telomeric repeat. These results indicate that in *U. maydis* the same segment of telomere-associated sequences is located adjacent to the telomeric repeat in many or all chromosomes, which suggests that it may have a common role in chromosome function.

Keywords: *Ustilago maydis*, telomere, chromosome function

INTRODUCTION

In the last few years a dramatic increase of information has been generated in the molecular analysis of telomeres. These specialized DNA–protein complexes located at the chromosomal termini are required to stabilize the chromosomes and for the complete replication of DNA at the chromosome end. The telomere consists of tandemly repeated copies of a DNA sequence which contains clusters of G residues oriented 5' to 3' towards the chromosome end. In telomeres of *Arabidopsis*, human, *Tetrahymena* and *Chlamydomonas*, among other eukaryotes, this sequence is perfectly repeated with little or no divergence. In others, like *Saccharomyces cerevisiae* and *Dictyostelium*, variations of a primary pattern are repeated. A repeated sequence is characteristic for each species and it is assumed to be present in all the chromosomes (Blackburn, 1991; Kipling & Cooke, 1992; Zakian, 1989). An identical telomere repeat unit can be present in divergent species, ranging from protozoa to plants and humans. Also, telomeres from one species can stabilize

linear molecules in another one, even if their telomeric sequences are different (Zakian, 1989). The length of this repeated unit varies between species; in humans, differences in length have been observed depending on the cell type or on donor age (Harley *et al.*, 1990; Hastie *et al.*, 1990). In ciliated protozoa, synthesis of the G-rich strand is catalysed by an enzyme activity named telomere terminal transferase or telomerase. Telomerases are ribonucleoprotein enzymes, in which the RNA component contains sequences complementary to the telomeric repeat for which it is the template (Blackburn, 1992). Mutations in a gene that is thought to code for a component of the telomerase in *S. cerevisiae*, or mutations on the telomerase RNA gene of *Tetrahymena*, can lead to dramatic phenotypes: telomere shortening, chromosomal instability and cell death (Lumblad & Szostak, 1989; Yu *et al.*, 1990).

Studies in *S. cerevisiae* have determined that the only DNA sequence that is required for telomere function is the simple telomeric repeat. Although middle-repetitive DNA sequences are also often found adjacent to the telomere repeat, these sequences are not required for telomere function. For these sequences, called telomere-associated sequences, roles in recombination, replication and chromosome healing have been suggested; in *S. cerevisiae* these sequences are highly recombinogenic and

Abbreviations: ARS, autonomously replicating sequence; PFGE, pulsed-field gel electrophoresis; UTAS, *Ustilago* telomere-associated sequences.

The GenBank accession numbers for the sequences reported in this paper are X77242 and X77243.

contain putative DNA replication origins (Zakian, 1989). Telomere-associated sequences can extend from a few to hundreds of kilobases and have a polymorphic distribution. In *S. cerevisiae* two types of telomere-associated sequences, called X and Y', are found. Y' is a 6.7 kb highly conserved element present in 0 to 4 copies at the telomere region, and X is a 0.3 to 3.75 kb less conserved and less well characterized element (Chan & Tye, 1983a, b). In humans, a few kilobases of telomere-associated sequences have been sequenced. These sequences are polymorphic in the genome and harbour a complex mixture of repetitive elements (Brown *et al.*, 1990). In other organisms, such as *Plasmodium falciparum*, the telomere-associated repeats can extend for about 100 kb (Foote & Kemp, 1989).

We are interested in the analysis of chromosome structure in *Ustilago maydis*, a basidiomycetous fungus that causes the smut disease in maize. This fungus has been used as a system to study genetic recombination (Holliday, 1974), DNA repair and replication (Holliday, 1974) and host-pathogen interactions (Banuett & Herskowitz, 1989; Kronstad & Leong, 1989; Schultz *et al.*, 1990). *U. maydis* has several features that facilitate molecular genetic studies. It can be grown as a yeast-like uninucleate haploid in simple chemically defined media. Efficient transformation systems have been described and gene disruption by homologous recombination has been achieved (Fotheringham & Holloman, 1989; Kronstad *et al.*, 1989; Tsukuda *et al.*, 1988; Wang *et al.*, 1988). *U. maydis* has a haploid chromosome number of approximately 20 in the size range of 300 to more than 2000 kb, which allows the use of pulsed-field gel electrophoresis (PFGE) for karyotyping (Kinscherf & Leong, 1988). In this report we describe the molecular cloning of the telomeric repeat and of a segment of telomere-associated sequences from this fungus.

METHODS

Strains and DNA manipulation. The *Escherichia coli* strain was XL-1 Blue (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI^q ZΔM15 Tn10(Tet^r)*]) from Stratagene. The *Saccharomyces cerevisiae* strain was YPH252 (MATα *ura3-52 lys2-801 ade2-101 leu2Δ1,112 his3Δ200 trp1-Δ1*), kindly provided by P. Hieter, Johns Hopkins University. The *Ustilago maydis* strain was UCM104 *a1b2 leu1*, kindly provided by William Holloman, Cornell University. Restriction enzymes and T4 DNA ligase were purchased from Gibco-BRL and ENZIBIOT; *Bal31* was from Boehringer Mannheim. Plasmid isolation by the alkaline procedure and *E. coli* transformation by CaCl_2 were performed as described by Sambrook *et al.* (1989). The DNA sequence was determined using the DNA Sequencing Kit, Sequenase version 2.0, from United States Biochemical. Single-stranded DNA for sequencing was prepared by the ssDNA template preparation procedure from Promega.

Isolation of *U. maydis* DNA. A modification of a procedure for yeast DNA isolation (Sherman *et al.*, 1986) was used. Protoplasts were obtained from a cell pellet harvested from a 1 l culture by incubating cells in SCS (1 M sorbitol, 20 mM sodium citrate pH 5.8, 10 mM EDTA) containing 20 mg Novozym (Novobiolabs) ml^{-1} for 30 min. Protoplasts were then washed in SCS and resuspended in 50 ml 50 mM Tris/HCl (pH 7.4), 20 mM EDTA. SDS (5 ml of a 10%, w/v, solution) was added, and the

mixture incubated at 65 °C for 30 min. Potassium acetate (15 ml of a 5 M solution) was added and the suspension was incubated for a further 60 min on ice. The cleared lysate was ethanol-precipitated, RNase-treated, phenol/ CHCl_3 -extracted, ethanol-precipitated again, and the DNA was spooled out with a glass rod. The DNA was then resuspended in TE (10 mM Tris/HCl pH 8, 1 mM EDTA). This procedure was also scaled down for 10 ml cell cultures.

***Bal31* digestion of genomic DNA.** *U. maydis* genomic DNA ($10 \mu\text{g ml}^{-1}$) was digested for various periods of time with nuclease *Bal31* (0.25 units ml^{-1}) at 30 °C, in a solution containing 12 mM CaCl_2 , 24 mM MgCl_2 , 200 mM NaCl, 1 mM EDTA and 20 mM Tris/HCl (pH 8.0). Reactions were stopped by adding EGTA to a final concentration of 20 mM. DNA was extracted with phenol/ CHCl_3 and ethanol-precipitated. DNA was then digested with either *Sau3A*I or *Bam*HI and size-fractionated by agarose gel electrophoresis.

Construction and screening of a telomere-enriched library. The construction of the telomere-enriched library is depicted in Fig. 1. *U. maydis* DNA (60 μg) was digested for 30 s with *Bal31*, and the ends were filled in with the Klenow fragment of DNA polymerase followed by digestion with *Sau3A*I. DNA fragments of 500–1000 bp were size-selected on a 1% (w/v) agarose gel and eluted from the gel using the Prep-A-gene DNA purification kit from Bio-Rad. Purified DNA was then ligated at high concentration to double-digested *Bam*HI–*Hinc*II Bluescript-KS[−] vector (Stratagene), using a 10-fold molar excess of vector to genomic DNA. The ligation mixture was then digested

Fig. 1. Strategy for telomere cloning: construction of a *U. maydis* chromosome-end-enriched library. High molecular mass DNA was briefly treated with *Bal31* nuclease and then with Klenow enzyme to ensure the creation of blunt-ended sites for cloning. *Sau3A*I fragments of 500–1000 bp were size-selected in agarose gels and cloned into the Bluescript-KS[−] vector double-digested with *Hinc*II and *Bam*HI. After ligation the mixture was transformed into *E. coli* strain XL-1 Blue (for details see Methods).

with *Mbo*I and *Bam*HI, religated at a low DNA concentration and transformed into *E. coli* XL-1 Blue; *Mbo*I does not cut within vector sequences because the vector was prepared in an *E. coli dam*⁺ host. Colonies were screened by hybridization on nylon membranes (Hybond-N, Amersham) and lysed according to the manufacturer's instructions. Membranes were prewashed in a solution containing 1% SDS, 1 M NaCl, 50 mM Tris/HCl pH 7.5, and then prehybridized at 55 °C in the same solution containing 25 µg tRNA ml⁻¹. Hybridization was done in a 1% SDS, 10% dextran sulfate, 25 µg tRNA ml⁻¹ solution at 55 °C. The probe, at a concentration of 5×10^5 c.p.m. ml⁻¹, was the *Pst*I-*Bam*HI insert from the *Arabidopsis thaliana* clone pAtT4 (Richards & Ausubel, 1988); a Random Primer kit from Amersham was used for labelling. After hybridization, filters were washed in 2× SSPE/1% SDS at room temperature, then in 0.5× SSPE/0.1% SDS at 60 °C (1× SSPE is 0.15 M NaCl, 0.01 M sodium phosphate, 1 mM EDTA).

PFGE. To prepare DNA-agarose plugs from *U. maydis*, cells grown in 20 ml MC medium (Holliday, 1974) to OD₆₀₀ 1.2 (approx. 10^7 cells ml⁻¹) were washed twice in 50 mM EDTA and resuspended in 450 µl SCE (1 M sorbitol, 20 mM sodium citrate pH 5.8, 10 mM EDTA). Novozyme (0.5 µg ml⁻¹) was added, and 750 µl of a 1% solution of low-melting-point agarose (BRL) prepared in 125 mM EDTA (pH 7.5). The mixture was allowed to solidify on ice for 10 min. The solidified mixture was incubated first in LET buffer (500 mM EDTA, 10 mM Tris/HCl pH 7.5, 30 mM 2-mercaptoethanol) at room temperature for 12 h and then in LDS buffer (1%, w/v, lithium dodecyl sulfate, 100 mM EDTA, 10 mM Tris/HCl pH 8.0) at 50 °C for 24 h (Southern *et al.*, 1987). DNA-agarose plugs were kept at 4 °C in 50 mM EDTA. PFGE was performed on a Rotaphor unit (Biometra Rotaphor type IV). Electrophoresis was carried out in 1% agarose gels (20 × 20 cm) at 13 °C in 0.5× TBE buffer (1× TBE is 0.1 M Tris, 0.1 M boric acid, 2 mM EDTA). Conditions used for separation of *U. maydis* chromosomes were 200 V for 26 h; the pulse time decreased linearly from 120 to 30 s and the angle between the orientation of the homogeneous electronic field decreased linearly from 110° to 99°.

Southern hybridization. Southern blotting was done on nylon membranes (Hybond N⁺, Amersham) (Church & Gilbert, 1984). Hybridizations were carried out in 0.5 M Na₂HPO₄ (pH 7.2), 7% SDS, 1 mM EDTA, 1% (w/v) BSA at a probe concentration of 10^6 c.p.m. ml⁻¹. Low-stringency hybridization was done at 55 °C followed by washes with 2× SSPE, 1% SDS at 55 °C. High-stringency hybridization was at 65 °C followed by washes with 0.2× SSPE, 0.1% SDS at 65 °C. For removal of probes a solution of 0.1% SDS and 0.1× SSPE was boiled, poured on the membrane and allowed to cool at room temperature; this treatment was repeated at least twice.

RESULTS AND DISCUSSION

Identification of *U. maydis* telomeric DNA

The conservation of telomere sequences among divergent eukaryotes has facilitated the characterization of telomeric DNA from new species. Telomeric DNA from several organisms has been identified and cloned by using telomeric repeats from non-related organisms as probes. The telomeric repeat from the plant *Arabidopsis thaliana* [(TTTAGGG)_n, clone pAtT4] (Richards & Ausubel, 1988) has been used to identify telomere DNA from plants (Ganal *et al.*, 1991) and to clone telomere sequences from species such as *Chlamydomonas reinhardtii* (Petracek *et*

al., 1990). To test whether the *A. thaliana* telomeric repeat cross-hybridized with *U. maydis* DNA, we digested high molecular mass *U. maydis* DNA for progressively increasing lengths of time with *Bal*31 followed by digestion with *Sau*3AI or *Bam*HI. Analysis of the digests by electrophoresis on agarose gels, transfer to nylon membranes, and hybridization to the insert of the *A. thaliana* pAtT4 clone showed a shift of the hybridization signal towards the lower molecular mass (Fig. 2a). This result suggests that the *A. thaliana* telomere repeat cross-hybridizes with the ends of *U. maydis* chromosomal DNA molecules. This type of shift was not observed when the same filter was probed with a non-repeated probe (Fig. 2c).

Cloning and sequence analysis of the *U. maydis* telomeric repeat

Telomeric DNA is not usually represented in genomic libraries. End-enriched libraries have to be constructed to clone the ends of the chromosomes (Richards & Ausubel, 1988; Van der Ploeg *et al.*, 1984). As shown in Fig. 2(a), pAtT4 hybridized to a smear on *U. maydis* DNA digested with *Sau*3AI, suggesting that a *Sau*3AI site is present close to the end of several of the *U. maydis* chromosomes. We size-selected 0.5–1.0 kb fragments of *U. maydis* DNA digested with this enzyme to construct a telomere-enriched library (see Fig. 1). Screening of 8000 clones by hybridization to the pAtT4 probe revealed three positive clones. These clones contained a 600 bp DNA insert which strongly hybridized to the *A. thaliana* telomeric repeat. DNA sequence analysis of the three clones, pUT1, pUT2 and pUT3, revealed a 6 bp repeat extending from the blunt-end cloning site (Fig. 3b). The sequence CCCTAA was perfectly repeated in the three clones; pUT1 and pUT2 each had 37 tandemly repeated copies and pUT3 had 39 tandemly repeated copies. The complementary sequence TTAGGG correlates with their location towards the 3' end of the chromosome, as has been also found in other organisms. As expected for telomeric DNA, the sequence contained in these clones is sensitive to exonuclease *Bal*31 digestion (Fig. 2a); it is remarkably similar to the *A. thaliana* telomere repeat which was used as a probe, and the G-rich strand is oriented towards the 3' end of the chromosome. The DNA sequence of this telomeric repeat is identical to the sequence found in humans and other vertebrates (Meyne *et al.*, 1989; Moyzis *et al.*, 1988), in *Physarum* (Forney *et al.*, 1987), *Trypanosoma* (Blackburn & Challoner, 1984; Van der Ploeg *et al.*, 1984) and *Neurospora* (Schechtman, 1990), where the telomere sequences consist of tandem repeats of the fixed unit TTAGGG.

Sequence analysis of the telomere-associated sequences

In addition to the telomeric repeat, the adjacent regions also had stretches of repeated sequences. These sequences are referred to as telomere-associated sequences. No function has yet been identified for telomere-associated sequences, although roles in replication, healing of broken chromosomes after radiation damage or protection of

Fig. 2. *Bal31* sensitivity of *Sau3AI* and *BamHI* fragments identified by the *A. thaliana* telomeric repeat and the *U. maydis* UTAS probes. *U. maydis* DNA was digested for 0 (lane 1), 5 (lane 2), 10 (lane 3), 15 (lane 4) and 20 (lane 5) min with *Bal31* nuclease and then digested with *Sau3AI* (left lanes) or *BamHI* (right lanes). DNA was size-fractionated by agarose gel electrophoresis and transferred to a nylon membrane. The blot was first probed with the *A. thaliana* telomeric sequence from clone pAT4 (a), rehybridized to the *U. maydis* UTAS region, a *Sau3AI-HincII* insert from clone UT1 (see Fig. 3) (b) and then rehybridized again to the *U. maydis* Hsp70 sequences obtained from plasmid pCM54 (Tsukuda *et al.*, 1988) (c). The blot shown in (a) was hybridized and washed at low stringency, those in (b) and (c) at high stringency. The probe was removed after each hybridization. The positions of molecular size markers (1 kb ladder from Gibco-BRL) are shown on the left.

chromosomes from degradation have been proposed (Zakian, 1989). The fact that the smallest yeast chromosome lacked detectable amounts of the telomere-associated sequences X and Y' and that large chromosomes seem to contain these elements suggests that they are required for stable maintenance of large natural chromosomes (Zakian & Blanton, 1988). DNA sequence analysis of the region adjacent to the telomere in clones pUT1, pUT2 and pUT3 revealed an almost identical sequence in the three clones: pUT1 and pUT2 were identical and pUT3 had 90% identity (Fig. 3a). Although the three *U. maydis* clones were isolated as independent clones, two have an identical DNA sequence; at this point we can not tell whether these two correspond to the same telomere or if they come from different chromosome ends. The 376 bp of this sequence show some noteworthy features (Fig. 3a, b): a 198 bp G-rich region (42%) and a 87 bp G-rich region flank a 91 bp A+T rich region (88%). We will refer to this sequence as UTAS, for *Ustilago* telomere-associated sequences. In other species, one characteristic of telomere-associated sequences is their polymorphic distribution (Zakian, 1989). In *S. cerevisiae* and in humans these sequences are organized as a series of middle-repeated sequences, which are found in some but not all the chromosomes, with their distribution varying among different chromosomes (Brown *et al.*, 1990; Zakian & Blanton, 1988). With the analysis of our clones we can not yet determine the extent of the telomere-associated elements in *U. maydis*. The cloning and DNA sequence

analysis of larger segments of DNA from the telomere region towards the centromere will define more precisely the structure of the telomere-associated elements in *U. maydis*.

Interestingly, adjacent to the telomere repeat in the *U. maydis* UTAS there is an A+T-rich region of some 90 bp. Autonomously replicating sequences (ARSs) have been also found associated with the yeast X and Y' elements (Chan & Tye, 1980). Yeast ARSs are some 100 bp in length and have a high A+T content; all ARSs contain one or more copies of an 11 nucleotide consensus sequence (Newlon, 1988). Thus, it could be speculated that the *U. maydis* UTAS could have an ARS function. ARSs have already been isolated from *U. maydis* (Tsukuda *et al.*, 1988). These sequences, called UARS1, were identified by searching for plasmids containing random genomic fragments which increased the frequency of transformation. No obvious sequence homology could be detected between UARS1 and UTAS (data not shown). Thus, if UTAS have a function in chromosome replication it may be as a special type of replication origin for chromosome termini, although other types of function can be hypothesized.

Chromosomal distribution of the telomeric repeat and of telomere-associated sequences

It is assumed that telomeric repeated sequences are found in all of the chromosome ends of an organism, whereas

Fig. 3. DNA sequence of the *U. maydis* telomeric region. (a) Sequences of the telomere-associated sequences in clones pUT1 and pUT3 (clones pUT1 and pUT2 have an identical sequence). The sequence is written in the usual 5' to 3' direction towards the chromosome end. The *Sau3AI* site of the insert was used for cloning into the *Bam*HI site of the vector; the G nucleotide of the site corresponds to the first nucleotide of the sequence. The fusion of the blunt-end cloning site of the vector to the telomeric sequences is not shown. |, Nucleotide identical to the nucleotide above; *, missing nucleotide. (b) Primary DNA sequence of the telomere repeat in clone pUT1, determined by the dideoxy procedure. (c) Schematic illustration of the telomeric region. The stippled area corresponds to G-rich regions: nucleotides 1–87, G = 46%, and nucleotides 179–384 (198 bp) G = 42%. An A+T-rich region, nucleotides 88–178, is shown in white, T = 52% and A = 31%, and an area corresponding to the telomeric repeat is shown in black.

the telomere-associated sequences are often found at some but not all the chromosome ends (Zakian, 1989). To confirm that the *A. thaliana* telomeric cross-hybridizes with all of the *U. maydis* chromosomes and to examine the distribution of the UTAS we initially used PFGE. As probes we used the *A. thaliana* telomeric repeat and a 330 bp restriction fragment which contains the UTAS region and no telomeric repeat. Although clusters of bands in the range 600 to ≥ 1100 kb were not resolved when *U. maydis* chromosomes were separated by PFGE, the result showed that the telomeric repeat and the UTAS hybridized to all of the *U. maydis* chromosomes that could be distinguished after ethidium bromide staining (Fig. 4a, lanes 2 and 3). The fact that the intensity of hybridization with telomeric or UTAS probes was comparable, both between the probes and to the ethidium bromide staining, supports this result.

To determine whether UTAS were found at the chromo-

somal end adjacent to the telomeric repeat, *U. maydis* DNA was digested separately with three restriction enzymes, electrophoresed on an agarose gel, and hybridized first to the telomeric repeat and then rehybridized to the UTAS probe. At least 25 fragments hybridized for each digestion. In each of the three digestions the pattern of hybridization was very similar with either probe. The fact that the same size fragments hybridize with the two probes suggests that they are contained within the same fragment. These results suggest that the UTAS region is found in all of the *U. maydis* chromosomes at a position adjacent to the telomeric repeat. Some differences can be seen, though: *Eco*RI fragments of about 7.0 and 3.0 kb, *Bam*HI fragments of about 6.0, 4.5 and 3.0 kb, and *Hind*III fragments of about 13.0 and 6.8 kb (Fig. 4b).

In various species telomere-like sequences can be found at non-telomeric positions in the chromosome. They can be found close to the telomeric region as in *S. cerevisiae*, where

Fig. 4. Distribution of telomeric and telomere-associated sequences on the *U. maydis* chromosomes. (a) DNA-agarose plugs were prepared from *S. cerevisiae* (lane M, used as a molecular size marker) and from *U. maydis* strain UCM104. Chromosomes were subjected to PFGE as described in Methods. Several lanes of UCM104 were separated and transferred to a nylon membrane: lane 1, ethidium bromide staining; lane 2, probed with the *A. thaliana* telomere repeat; lane 3, probed with the *U. maydis* UTAS region. (b) *U. maydis* DNA was prepared from strain UCM104, digested to completion with *EcoRI* (first two lanes), *BamHI* (third and fourth lanes) and *HindIII* (last two lanes). DNAs were size-fractionated by agarose gel electrophoresis and transferred to a nylon membrane. The blot was probed with the *A. thaliana* telomere repeat (lanes

short tracts of telomeric sequences are at the junctions of telomere-associated elements (Walmsley *et al.*, 1984). In vertebrates and plants telomere-like sequences have also been found at the centromere regions (Allshire *et al.*, 1988; Richards *et al.*, 1991). Telomere-like sequences are insensitive to *Bal31* nuclease; their electrophoretic mobility is not affected in a *Bal31* kinetic analysis when probed with a telomeric repeat (Richards *et al.*, 1991). In the *Sau3AI* digestion of *U. maydis* DNA hybridized with pAtT4 there was a fragment of approximately 1.0 kb which was *Bal31* insensitive. A similar analysis performed with the UTAS region (Fig. 2b), indicated that there are at least four bands (0.8 kb, 0.9 kb and two of 1.7 kb) which are *Bal31* insensitive. These results suggest that telomere-like and UTAS-like sequences are also located in non-telomeric regions in the *U. maydis* genome. Thus, it would be interesting to investigate further the chromosomal location of telomere-like and UTAS-like sequences in *U. maydis*.

ACKNOWLEDGEMENTS

We wish to thank Eric Richards and William Holloman for plasmids and strains, Beatriz Jiménez for DNA sequence analysis and Gabriela Olmedo for critical review of the manuscript. This work was supported in part by research grants 0024N and 0072N from Consejo Nacional de Ciencia y Tecnología and 334.9 from Consejo del Sistema Nacional de Educación Tecnológica, México, to P.G.

REFERENCES

- Allshire, R. C., Gosden, J. R., Cross, S. H., Cranton, G., Raut, D., Sugawara, N., Szostak, J. W., Fantes, P. A. & Hastie, N. D. (1988). Telomeric repeat from *T. thermophila* cross hybridizes with human telomeres. *Nature* **332**, 650–659.
- Banuett, F. & Herskowitz, I. (1989). Different *a* alleles of *Ustilago maydis* are necessary for maintenance of filamentous growth but not for meiosis. *Proc Natl Acad Sci USA* **86**, 5878–5882.
- Blackburn, E. H. (1991). Structure and function of telomeres. *Nature* **350**, 569–573.
- Blackburn, E. H. (1992). Telomerases. *Annu Rev Biochem* **61**, 113–129.
- Blackburn, E. H. & Challoner, P. B. (1984). Identification of a telomeric DNA sequence in *Trypanosoma brucei*. *Cell* **36**, 447–457.
- Brown, W. R. A., MacKinnon, P. J., Villasante, A., Spurr, N., Buckle, V. J. & Dobson, M. J. (1990). Structure and polymorphism of human telomere-associated DNA. *Cell* **63**, 119–132.
- Chan, C. S. M. & Tye, B.-K. (1980). Autonomously replicating sequences in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **77**, 6329–6333.
- Chan, C. S. M. & Tye, B.-K. (1983a). A family of *Saccharomyces cerevisiae* repetitive autonomously replicating sequences that have very similar genomic environments. *J Mol Biol* **168**, 505–523.
- Chan, C. S. M. & Tye, B.-K. (1983b). Organization of DNA sequences and replication origins at yeast telomeres. *Cell* **33**, 563–573.

labelled 1), the probe was then removed and the membrane was rehybridized with the *U. maydis* UTAS probe (lanes labelled 2). Probes are the same as for Fig. 2.

- Church, G. M. & Gilbert, W. G. (1984). Genomic sequencing. *Proc Natl Acad Sci USA* **81**, 1991–1995.
- Foote, S. J. & Kemp, D. J. (1989). Chromosomes of malaria parasites. *Trends Genet* **5**, 337–342.
- Forney, J., Henderson, E. R. & Blackburn, E. H. (1987). Identification of the telomeric sequence of the acellular slime molds *Didymium iridis* and *Physarum polycephalum*. *Nucleic Acids Res* **15**, 9143–9152.
- Fotheringham, S. & Holloman, W. K. (1989). Cloning and disruption of *Ustilago maydis* genes. *Mol Cell Biol* **9**, 4052–4055.
- Ganal, M. W., Lapitan, N. L. V. & Tanksley, S. D. (1991). Macrostructure of the tomato telomeres. *Plant Cell* **3**, 87–94.
- Harley, C. B., Futcher, A. B. & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature* **345**, 458–460.
- Hastie, N. D., Dempster, M., Dunlop, M. G., Thompson, A. M., Green, D. K. & Allshire, R. C. (1990). Telomere reduction in human colorectal carcinoma and with ageing. *Nature* **346**, 866–871.
- Holliday, R. (1974). *Ustilago maydis*. In *Handbook of Genetics*, pp. 575–595. Edited by R. C. King. New York: Plenum Press.
- Kinscherf, T. G. & Leong, S. A. (1988). Molecular analysis of the karyotype of *Ustilago maydis*. *Chromosoma* **96**, 427–433.
- Kipling, D. & Cooke, H. J. (1992). Beginning or end? Telomere structure, genetics and biology. *Hum Mol Genet* **1**, 3–6.
- Kronstad, J. W. & Leong, S. A. (1989). Isolation of two alleles of the *b* locus of *Ustilago maydis*. *Proc Natl Acad Sci USA* **86**, 978–982.
- Kronstad, J. W., Wang, J., Covert, S. F., Holden, D. W., McKnight, G. L. & Leong, S. A. (1989). Isolation of metabolic genes and demonstration of gene disruption in the phytopathogenic fungus *Ustilago maydis*. *Gene* **79**, 97–100.
- Lumblad, V. & Szostak, J. W. (1989). A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* **57**, 633–643.
- Meyne, J., Ratliff, R. L. & Moyzis, R. K. (1989). Conservation of the human telomere sequence TTAGGG_n among vertebrates. *Proc Natl Acad Sci USA* **86**, 7049–7053.
- Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meyne, J., Ratliff, R. L. & Wu, J.-R. (1988). A highly conserved repetitive DNA sequence, (TTAGGG)_n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* **85**, 6622–6626.
- Newlon, C. S. (1988). Yeast chromosome replication and segregation. *Microbiol Rev* **52**, 568–601.
- Petracek, M. E., Lefebvre, P. A., Silflow, C. D. & Berman, J. (1990). *Chlamydomonas* telomere sequences are A + T-rich but contain three consecutive GC base pairs. *Proc Natl Acad Sci USA* **87**, 8222–8226.
- Richards, E. J. & Ausubel, F. M. (1988). Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. *Cell* **53**, 127–136.
- Richards, E. J., Goodman, H. M. & Ausubel, F. M. (1991). The centromere region of *Arabidopsis thaliana* chromosome 1 contains telomere-similar sequences. *Nucleic Acids Res* **19**, 3351–3357.
- Sambrook, J. E., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning, a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schechtman, M. G. (1990). Characterization of telomere DNA from *Neurospora crassa*. *Gene* **88**, 159–165.
- Schulz, B., Banuett, F., Dahl, M., Schlesinger, R., Schäfer, W., Martin, T., Herskowitz, I. & Kahmann, R. (1990). The *b* alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. *Cell* **60**, 295–306.
- Sherman, F., Fink, G. R. & Hicks, J. B. (1986). *Methods in Yeast Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Southern, E. M., Brown, W. R. A. & Fletcher, D. S. (1987). A model for the separation of large DNA molecules by crossed field gel electrophoresis. *Nucleic Acids Res* **15**, 5925–5943.
- Tsukuda, T., Carleton, S., Fotheringham, S. & Holloman, W. K. (1988). Isolation and characterization of an autonomously replicating sequence from *Ustilago maydis*. *Mol Cell Biol* **8**, 3703–3709.
- Van der Ploeg, L. H. T., Liu, A. Y. C. & Borst, P. (1984). Structure of the growing telomeres of trypanosomes. *Cell* **36**, 459–468.
- Walmsley, R. W., Chan, C. S. M., Tye, B.-K. & Petes, T. D. (1984). Unusual DNA sequences associated with the ends of yeast chromosomes. *Nature* **310**, 157–160.
- Wang, J., Holden, D. W. & Leong, S. A. (1988). Gene transfer system for the phytopathogenic fungus *Ustilago maydis*. *Proc Natl Acad Sci USA* **85**, 865–869.
- Yu, G.-L., Bradley, J. D., Attardi, L. D. & Blackburn, E. H. (1990). *In vivo* alteration of telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. *Nature* **344**, 126–132.
- Zakian, V. A. (1989). Structure and function of telomeres. *Annu Rev Genet* **23**, 579–604.
- Zakian, V. A. & Blanton, H. M. (1988). Distribution of telomere-associated sequences on natural chromosomes in *Saccharomyces cerevisiae*. *Mol Cell Biol* **8**, 2257–2260.

Received 16 July 1993; accepted 2 December 1993.