Organization of Chromosome Ends in Ustilago maydis. RecQ-like Helicase Motifs at Telomeric Regions

Patricia Sánchez-Alonso and Plinio Guzmán

Department of Genetic Engineering of Plants, Centro de Investigación y de Estudios Avanzados del IPN,
UNIDAD IRAPUATO, Irapuato, Gto., 36500 México

Manuscript received September 5, 1997
Accepted for publication November 12, 1997

ABSTRACT

In this study we have established the structure of chromosome ends in the basidiomycete fungus Ustilago maydis. We isolated and characterized several clones containing telomeric regions and found that as in other organisms, they consist of middle repeated DNA sequences. Two principal types of sequence were found: UTASa was highly conserved in nucleotide sequence and located almost exclusively at the chromosome ends, and UTASb was less conserved in nucleotide sequence than UTASa and found not just at the ends but highly interspersed throughout the genome. Sequence analysis revealed that UTASa encodes an open reading frame containing helicase motifs with the strongest homology to RecQ helicases; these are DNA helicases whose function involves the maintenance of genome stability in Saccharomyces cerevisiae and in humans, and the suppression of illegitimate recombination in Escherichia coli. Both UTASa and UTASb contain a common region of about 300 bp located immediately adjacent to the telomere repeats that are also found interspersed in the genome. The analysis of the chromosome ends of U. maydis provides information on the general structure of chromosome ends in eukaryotes, and the putative RecQ helicase at UTASa may reveal a novel mechanism for the maintenance of chromosome stability.

The ends of eukaryotic chromosomes harbor specialized structures that are essential for chromosome maintenance. These regions consist mostly of repeated DNA sequences, with the ends capped by the telomeres. Telomeres protect and track chromosomes for integrity, permit the complete replication of the terminal regions of the chromosomes, and participate in the correct separation of sister chromatids during mitosis (Zakian 1996; Kirk et al. 1997). Telomeres most commonly consist of tandemly repeated short DNA sequences (5 to 8 bp in length), containing clusters of G residues oriented 5' to 3' toward the chromosome end. These short repeats are highly preserved in evolution since identical sequences are found in the telomeres of divergent organisms (Zakian 1996). Large repeated sequences of the non-long terminal repeat retropon/ type family also have been found as chromosome ends; these are rare, however, and have been found only at the chromosome ends of Drosophila (Levis et al. 1993).

Regulation of telomere length during development and the cell cycle is essential; alteration of telomere length affects cell survival (Greider 1996). Knowledge of the various elements necessary to synthesize and maintain telomeres, and to regulate telomere length is now emerging. One such element is telomerase, a telomere-specific ribonucleoprotein polymerase that synthesizes the telomeric repeats. The RNA component in this enzyme contains sequences complementary to the telomeric repeat for which it is the template (Blackburn 1992). Components of the telomerase have been isolated from various organisms; genes encoding protein components, initially isolated from ciliated protozoa (Collins et al. 1995), have some homologs in mammals (Harrington et al. 1997; Nakayama et al. 1997). A catalytic component of the telomerase, encoding reverse transcriptase motifs, has also been isolated from S. cerevisiae, euplotes, Schizosaccharomyces pombe, and humans (Lingner et al. 1997; Counter et al. 1997; Nakamura et al. 1997; Meyerson et al. 1997). Likewise, the RNA components have been cloned from Saccharomyces cerevisiae, Kluveromyces lactis, mammals and from several ciliated protozoa (Singer and Gottschling 1994; M cazuela and Blackburn 1995; Blasco et al. 1995; Greider 1996). The mechanism controlling telomere length also seems to have common features in non-related eukaryotes. For instance, proteins that bind to the telomeric repeats in S. cerevisiae, S. pombe, and humans, negatively regulate telomere elongation (Marcand et al. 1997; Cooper et al. 1997; van Steensel and de Lange 1997). These proteins have a similar DNA-binding domain and are thought to operate by preventing elongation of the telomere repeat (Shore 1997).

A complex mixture of middle repeated DNA se-
quences is commonly found adjacent to the telomeric repeats (Zakian 1996). These sequences, which are known as telomere-associated sequences (TAS), are highly polymorphic in both length and distribution in the genome, and are not as conserved as the short telomeric repeats. There is growing information about the organization of TAS. In S. cerevisiae, Plasmodium and humans (Zakian 1996; Dore et al. 1990; Brown et al. 1990), these sequences consist of a series of middle repeated sequences, which are found in some but not all the chromosomes of the same organism, where they often also display a varying distribution between different chromosomes. In S. cerevisiae, two types of telomere associated sequences, called X and Y, are mainly found. Y has been well characterized; it is a highly conserved element present as a single copy or as tandemly repeated copies at the telomeres of some of the chromosomes and it occurs in two classes of 5.3 or 6.7 kb (Chan and Tye 1983a; Chan and Tye 1983b; Zakian and Blanton in press). X is a composite of sequences less conserved with a core of 475 bp that is present in all chromosome ends (Louis et al. 1994; Pryde et al. 1995; Louis 1995). Plasmodium falciparum TAS contain repetitive elements extending over 60 kb from the telomere repeats. The arrangement of the repetitive elements is preserved in several of the chromosome ends, suggesting a conserved structural organization in this region (Corcoran et al. 1988). In humans, TAS contain a diverse array of repeated sequences, which show a polymorphic distribution in the genome and distinct domains have been predicted in such regions (Brown et al. 1990; Flint et al. 1997). Comparison of TAS shows evidence of structural similarities in such regions among distant species; distinct equivalent domains have been inferred from both human and yeast (Flint et al. 1997). Little is known about the function of TAS, although it has been suggested that they have an active role in modulating telomere function. Studies in S. cerevisiae indicate that under circumstances of loss of the terminal repeat, TAS are rearranged and amplified within the telomeric regions, restoring telomere function (Lundblad and Blackburn 1993). In P. falciparum and other intracellular protozoan parasites, gene conversion between the telomere-proximal antigenic genes has been described as a strategy to enhance genetic variation (Borst and Rudenko 1994; Hernandez-Rivas et al. 1997). In these species TAS also promote chromosome pairing and facilitate meiotic recombination (de Bruin et al. 1994).

We have adopted the fungus U. maydis, a basidiomycete that causes the smut disease in maize, as a system for genome organization studies. U. maydis possesses various of the advantages of the favorite model S. cerevisiae for this type of analysis. The size of the genome and the haploid chromosome number is similar to that of S. cerevisiae and molecular genetic approaches to address many biological phenomena are routinely pursued in this fungus (Kirschner and Leong 1988; Wang et al. 1988; Kronstad et al. 1989; Fotheringham and Holloman 1989; Bolker et al. 1995). We have previously characterized segments of chromosome ends in U. maydis. The sequence TTAGGG, which is a common telomeric repeat present in many other eukaryotes, ranging from protozoans to humans, was found tandemly repeated at least 37 times at the chromosome termini in U. maydis. In addition, a 376-bp segment of Ustilago TAS (UTAS) was isolated and shown to be immediately adjacent to the telomeric repeat in many or all of the chromosomes (Guzman and Sanchez 1994). To pursue our work on the structure of U. maydis chromosomes, we have further characterized the terminal regions. Here, we report the identification of two principal types of UTAS in U. maydis. In one of them, a novel feature that may have a role in chromosome structure and function was found: an element encoding a helicase showing homology to RecQ helicases.

**MATERIALS AND METHODS**

**Strains and media:** Escherichia coli strain XL1-blue MRF’(F’ lacIq recA1 endA1 gyrA96 thi-1 supE44 relA1 lacI proAB lacI q ZAM15 Tn10) from Stratagene (La Jolla, CA) was used as host for plasmids and lambda phage clones. LB media supplemented with carbenicillin (50 μg/ml) were used to grow E. coli strains. U. maydis strain FB2a2b2 (Bauuett and Herskowitz 1989) was provided by Flora Bauuett, University of California in San Francisco, and 12, a wild isolate, by Octavio Paredes at CINVESTAV, Unidad Irapuato. U. maydis strains were grown in YEPS media (1% yeast extract, 1% peptone, and 1% sucrose) at 30°C or in minimal medium (Holliday 1974).

**Isolation of clones containing telomeric DNA:** A chromosome-end enriched library was constructed and screened as previously described (Guzman and Sanchez 1994). EcoRI-generated restriction fragments of U. maydis DNA were selected to generate the library. These DNA fragments were ligated at high concentration to double digested and alkaline phosphatase-treated EcoRI HinfI Bluescript KS vector (Stratagene), using a 10-fold molar excess of vector to genomic DNA. The ligation mixture was then transformed into E. coli XL1-blue MRF’. The telomeric repeated sequence TTAGGG, at a concentration of 5 × 10⁶ cpm/ml, was used as a probe to screen the chromosome-end enriched library. To isolate non-telomeric fragments related to the junction fragments between TAS and the telomeric repeat a genomic XEMBL3 library of U. maydis (kindly donated by R. Kahmann) were screened with probe UT1-a (see Figure 1).

**DNA manipulation and Bal31 sensitivity essays:** Procedures for the preparation of DNA from U. maydis and for the Bal31 treatments were previously described (Guzman and Sanchez 1994). Southern blotting was done with nylon membranes (Hybond N+; Amersham) and hybridizations were carried out in a 0.5 M Na2HPO4 (pH 7.2), 7% SDS, 1 mm EDTA, and 1% BSA solution at a probe concentration of 10⁶ cpm/ml. Hybridizations were done 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 to room temperature; this treatment was repeated at least twice.

**DNA sequence determination and analysis:** The DNA sequence was determined using Sequenase 2.0 (United States Biochemical, Cleveland, OH). Single stranded DNA for se-
were stained with ethidium bromide (1 mg/ml) for 30 min, then destained in distilled water. The DNA bands were visualized with a UV transilluminator. *U. maydis* chromosomes were sized by comparison with *S. cerevisiae* chromosomes, purchased from Bio-Rad Laboratories.

**RESULTS**

Two principal classes of chromosome ends are present in *U. maydis*: In the course of the characterization of telomeric regions from *U. maydis*, we isolated a segment of about 380 bp of a telomere-associated sequence (UTAS), which was found to be immediately adjacent to the telomeric repeats in many or all of the chromosome ends (Guzmán and Sánchez 1994); we speculated that this segment of UTAS would be part of a larger structural component of chromosome ends. Since we were interested in examining in detail the chromosome ends of *U. maydis*, we set out to isolate and characterize a larger segment of UTAS from various chromosomes, which would permit us to define the basic structure of these regions. For this purpose, a library enriched for chromosome ends was constructed from the standard laboratory strain FB2. Twenty-five of about 2400 clones hybridized to telomere repeats. Analysis of 15 of these clones, containing DNA inserts ranging from 1.8 to 2.8 kb, showed that they also hybridized to a region of UTAS located immediately adjacent to the telomeric repeat (probe UT1-a, Figure 1). This result confirmed that the telomeric repeat and the adjacent UTAS were included in the same DNA segment in many of the chromosome ends. The restriction map of five such clones, named UT4 to UT8, and the location of some of the probes used in this study are shown in Figure 1. Using DNA segments from various regions of the cloned UTAS as probes, we found that some of them only hybridized to certain sets of clones (data not shown). Two segments named UT4-a and UT6-b, were selected as probes for further analysis of the chromosome ends; UT4-a hybridized to UT5 but not to UT6, UT7, or UT8, and UT6-b hybridized to UT7 and UT8, but not to UT4 or UT5.

**Organization of UT4-a and UT6-b sequences in the *U. maydis* genome:** To determine the organization of UTAS we initially carried out a series of Southern blot analyses on three different restriction digests of genomic DNA. Since in previous work genetic variation between *U. maydis* strains was detected using probes derived from chromosome ends, we decided to include in the analysis two strains from a different source (Sánchez-Alonso et al. 1996). One strain was FB2, a standard laboratory strain and the other was strain I2, a wild isolate. The two probes from UTAS, UT4-a, and UT6-b, showed different patterns of hybridization (Figure 2). UT4-a detected a prominent signal with DNA from FB2 in each of the three digests and about four signals of minor intensity in two of the digests (Figure 2, left panel). These results suggest that multiple copies
Figure 2.—Analysis of the distribution of UT4-a and UT6-b sequences in the *U. maydis* genome. DNA preparations from strains FB2 and I2 were digested to completion with restriction enzymes: *Hin* II (H), *Pst* I (P), *Eco* RI (E). DNA was size-fractionated by agarose gel electrophoresis and transferred to a nylon membrane. The blot was hybridized under high stringency conditions to probe UT-4a and then to probe UT-6b after stripping. The removal of the first probe was verified by exposure of the membrane to X-ray film. The positions of the molecular size markers (1 kb ladder from GIBCO BRL, Grand Island, NY) are shown on the left.

of UT4-a, most of them of very similar length, were detected in strain FB2 (see below). In a similar manner, a single signal that seems to coincide in size with the prominent signal observed with FB2, was detected for all digests of DNA from strain I2 (Figure 2, second panel). Analysis of the pattern with UT6-b indicates that this probe detected multiple hybridizing signals in a wide range of sizes and of different intensities in DNA both from strain FB2 and strain I2 (Figure 2, third and fourth panels); in general, the intensity of the signals was stronger for DNA from FB2 than from I2. These Southern blot results suggest that UT4-a and UT6-b are two classes of repeated DNA sequences that show differences in copy number and distribution within the genome as well as between strains.

*Bal*31 sensitivity analysis was then performed to ascertain the location of the probes at the chromosome ends. *U. maydis* DNA was progressively digested for increasing lengths of time with the exonuclease *Bal*31 followed by digestion with *Eco* RI. Southern blot analysis revealed that a shift of the hybridization signal toward a lower molecular mass occurred for two of the signals detected with FB2 when the UT4-a probe was used. The shift occurred in the most prominent signal (3-4 kb region in Figure 3A, left panel) and in one of the minor signals (1.2 kb region in Figure 3A, left panel); the single signal detected for strain I2 was also sensitive to the exonuclease (Figure 3B, left panel). These results suggest...
Figure 4.—Location of UT4-a and UT6-b sequences on the U. maydis chromosomes. Chromosome preparations of U. maydis strains FB2 (first three lanes) and I2 (last three lanes) were subjected to PFGE in a CHEF apparatus; separated chromosomes were transferred to a nylon membrane. Blots were hybridized under high stringency conditions with probes UT4-a, UT6-b, and TTAGGGn, as indicated. The sizes in kilobase pairs of the U. maydis chromosomes is shown on the left; they were estimated by comparison to the size of the S. cerevisiae chromosomes and to the lambda ladder (GIBCO BRL).

that UT4-a sequences are mainly located at the chromosome ends in both U. maydis strains. In addition, strain FB2 contains at least five copies of the sequence that are insensitive to the exonuclease, indicating that they are not located at the chromosome termini. Sensitivity analysis with UT6-b sequences showed a different result. In this case, only a few fragments showed a shift toward the lower molecular mass with FB2 and with I2 (Figure 3, A and B, second panels, pointed by an arrow). This result suggests that most of the fragments detected by UT6-b are interspersed in the genome and not exclusively at the chromosome termini. As controls to assess the proficiency of the Bal31 digests, the filters were probed with the telomeric repeat and then with rDNA, a non-telomeric probe. With the first probe, most of the hybridizing signal rapidly disappeared after 10 minutes in both strains (Figure 3, A and B, third panels) and with the non-telomeric probe, the shift toward a lower molecular weight was not observed (Figure 3, A and B, last panels).

The location of UT4-a and UT6-b on the U. maydis chromosomes was then determined. Southern blot analysis using these two probes was performed on the electrophoretic karyotype of FB2 and I2 (Figure 4). The two strains displayed differences in the pattern of hybridization. While at least fifteen chromosomes hybridize with the UT4-a probe on DNA from strain FB2 (Figure 4, lane 1), only two showed a signal with DNA from I2 (Figure 4, lane 4; second smallest and largest bands). This observation indicates that most of the signals that UT4-a detects for strain FB2 (Figure 2A, right panel) and for strain I2 (Figure 2B, right panel), consist of various copies of a highly related sequence. Densitometric analysis of the hybridizing chromosomes indicates that the intensity of the signals obtained with UT6-a and the telomeric repeat probes was comparable, suggesting that a similar number of copies of UT4-a are present in each of the hybridizing chromosomes (data not shown).

UT6-b detects sequences that were present in most of the chromosomes in the two U. maydis strains tested. Densitometric analysis of the hybridizing chromosomes showed differences in the intensity of the hybridizing signals among some of the chromosomes in the same strain when compared to the TTAGGGn probe (compare the three smallest chromosomes, Figure 4, second and third lanes), suggesting that a different number of copies of UT6-b are present in each of the hybridizing chromosomes (data not shown). This preceding analysis on the organization of UT4-a and UT6-b sequences, and subsequent nucleotide sequence determination, indicate that these two probes are part of two classes of UTAS. Hence forth, we will refer to these two classes of chromosome ends showing homology to UT4-a and UT6-b as UTASa and UTASb, respectively.

DNA sequence analysis of UTASa: DNA sequence analysis of the UTASa clones, UT4 and UT5, showed a 90% identity between them in the overall 2.7-kb insert; this result supports the assumption that UTASa consists of highly homologous sequences. Some minor differences are observed that correspond to two deletions of fifteen and seven nucleotides in UT5 (positions 1610-1624 and 2694-2700, respectively; see Figure 5) and to four-single nucleotide deletions occurring in the last 400 bp of UT4. Conceptual translation of the sequences revealed open reading frames encoded in the clones that are not altered by the differences observed between the two sequences (see below); these ORFs may be part of longer ORFs whose complete sequence is not included within the cloned fragment.

An ORF containing helicase domains homologous to the RecQ family of DNA helicases is encoded in UTASa: Examination of sequence homology of the predicted product of translation found in UTASa to sequences in the databases revealed that it encodes a putative helicase. The homology is composed of the seven canonical sequences that are conserved among helicases, including domain I, which corresponds to the A motif of ATases, domain II, which is probably involved in ATP binding and/or ATP hydrolysis, and domain VI, which may participate in nucleic acid interaction (Gorbalenya et al. 1989; Matson et al. 1994). This comparison with the databases showed that the best homologies are obtained with RecQ helicases, a particular class of DNA...
Figure 5.—DNA sequence of UT4As chromosome ends. The alignment of the nucleotide sequences of UT4 and UT5 is shown. The sequence is written in a 5’ to 3’ direction towards the chromosome end, which corresponds to the telomeric repeats; identity is indicated by |, a missing nucleotide by (*). Open reading frames of 758 residues in UT4 and of 753 in UT5 initiate at the 5’-end of the sequence and end at the boxed stop codon located at nucleotide 2273. The number on the right indicates the position of the nucleotide residue. Sequences inferred to be at the beginning of a region conserved in almost all chromosome ends are underlined with a broken line. UT4 and UT5 have accession numbers AF030885 and AF030886 in the GenBank database, respectively.
helicases that include gene products from diverse organisms including E. coli (Irino et al. 1986), S. cerevisiae (Gangloff et al. 1994; Watt et al. 1995), as well as recently described genes involved in Bloom and Werner syndromes in humans (Ellis et al. 1995; Yu et al. 1996) (Figure 6).

DNA sequence analysis of UTASb: The nucleotide sequence of three UTASb clones was determined. The insert that hybridized to UT6-b was different in size for each of the clones; about 2.8 kb for UT6, 2.0 kb for UT7 and 1.6 kb for UT8. The DNA sequence analysis revealed that UT7 and UT8 were very similar, having 87% identity in the end-most 1.4 kb of UTAS. The analysis of the nucleotide sequence and of short ORFs detected by the conceptual translation of the sequences revealed that UT7 and UT8 were very similar, having 87% identity in the end-most 1.4 kb of UTAS.

UT6 also shows three duplicated sequences within the insertion fragments (marked as 1, 2, and 3 in Figure 7), and a 262 nucleotide deletion at the chromosome end when compared to UT8. Interestingly, the sequence CCTAACCTAACCCTAA (position 718–736, Figure 7), which coincides with complementary telomeric repeats, is interrupted by two DNA insertions in UT6; one is 65 nucleotides long (position 717 to 782, Figure 7) and the other is 865 nucleotides long (position 1620 to 2485, Figure 7).
and that UTASb sequences are also found interspersed in the genome. A general organization of the U. maydis chromosome-end regions is depicted in Figure 8.

**Identification of UTAS interspersed in the genome:**
Comparison of the nucleotide sequence of the five UT clones revealed a region highly conserved in all of them. This region is located immediately adjacent to the telomeric repeat and extends for about 300 bp (from the broken line to the end of the sequence on Figures 5 and 7) and in previous work, similar sequences were inferred to occur in non-telomeric regions of the genome (Guzmán and Sánchez 1994). To establish the relationship between the sequences located in telomeric and non-telomeric regions we set out to isolate sequences from non-telomeric regions. We screened a genomic λEMBL3 library of U. maydis with a probe containing the conserved region of UTAS (probe UT1-a, Figure 1). We found that about 1% of the screened clones hybridized to this probe, indicating that as in the case of UT4-a and UT6-b this particular sequence was highly represented in the U. maydis genome. Since the λEMBL3 library was generated by Sau3A1 partial digestion of DNA, Sau3A1 fragments containing telomeric DNA were expected to be under-represented; in fact, no positive clone was found to hybridize to telomeric repeats (data not shown). Restriction digestion and Southern hybridization analysis of ten positive clones revealed a different pattern for each one and a common 200-bp PstI fragment which hybridized to the UTAS probe (data not shown). From four of the clones this 200-bp fragment was cloned and its nucleotide sequence determined; these clones were named UTL1, UTL2, UTL3, and UTL4, for UT-Like sequences. The alignment between these four clones showed a high degree of identity between them over the length of the PstI segment and with a region of UTAS of the U. clonies; the homologous region with UTAS was in the PstI segment adjacent to the telomeric repeat (Figure 9). These observations indicate that a similar type of repeated element is found adjacent to the telomeric repeat in Ustilago chromosomes and interspersed in the genome, suggesting that these sequences have a common origin and that they are important structural components of the U. maydis genome. Further examination of the nucleotide sequence of these regions reveals possible sequence duplications that in some of the clones may correspond to tandem arrays (marked as 1, 2, and 3 in Figure 9).

**DISCUSSION**

The analysis of TAS has been addressed for only a few organisms. These regions of the chromosomes consist commonly of middle repetitive DNA sequences that may vary in their copy number and occurrence in the genome of an individual, and between individuals of the same species. Our analysis of clones containing chromosome ends indicates that this is also the case in U. maydis. In this work we described two primary classes of TAS from U. maydis, referred to as UTASa and UTASb. Since genomic clones carrying UTASa also hybridize to a probe of UTASb, the latter are probably present in all of the chromosome ends. UTASa, defines a repeated DNA sequence that is located almost exclusively at the chromosome ends and that is highly conserved in nucleotide sequence. UTASb defines a different type of repeated element that is highly abundant and also found interspersed in the genome. Analysis of the end-most regions reveals that break and fusion events have probably occurred in this region. Both classes of UTAS contain a common region of about 300 bp that is located immediately adjacent to the telomere repeats; this sequence is present at almost all of the chromosome ends and is also found interspersed in the genome. Additional DNA sequence analysis of the chromosome termini and characterization of other chromosome termini will determine the length of the repeated elements described in this work and their structure and organization in the U. maydis genome.

Common features between UTASa and the S. cerevisiae
Figure 9.—DNA sequence alignment of UT and UTL clones. The complete sequence of the PstI-PstI fragment from four UTL clones is aligned to the corresponding region in the UT clones; clone UT6 was not included in the alignment because sequence rearrangements have probably occurred in this clone (see text, and Figure 7). The sequence from PstI to the telomeric end from UT-1, UT-3, UT-4, UT-5, UT-7, and UT-8 is shown ending with TTAGGG repeats. Positions of identity in at least 5 of the 10 clones or in at least 2 of the UTL clones are shadowed. The numbers 1, 2, and 3 denote repeated sequences predicted from the alignment. In region 1, 15 out of 18 residues are identical and in region 2, 11 of 12 are identical. Region 3 corresponds to four tandem duplications of an eight bp sequence; the third and fourth repeats are identical and when compared to the first two, 6 out of 8 nucleotides are identical. UTL1, UTL2, UTL3, and UTL4 have accession numbers AF030890, AF030891, AF030892, and AF030893 in the GenBank database, respectively.

Y′ sequences can be predicted. For instance, the UTASa element is highly conserved in nucleotide sequence, it is preferentially located at the chromosome ends, it is found in some but not all chromosomes and it varies in copy number and chromosome location among strains. These are all features shared with the S. cerevisiae Y′ sequences (Zakian and Blanton 1988; Louis and Haber 1992). Another significant observation is that both Y′ and the UTASa encode an ORF and for both, the predicted amino acid sequence revealed the seven conserved domains found in helicases (Louis and Haber 1992). No significant homology of the helicase at Y′ to the helicase present at UTAS was detected. However, these helicases may be related, since both show the DExH box in domain II with the corresponding variation QxxGRxxR in domain VI. This reciprocity has been previously suggested in helicases; polypeptides with the DExD box show the sequence HxxGRxxR in domain VI whereas those presenting DExH show the sequence QxxGRxxR (Gorbalenya et al. 1989). All these similarities provide evidence that UTASa and Y′ may have equivalent roles in the U. maydis and S. cerevisiae genomes. The finding of a similar type of element at the chromosome termini of these two fungi is a meaningful feature, suggesting that the location of helicases at the chromosome ends may have structural and functional significance. The finding of a polymorphic distribution of UTASa sequences and variation in copy number of this sequence among strains, indicates that the helicase is not essential for the functioning of individual chromosomes. One can speculate that this type of telomeric region. It would be interesting to determine whether elements with the properties of Y′ and UTASa are present at the chromosome ends of other organisms.

The helicase found at UTASa is most closely related to the RecQ family of DNA helicases. Analysis of the phenotypes associated with recQ helicases suggest that the function of this type of helicase is conserved from bacteria to mammals. Members of this family that have
been isolated are likely to be involved in maintenance of genome stability; these are the S. cerevisiae SG51 gene (Watt et al. 1996) and the human BLM and WRN genes, for Bloom’s and Werner’s syndromes, respectively (Ellis et al. 1995; Yu et al. 1996). An abnormal increase of mitotic recombination is observed in S. cerevisiae sg51 mutants and in Bloom’s syndrome cell lines; an abnormal replication and diverse levels of chromosomal translocations and deletions are observed in Werner’s syndrome cell lines. The function of the E. coli recQ gene correlates with these observations, since it has been shown to be a suppressor of illegitimate recombination (Hanada et al. 1997). Premature aging phenotypes are also associated with alterations in some of these helicases; individuals with the Werner’s syndrome show several symptoms of premature aging and the S. cerevisiae sg51 mutants display alterations that can be correlated with aging disorders (Sinclair et al. 1997). It could be speculated that the helicases encoded at UTASa may have a role in maintaining genome stability. A single helicase gene expressed at a given time might be sufficient for this function. In such a case, the distinct location of this recQ-like helicase at chromosome ends may be of functional importance. The function of the helicase at UTASa may be related to that of SG51 and WRN gene products, but having a specific mechanism to regulate its expression. The UTASa helicase may be subject to a telomere-position effect, with its expression being modulated by the silencing mechanism occurring at the telomere region; such a mechanism would not function under normal conditions, but would be turned on under circumstances of defective telomere maintenance. It has been shown in Drosophila, S. cerevisiae, S. pombe, and trypanosoma genes that are transcriptionally active in an endogenous location of the chromosome become transcriptionally inactive when placed near the telomere (Levis et al. 1985; Gottsching et al. 1990; Nimmo et al. 1995; Horn and Cross 1995).

In conclusion, the analysis of telomere associated sequences in U. maydis provides information on the structure and potential functions of these chromosomal regions. A novel feature that may be important for chromosome function is predicted; a putative helicase of the RecQ family. These observations are consistent with the general knowledge that the structure of TAS consists of middle repeated sequences and their involvement in recombination events. Further characterization of the putative RecQ-like helicase present in UTASa will reveal more information on the possible role of UTAS in a mechanism to ensure chromosome stability and on its role structuring telomeric regions.

We thank Flora Banuett and Octavio Paredes for Ustilago strains, Regina Kahmann for the Ustilago genomic library in AEMBL3, Beatriz Jiménez and Guillermo Corona for DNA sequencing, Cristina García de la Cueva for technical assistance, and Gabriel Olmedo, June Simpson, William K. Holloman, and an anonymous reviewer for critical analysis of the work and comments on the manuscript. P.S. acknowledges a scholarship from CONACyT México. This work was supported in part by grants from CONACyT, México to P.G.


