Evolution and Organization of a Highly Dynamic, Subtelomeric Helicase Gene Family in the Rice Blast Fungus *Magnaporthe grisea*

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ABSTRACT

Sequence analysis of a 13-kb telomeric region in O-137, a rice pathogenic isolate of *Magnaporthe grisea*, uncovered a novel gene, designated *TLH1* (*t*elomere-*l*inked *h*elicase 1). The *TLH1* gene is a member of a gene family, and the sequences flanking this gene family have also been amplified. Genetic mapping showed that most members of the *TLH* gene family are tightly linked to the telomeres. A physical mapping technique, termed RecA-mediated Achilles' heel cleavage, and cloning and sequencing of two additional telomeres of O-137 associated with the *TLH* gene family confirmed that most members of the *TLH* gene family are located within 10 kb from the telomeric repeat. A survey of *M. grisea* strains from diverse hosts revealed that the gene family is ubiquitously present among rice pathogens, but is absent from almost all isolates of hosts other than rice. The gene family appears to be highly dynamic, undergoing frequent deletion/amplification events. Given the presence of similar helicase gene families in chromosome ends of *Saccharomyces cerevisiae* and *Ustilago maydis*, the initial association of helicase genes with fungal telomeres might date back to very early stages of the fungal evolution.

WORLDWIDE, rice blast caused by the filamentous fungus *Magnaporthe grisea* (Hebert) Barr. (anamorph, *Pyricularia grisea* Sacc.) is one of the most economically devastating crop diseases (Valent and Chumley 1994). Management of rice blast via breeding blast-resistant varieties has had only short-term success due to the frequent breakdown of resistance under field conditions (Valent and Chumley 1994). Frequent appearance of new races (or pathotypes) of the fungus that are capable of infecting previously resistant varieties has been proposed as the principal cause for the loss of resistance (Ou 1980). Although the degree of race variation in *M. grisea* remains a controversial subject (Ou 1980; Bonman *et al.* 1987), molecular analysis of its genes controlling host specificity has begun to provide new insights into potential mechanisms underlying race variation (Sweigard *et al.* 1995; Orbach *et al.* 2000; Kang *et al.* 2001; Farman *et al.* 2002).

Rice pathogen O-137, a field isolate from China, is unable to infect a number of rice varieties, including Tsuyuake and Yashiro-mochi, because it carries the avirulence genes *AVR1-TSUY* and *AVR-Pita*, respectively (Valent and Chumley 1991). However, O-137 and its progeny frequently produce spontaneous mutants that are virulent on each of these varieties. The gain-of-virulence mutants of *M. grisea* on Yashiro-mochi have been shown to have a diverse array of mutations in *AVR-Pita*, including point mutations, deletions (ranging in size from 100 bp to >12.5 kb), and an insertion by transposon Pot3 (Orbach *et al.* 2000; Kang *et al.* 2001). Because the gene is located entirely within the most distal region (1.5 kb) of a chromosome, with its open reading frame (ORF) ending just 48 bp upstream of the telomeric repeat (Orbach *et al.* 2000), some of these mutations altered the telomere linked to *AVR-Pita*. Similarly, *AVR1-TSUY* is very closely linked to a chromosome end and appears to mutate spontaneously at a high frequency (Valent and Chumley 1994; Kang *et al.* 2000); spontaneous mutants of O-137, which are virulent on rice variety Tsuyuake, also exhibit frequent alteration or disappearance of a specific telomere (Kang *et al.* 2000; Kang 2001).

Additional host specificity genes in *M. grisea*, including *AVR1-Ku86*, *AVR1-MedNoi*, and *PWL1*, are also telomere linked (Kang *et al.* 1995; Dioh *et al.* 2000), suggesting that the presence of these genes in highly dynamic chromosome ends may provide a selective advantage to *M. grisea* by allowing it to adapt rapidly to new rice varieties. The association of telomeres with host specificity genes is not unique to *M. grisea*. *Phytophthora infestans*, the causal agent of late blight on potato and tomato, also carries several avirulence genes close to its telomeres (van der Lee *et al.* 2001). In addition, animal pathogenic microorganisms, such as *Plasmodium falciparum* (malaria pathogen) and *Pneumocystis carinii* (pneumonia pathogen), contain a large family of genes encoding major surface antigens in their telomeric regions

Sequence data from this articlehave been deposited with the EMBL/GenBank Data Libraries under accession nos. AY077623 (*TLH1* locus), AY077624 (pSK529), and AY077625 (pSK515).

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(Wada and Nakamura 1994; Henandez-Rivas *et al.* 1997), and frequent recombinations among these genes generate a large number of variants (Henandez-Rivas *et al.* 1997; Freitas-Junior *et al.* 2000). These observations support the hypothesis that telomeric locations may serve as a home for those genes whose frequent variation can confer an adaptive advantage to various pathogens in evading host recognition.

For a better understanding of the mechanisms underpinning the dynamics of chromosome ends in *M. grisea*, further characterization of the organization and structure of these regions is needed. In this work, we cloned and characterized three telomeres from O-137, which are associated with a novel, telomere-linked gene family. We report here the structure, distribution, putative function, and potential mechanisms underlying the evolution and dynamics of this gene family.

MATERIALS AND METHODS

Strains and growth conditions: Host and geographic origins of *M. grisea* field isolates used in this study are listed in Table 1. Laboratory strain 70-15 is a progeny from a cross between Guy11 and 66-10 (Chao and Ellingboe 1991). Ten generations of monoconidial cultures originated from 70-15 were produced in the following manner: For the first 2 generations, we picked three random spores from each culture growing on oatmeal agar to initiate new cultures for the next round of monoconidiation (nine cultures at the second generation). For the third generation, we isolated two spores from each of the nine cultures. From the fourth to the tenth generations, we picked a single spore from each culture, resulting in a total of 156 monoconidial cultures. *Escherichia coli* strain XL1-blue MRF′ was used for maintaining plasmids. The telomere-enriched λ library (Kang 2001) was screened using *E. coli* strain KW251 as a host.

Nucleic acid analyses: Genomic DNA was prepared and purified by CsCl gradient centrifugation as previously described (Kang *et al.* 2001), except that no CsCl gradient purification step was done for the monoconidial cultures derived from 70-15. The construction of a telomere-enriched λ library was previously described (Kang 2001). For Southern hybridizations, restricted genomic DNA was subjected to electrophoresis in 0.7% agarose gels using 1× TAE (40 mm Tris-acetate, pH 8.5, and 2 mm EDTA) buffer and blotted to Hybond N+ membranes (Amersham, Piscataway, NJ). Blots were hybridized with ^{32}P-labeled probes generated by random priming. Following hybridization at 65°, blots were washed twice (30-min washing at 65° in 2× SSPE, 0.1% SDS followed by 30-min washing at 65° in 0.1× SSPE, 0.1% SDS). For low-stringency hybridizations, blots were hybridized at 55°, followed by two 30-min washings at 55° in 2× SSPE, 0.1% SDS.

The DNA fragments used as hybridization probes (Figure 1) were prepared by either PCR (A–E) or digestion with restriction enzymes (probe TLH). With the exception of probe E, a 13-kb telomeric fragment in λGEM12 (Figure 1) was used as a template for PCR. The template for probe E was pSK224, a 1.4-kb *Hin*dIII-*Xho*I subclone in pBCSK. Primer sequences for individual probes are: probe A, 5′-CCCGCCGGTACGACC GTG-3′ and 5′-AAACGCGCTTGCCTCGTC-3′; probe B, 5′-ACC CCCAATTTTAATGCG-3′and 5′-GTAAGTATTAACAATTTGT-3′; probe C, 5′-TTTGTATCGTCCAACGAT-3′ and 5′-CGCGCC GAAAATCCGAAT-3′; probe D, 5′-TCGGCTCCAACTTCTCGT-3′ and 5′-CGGTCTTGTGTAGTGACA-3′; and probe E, T3 and 5′-CGCTTGCGGCCAGCATCG-3′. PCR amplification was carried out in a 50-μl reaction mixture consisting of 0.5 μl (2.5 units) of Ampli*Taq* DNA polymerase (Perkin Elmer, Boston), 5 μl 10× PCR buffer, 5 μl 10 mm dNTP mix, 1 μl each primer (20 pmol/μl), and 1 μl DNA template (0.2 μg/μl). PCR conditions included an initial denaturation at 96° for 1 min, 25 cycles of 94° for 1 min/50° for 15 sec/72° for 2 min, and a final 2-min elongation at 72°. PCR was performed in a PTC-100 DNA thermal cycler (MJ Research, Waltham, MA). The QIAquick spin column (QIAGEN, Valencia, CA) was used to purify PCR or restriction products.

Total RNA from strain O-137 was extracted from mycelia and infected plant materials using the TRI reagent protocol (MRC, Cincinnati). Poly(A) RNA was purified from total RNA using the Oligotex resin (QIAGEN). Total RNA was also isolated from perithecia derived from a cross between 70-6 and 70-15 (Chao and Ellingboe 1991) and from cultures of 70-15 that had been subjected to various stress conditions. RNA samples were separated on denaturing formaldehyde-agarose

TABLE 1

***M. grisea* field isolates used in this study**

Isolates	Host of origin	Country of origin
G-221	*Pennisetum clandestinum*	Japan
G-48	*Setaria italica*	United States
G-219	*Panicum maximum*	India
G-220	*Panicum repens*	India
G-58	*Panicum repens*	Philippines
G-200	*Eleusine indica*	Ivory Coast
G-167	*Eleusine indica*	Philippines
G-77	*Eleusine indica*	Philippines
G-26	*Eleusine indica*	Japan
G-172	*Eleusine coracana*	Uganda
G-22	*Eleusine coracana*	Japan
G-17	*Eragrostis curvula*	Japan
G-158	*Triticum* × *Secale*	Brazil
T29	*Triticum*	Brazil
T-5	*Triticum*	Brazil
O-27	*Oryza sativa*	United States
O-42	*Oryza sativa*	Japan
O-135	*Oryza sativa*	China
O-137	*Oryza sativa*	China
O-142	*Oryza sativa*	China
O-172	*Oryza sativa*	United States
O-188	*Oryza sativa*	United States
O-190	*Oryza sativa*	Korea
O-219	*Oryza sativa*	Ivory Coast
O-222	*Oryza sativa*	Guinea
O-224	*Oryza sativa*	Nigeria
O-225	*Oryza sativa*	Philippines
O-250	*Oryza sativa*	India
O-256	*Oryza sativa*	South Africa
O-261	*Oryza sativa*	Nepal
O-281	*Oryza sativa*	Egypt
O-284 (Guy11)	*Oryza sativa*	French Guyana
O-312	*Oryza glaberrima*	Cameroon
O-313	*Oryza glaberrima*	Ivory Coast
O-314	*Oryza glaberrima*	Senegal
O-315	*Oryza longistaminata*	Cameroon
O-316	*Oryza longistaminata*	Senegal
O-318	*Oryza longistaminata*	Ivory Coast

gels and subsequently transferred to nylon membranes (Hybond-XL; Amersham Pharmacia, UK) for hybridization.

***Rec*A-mediated Achilles' heel cleavage of the *TLH* gene family:** RecA-mediated Achilles' heel cleavage (RecA-AC) was performed as previously described (Farman and Leong 1998), with minor modifications. The primer AC-TLH (5′-CGCAGT TGGGGTTGTTAGGTCGGAC**GATC**GCGGGGTGGGGGGT GCAAGTGGTGT-3′) was designed to span an *Mbo*I site (indicated by boldface letters) located 1060 bp downstream from the start codon of *TLH1* (*t*elomere *l*inked *h*elicase *1*). To produce RecA-oligonucleotide filaments, 2 μl of AC-TLH (1 pmol/μl) was mixed with 1 μl RecA (2 mg/ml; New England BioLabs, Beverly, MA), 0.5 μl magnesium acetate (10 mm), and 0.5 μl Tris-acetate (250 mm, pH 7.5). Subsequent to incubating the mixture at 37° for 60 sec, 1 μl of 0.1 mg/ml ATP[γS], a nonhydrolyzable analog of ATP, was added, and the reaction was incubated at 37° for an additional 10 min. One microliter genomic DNA (1 μg/μl) from O-137 or Guy11 was combined with 2.5 μl Tris-acetate (250 mm, pH 7.5), 2.7 μl magnesium acetate (40 mm), 0.3 μl dithiothreitol (1 m), 3 μl BSA (1 mg/ml), and 12.5 μl double-distilled H_2O to bring the total volume to 22 μl, which was then mixed with the RecA-AC-TLH filaments. After a 30-min incubation at 37°, the concentration of magnesium acetate in the reaction was adjusted to 8 mm by adding 3 μl magnesium acetate stock (40 mm). To methylate unprotected *Mbo*I sites on genomic DNA, after adding 0.5 μl *dam* methylase (8 units/μl; New England BioLabs) and 1 μl of S-adenosyl methionine stock (32 mm), the reaction mixture was incubated at 37° for 30 min. The methylase and RecA proteins were inactivated by incubating the mixture at 65° for 15 min. Subsequently, methylated genomic DNA was ethanol precipitated. After washing twice with 70% ethanol, the DNA precipitate was air dried at room temperature. To demonstrate the specificity of protection by the AC-TLH primer, a control RecA-AC reaction was performed in the absence of AC-TLH. Methylated genomic DNA was completely digested with *Mbo*I and subsequently separated on a 0.7% agarose gel for Southern analysis.

PCR amplification of the sequences between the *TLH* gene family and the telomeric repeat: PCR amplification was performed in a 25-μl reaction mixture containing 2 μl (10 pmol/μl) each of AC-TLH and TEL-Bgl (5′-ACAGCTATGAATGA GATCTAACCCTAACCCTAACCCTAA-3′), 0.5 μl template genomic DNA (0.2 μg/μl), 0.5 μl TaKaRa Z-Taq (PanVera, Madison, WI), 2 μl dNTP mix (2.5 mm of each dNTP), 2.5 μl 10× PCR buffer (10 mm Tris, pH 9.2; 25 mm KCl; 1.5 mm $MgCl_2$; and 15 mm $[NH_4]_2SO_4$), and 15.5 μl H_2O. The cycling steps included an initial denaturation for 2.5 min at 95°, four cycles of 1-min denaturation at 94°, 1-min annealing at 62°, and 10-min extension at 65°; 26 cycles of 30-sec denaturation at 94°, 1-min annealing at 62°, and 10-min extension at 65°; and a final extension for 10 min at 65°. PCR products separated on 0.7% agarose gels were isolated from the gels using spin columns (QIAGEN) and were cloned into pGem-T vector (Promega, Madison, WI) for sequence analysis.

DNA sequencing and analysis: Sequencing reactions were performed using the ABI Prism Big-Dye terminator sequencing kit (Applied Biosystems, Foster City, CA) and analyzed with an ABI377 sequencer. The BLASTX program was used to identify proteins related to the gene products encoded by *TLH1*. Protein sequence alignment was performed using the CLUSTAL method in the Lasergene software (DNASTAR, Madison, WI). The helicase sequences used for the phylogenetic analysis were obtained from GenBank. A phylogenetic tree based on the neighbor-joining method was constructed using PAUP (beta-version 4.0; Sinauer Associates, Sunderland, MA).

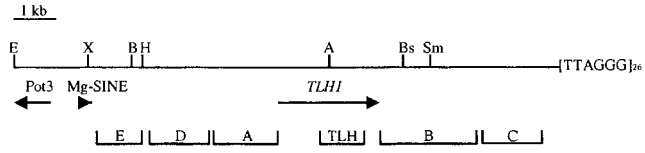

Figure 1.—Restriction map of the 13-kb telomeric fragment carrying the *TLH1* gene in O-137. Some of the unique restriction sites, including *Aat*II (A), *Bam*HI (B), *Bsg*I (Bs), *Eco*RI (E), *Hin*dIII (H), *Sma*I (Sm), and *Xho*I (X), are marked. The telomeric repeat is indicated by $(TTAGGG)_{26}$. The positions of probes A–E are indicated. The arrows beneath Pot3 and *TLH1* indicate the orientation of the sequences (5′ to 3′). The arrowhead to the right of Pot3 indicates Mg-SINE (5′ to 3′). Due to its small size (35 bp), a truncated copy of MGR619, located between Pot3 and Mg-SINE, was not indicated on the map.

RESULTS

Isolation and characterization of a 13-kb telomeric restriction fragment from *M. grisea* strain O-137: Two previously characterized chromosome ends of the rice-infecting isolate O-137 have different sequences, with the exception of their telomeric repeats (Orbach *et al.* 2000; Kang 2001). To further characterize the structure and organization at its chromosome ends, another telomeric region (13 kb) was cloned from a λ genomic library of O-137 enriched for telomeric fragments (Kang 2001). Sequence analysis of this region revealed the presence of two transposons, Pot3 and Mg-SINE, and a short repetitive element called MGR619 (Figure 1). The Pot3 transposon (Farman *et al.* 1996; Kang *et al.* 2001) is located distal to the 156-bp telomeric repeat, $(TTAGGG)_{26}$. A truncated copy (35 bp) of the ~80-bp-long repetitive DNA element MGR619 (Kang *et al.* 1995) was located 32 bp downstream from 5′ ITR of Pot3. An intact Mg-SINE transposon (Kachroo *et al.* 1995) was present 813 bp downstream from MGR619. In addition to these repetitive elements, a novel gene, designated *TLH1* on the basis of its telomeric location and significant similarity to the RecQ family of helicases from various organisms (see Figure 2), was present between the telomeric repeat and Mg-SINE. Another notable feature was a very low G/C content (33.4%) in the region between *TLH1* and the telomeric repeat (see Figure 5 for more information). In comparison, the *TLH1* gene and the remaining 13-kb region exhibited 59.3% G/C and 51.1% G/C, respectively.

The *TLH1* gene product exhibited similarity to members of the RecQ DNA helicase family: The *TLH1* gene is predicted to encode an 818-amino-acid protein that exhibits similarity to the RecQ family of DNA helicases from phylogenetically diverse organisms (Figure 2). Members of this family, including TLH1, share a conserved region of 300–350 amino acids corresponding to seven conserved helicase domains (Figure 2A). As shown in Figure 2B, TLH1 appears phylogenetically closest to that encoded by *UTASa*, a *Ustilago maydis* heli-

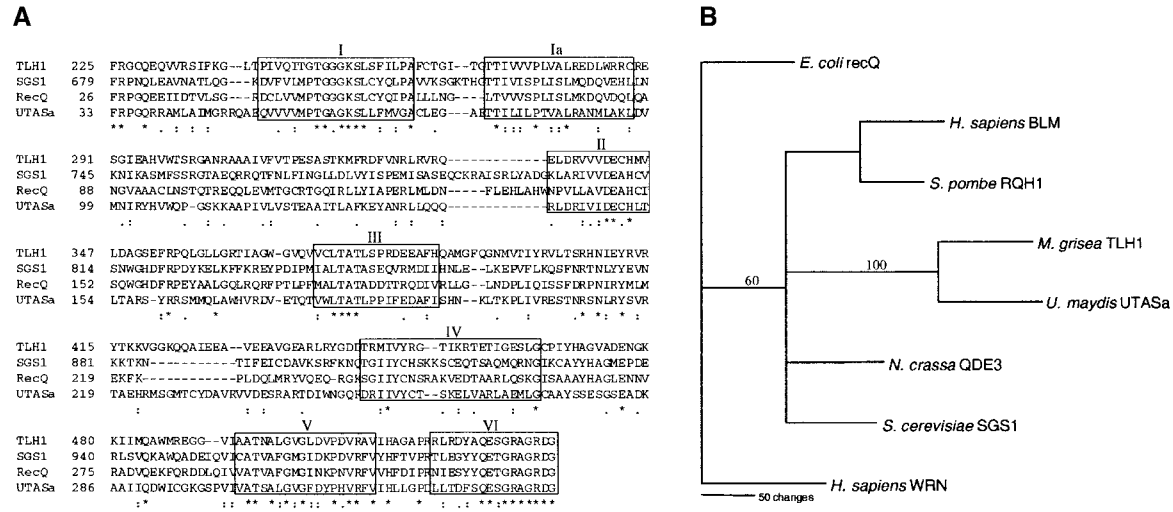

FIGURE 2.—Sequence comparison and phylogenetic relationship among members of the RecQ DNA helicase family. Both analyses were carried out using only the conserved helicase domains in the chosen proteins. (A) The names of proteins and amino acid positions are to the left of the sequences. The seven helicase domains (GORBALENYA *et al.* 1989) are boxed and indicated by Roman numerals. Identical amino acids are denoted by asterisks, and conservative changes are marked by dots. (B) The phylogenetic tree was constructed using the neighbor-joining method. Bootstrap values >50% in 500 repetitions are indicated at individual nodes of the tree.

case gene that is also closely linked to the telomere (SÁNCHEZ-ALONSO and GUZMÁN 1998).

The RecQ family of helicases can be divided into two classes on the basis of their sizes. Helicases WRN, BLM, and RECQ4 in humans (ELLIS *et al.* 1995; Yu *et al.* 1996; KITAO *et al.* 1999); SGS1 in *Saccharomyces cerevisiae* (WATT *et al.* 1995); QDE-3 in *Neurospora crassa* (COGONI and MACINO 1999); and RQH1 in *Schizosaccharomyces pombe* (STEWART *et al.* 1997) are 1300–1500 amino acids in length. Members of the other class of helicases including *E. coli* RecQ (NAKAYAMA *et al.* 1984) and *U. maydis* UTASa, are approximately one-half the size of the former group. On the basis of its size, TLH1 belongs to the latter group. However, like members of the former group, TLH1 contains a highly acidic patch (22 glutamic acid or aspartic acid out of 26 residues) at its amino terminus (residues 6–31). Such an acidic domain is absent in RecQ and UTASa.

The *TLH1* gene is a member of a gene family in *M. grisea*: When a Southern blot of restriction enzyme-digested genomic DNA of O-137 was hybridized with *TLH1*, three to seven bands were detected (Figure 3). Rice pathogen Guy11 also contained eight to nine bands hybridizing to *TLH1*. These results suggest that *TLH1* is a member of a gene family. To determine whether the regions flanking *TLH1* were also present in multiple copies, the blot was hybridized with five additional probes, representing different parts of the cloned 13-kb fragment (see Figure 1). Due to the high copy number of Pot3, MGR619, and Mg-SINE in the rice pathogen genome, the region containing these elements was excluded from this analysis. Although the whole 13-kb telomeric region appears to have been amplified in O-137 and Guy11, the copy number of the segment represented by probes D and E was lower than that of the rest of the region. Different probes, including the telomeric repeat, seemed to hybridize to the same bands (Figure 3), suggesting that the ends of other chromosomes in O-137 and Guy11 might also carry sequences homologous to *TLH1* and its flanking regions.

To determine when members of the *TLH* gene family are expressed, we performed a Northern analysis with poly(A) RNA isolated from O-137 cultures grown under three conditions (complete medium and carbon- or nitrogen-starved conditions) and poly(A) RNA extracted from two rice varieties, CO-39 and Saliceltik, infected with O-137 (four samples were collected from each variety every 2 days after infection). No hybridization signal was detected from any of the samples even after several days of exposure (data not shown). A parallel Northern analysis with RNA samples from strain 70-15 (a laboratory strain derived from a cross involving Guy11 as a parent) also failed to detect any *TLH* transcripts under a large number of growth and developmental conditions, including perithecial tissues at different developmental stages, heat and cold shocks, carbon- or nitrogen-starved conditions, and in the presence of various chemical agents that cause various forms of stress to the fungus (*e.g.*, methyl viologen, which generates reactive oxygen species). Transcripts from several other genes (such as *PWL2* and various transposons) were successfully detected on these blots (data not shown), indicating that the lack of hybridization signal by *THL1* as a probe was not caused by experimental problems.

Members of the *TLH* gene family are closely linked to the telomere in O-137 and Guy11: A mapping popula-

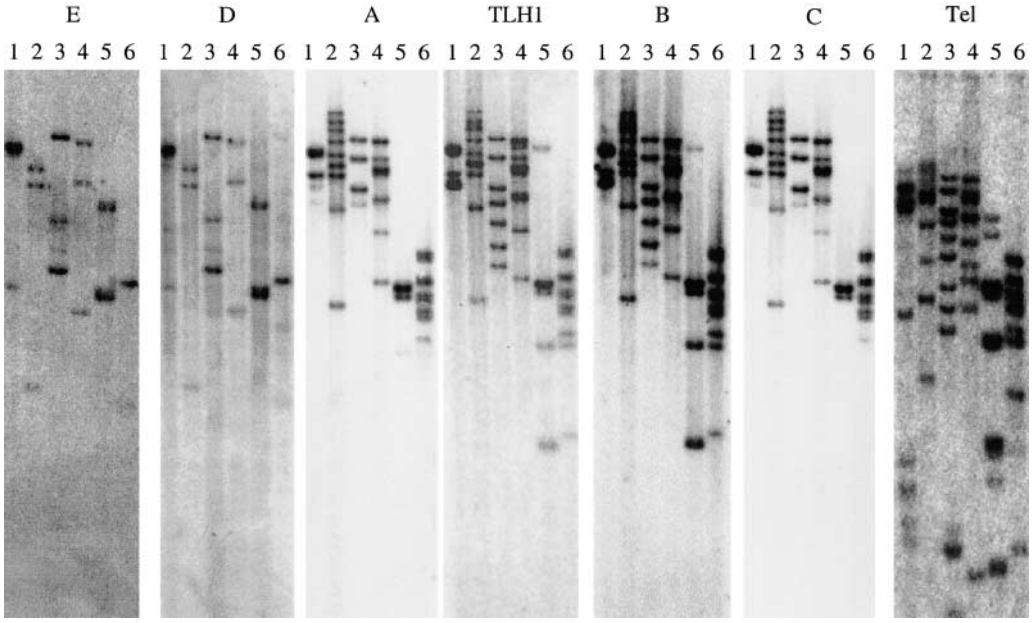

FIGURE 3.—Southern hybridization analysis of O-137 and Guy11 using *TLH1* and its flanking sequences as probes. Genomic DNA of O-137 (lanes 1, 3, and 5) and Guy11 (lanes 2, 4, and 6) was digested with *Eco*RI (lanes 1 and 2), *Hin*dIII (lanes 3 and 4), or *Sal*I (lanes 5 and 6). Blots containing the digested DNA were hybridized with the probes against *TLH1*, flanking regions A–E, or the telomeric repeat (see Figure 1 for locations of the hybridization probes).

tion derived from a cross between 6043 and 4224-7-8 (SWEIGARD *et al.* 1993) was used to map the *TLH* gene family. These mapping parents, carrying seven to nine members of the *TLH* gene family, originated from Guy11 and O-137, respectively (SWEIGARD *et al.* 1995). Restriction fragment length polymorphisms (RFLPs) associated with the gene family segregated with at least six telomeres. One of the RFLPs was linked to *AVR-Pita* (ORBACH *et al.* 2000). The close association between the *TLH* gene family and the telomere was confirmed by RecA-mediated Achilles' heel cleavage (RecA-AC). This technique is based on the specific cleavage of a predetermined restriction site on the genome following the protection of this site from genome-wide DNA methylation (KOOB *et al.* 1992; FARMAN and LEONG 1995). We designed a 55-nucleotide-long primer (AC-TLH) that spans an *Mbo*I site located 1060 bp downstream from the start codon of *TLH1*, to protect this site from being methylated by *dam* methylase. Following methylation in the presence of AC-TLH, all *Mbo*I sites in the O-137 and Guy11 genomes, with the exception of the one protected by AC-TLH, became methylated, thus preventing these sites from restriction by *Mbo*I. Subsequent digestion of the methylated genomic DNA with *Mbo*I liberated those telomeric fragments that are closely associated with the *TLH* gene family (Figure 4).

RecA-AC released seven fragments from Guy11 DNA, ranging in size from ~15 to 3.7 kb. Three fragments, ranging in size from 5.5 to 2.3 kb, were released from O-137 DNA. On the basis of the sequence of *TLH1*, RecA-AC was expected to produce a 5.5-kb fragment. However, the stronger hybridization signal at this fragment than that at the other two fragments suggested that two telomeric fragments of 5.5 kb were released from O-137 DNA. This possibility was subsequently confirmed by sequence analysis. When AC-TLH was omitted in RecA-AC reactions, these fragments failed to be released (Figure 4, lanes 3 and 7 for Guy11 and lanes 14 and 18 for O-137), confirming the specificity of protection from methylation by this primer. Hybridization of the released fragments to a telomeric repeat probe (Figure 4, lane 9 and not shown) confirmed that these fragments are telomeric. Probes B and C (see Figure 1) hybridized to most of these telomeric fragments, suggesting that other *TLH*-linked telomeres carry sequences homologous to *TLH1* and its flanking regions. Probe B, but not probe C, hybridized to the 15-kb fragment from Guy11 and the 2.3-kb fragment from O-137. According to our genetic mapping, one member of the gene family is tightly linked to *Avr-Pita*. However, none of the telomeric fragments released by RecA-AC hybridized to *Avr-Pita* (not shown).

Sequence analysis of two additional chromosome ends associated with the *TLH* gene family in O-137: To compare the sequence organization between the *TLH1* locus and other chromosome ends associated with the gene family, we amplified O-137 genomic DNA by PCR, using a pair of primers complementary to *TLH1* and the telomeric repeat (not shown). We subcloned two amplified fragments that correspond to the 5.5- and 2.3-kb telomeric fragments in Figure 4, resulting in pSK529 and pSK515, respectively (Figure 5). Sequence analysis of pSK529 confirmed that RecA-AC released two nearly identical telomeric fragments of 5.5 kb from O-137. The 2.3-kb telomeric fragment lacked the 3.1-kb region of the *TLH1* locus that immediately flanks the telomeric repeat, which explained the lack of hybridization to probe C. A 24-bp sequence unique to pSK515 was located between the telomeric repeat and the 919-bp region that was present in all three telomeric fragments.

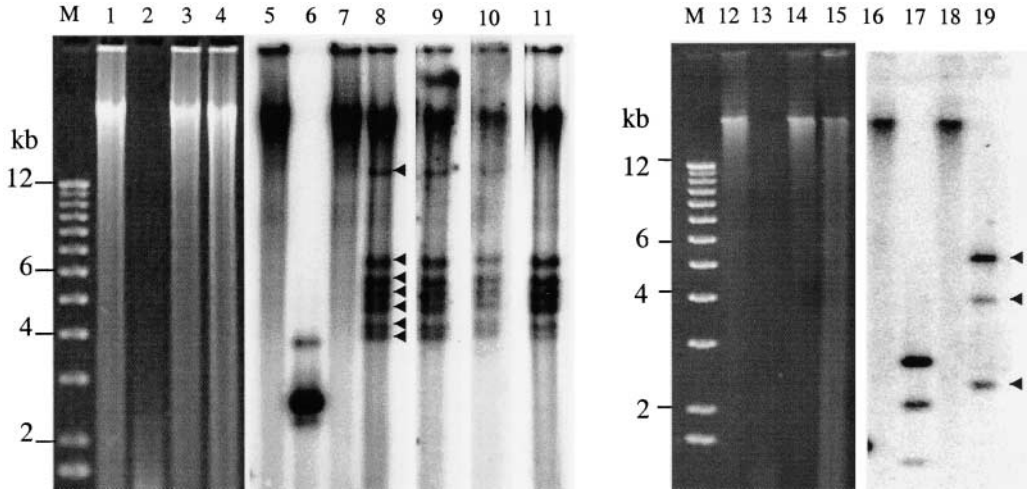

FIGURE 4.—RecA-mediated Achilles' heel cleavage (RecA-AC) of the *TLH* gene family in O-137 and Guy11. Lanes 1–4 are from the ethidium bromide-stained gel image of Guy11 genomic DNA treated in the following manner: lane 1, untreated genomic DNA; lane 2, untreated genomic DNA digested with *Mbo*I; lane 3, *Mbo*I-digested genomic DNA subsequent to methylation in the absence of AC-TLH; lane 4, *Mbo*I-digested genomic DNA subsequent to methylation in the presence of AC-TLH. Lanes 12–15 correspond to a gel image after treating O-137 genomic DNA in the same manner as the samples in lanes 1–4. The 1-kb ladder (Life Technologies, Rockville, MD) was used in the gel (indicated by M). The blotted gels were hybridized with TLH: lanes 5–8 for Guy11 and lanes 16–19 for O-137. Restricted DNA in lane 4 was subsequently hybridized with the following probes: telomeric repeat, lane 9; probe B, lane 10; probe C, lane 11. The arrowheads denote the telomeric fragments released by RecA-AC.

The 919-bp and 3.1-kb regions had a G/C content much lower (35 and 33%, respectively) than that of parts of the *TLH* genes shown in Figure 5 (57–58%).

Sequence comparison showed ~1.1% nucleotide divergence (15 and 16 polymorphic sites, respectively) from *TLH1* for members of the *TLH* gene family cloned in pSK515 and pSK529 (Figure 5). All polymorphic sites were located within the first 700 bp of the 1.4-kb region, and all 15 polymorphic nucleotides in pSK515 were also present in pSK529. These 15 sites were almost randomly distributed with respect to the three positions in the codon (6, 4, and 5 changes on the first, second, and third positions, respectively), resulting in 12 amino acid changes from TLH1. One of these changes introduced a stop codon in the *TLH* genes (at the amino acid residue 483) in pSK515 and pSK529. A polymorphic site unique to pSK529 occurred on the third position of a codon and caused a silent mutation.

Beyond the first 700 bp of the 1.4-kb region, the sequences were much more conserved. In pSK515, three sites were identified that differed from those in the 919-bp region immediately flanking the 3′ end of the *TLH1* gene (0.3% nucleotide divergence). This region was identical between the *TLH1* locus and the cloned sequence in pSK529. The remaining 3.1-kb region was also highly conserved, with only two polymorphic sites (one nucleotide deletion and an A to G change in pSK529) between the *TLH1* locus and pSK529.

The *TLH* gene family is almost exclusively present among isolates from rice: To determine if additional isolates from rice also carry members of the *TLH* gene family, 23 isolates from rice throughout the world were

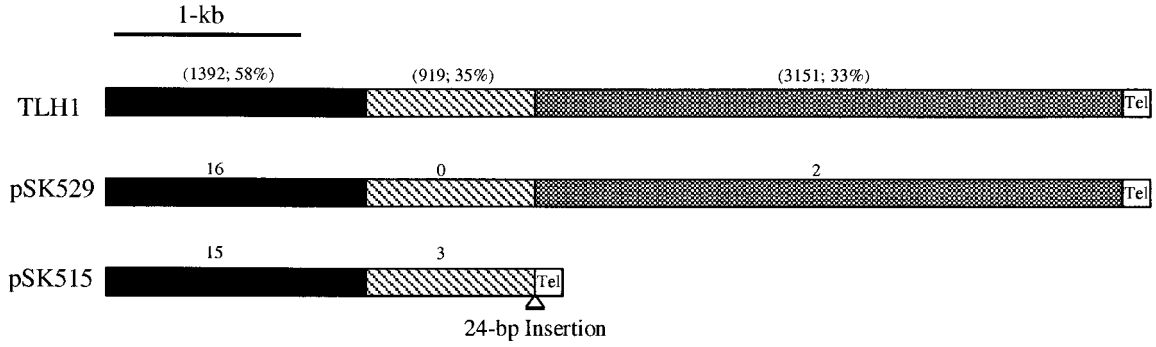

FIGURE 5.—Schematic illustration of the organization of three *TLH*-linked telomeres in O-137. TLH1 corresponds to part of the 13-kb telomeric fragment containing the *TLH1* gene (Figure 1). To better illustrate the degree of sequence variation among these telomeric fragments, the sequence of the *TLH1* locus was arbitrarily divided into three blocks, indicated by the different patterns. The size of each block (in base pairs) and corresponding G/C content are indicated above the blocks. The numbers above pSK529 and pSK515 denote the number of nucleotides polymorphic to the *TLH1* locus within each sequence block. The location of a 24-bp segment that is unique to pSK515 is indicated by a triangle (not to scale). The telomeric repeat sequence, not drawn to scale, is denoted by Tel.

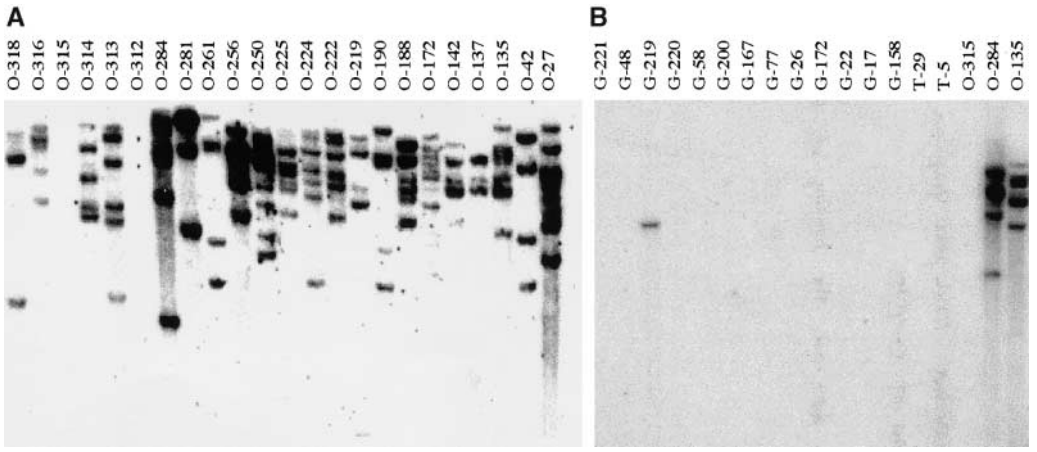

FIGURE 6.—Distribution of the *TLH* gene family among *M. grisea* isolates from diverse hosts. Host and geographic origin of these strains are listed in Table 1. *Eco*RI-digested genomic DNAs of rice isolates from many different geographic locations (A) and isolates from various hosts (B), including rice, were blotted and hybridized with *TLH1*.

surveyed. These results (Figure 6A) and those of a survey of >500 isolates collected from diverse rice varieties throughout Korea for >10 years (not shown) indicate that the *TLH1* gene family is ubiquitous in rice pathogenic isolates. Isolates representing rice pathogen populations in the United States (LEVY *et al.* 1991) also carried multiple members of the gene family (not shown). Only two strains, O-312 from *Oryza glaberrima* and O-315 from *O. longistaminata*, lacked sequences homologous to *TLH1*. The remaining isolates contained 3–10 members of the gene family. The two exceptions (O-312 and O-315) are genetically distinct from the rest of the rice isolates (S. KANG, unpublished data). Their mitochondrial genotypes and the very low copy number (<10) of MGR586, a repetitive DNA element that is present in >50 copies in other rice pathogens, suggest that O-312 and O-315 are more closely related to the group of isolates from wheat and triticale (which lack the gene family) than to the rice pathogens.

Almost all isolates from hosts other than rice lacked the *TLH1* gene family (Figure 6B). Among 15 isolates from other hosts, only 1, G-219 from *Panicum maximum*, contained a single *TLH1*-hybridizing DNA fragment. A survey of an additional 37 isolates from various hosts uncovered only 1 additional isolate (G-78 isolated from *Pennisetum polystachyon*) that also contained a single copy of *TLH1* (data not shown). Although these surveys included 7 isolates from various Pennisetum species and 3 isolates from Panicum species, all of these isolates lacked sequences homologous to the *THL1* gene probe (Figure 6B and not shown). Hybridization of the blots with *TLH1* under low-stringency conditions did not reveal any new bands, suggesting that most isolates from hosts other than rice lack the *TLH* gene family.

The *TLH* gene family is highly dynamic: The number of *TLH* genes varied substantially among rice pathogens (Figure 6A), suggesting that this gene family is highly dynamic in nature, undergoing frequent deletion/amplification events. To test this supposition, we surveyed *THL1* gene polymorphisms among a clonal (asexual) population of rice pathogens, including >100 strains that had been isolated in 1999 from various regions of Korea. Strains in this population exhibited 90% or higher identity among their DNA fingerprints generated by a repetitive DNA probe, MGR586 (LEVY *et al.* 1991), suggesting that all of these strains originated from a recent, common ancestor. On the basis of the copy number (two to five) and RFLPs associated with the gene family, we identified eight unique genotypes in this population (not shown), strongly supporting the highly dynamic nature of the gene family.

Given the apparent frequency in nature of changes in the *TLH* gene family, we decided to ascertain if such changes can also occur under laboratory conditions. We produced 18 asexual lineages from the laboratory strain 70-15 through 10 generations of successive monoconidiation, which resulted in 156 monoconidial cultures in total (see MATERIALS AND METHODS). We hybridized DNA from 18 strains from the tenth generation with the *TLH1* (Figure 7). Two of the strains contained a

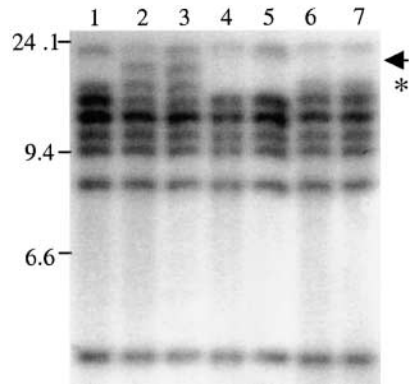

FIGURE 7.—Deletion and amplification events within the *TLH* gene family in culture. *Eco*RI-digested genomic DNA of isolate 70-15 (lane 1) and six monoconidial isolates derived from 70-15 after 10 generations of monoconidiation (lanes 2–7) were probed with *TLH1*: The arrowhead indicates a novel member of the gene family, gained in two isolates (lanes 2 and 3). The asterisk denotes a member of the family deleted in two isolates (lanes 4 and 5). Two isolates that had the same profile as 70-15 are shown in lanes 6 and 7.

novel fragment in addition to the seven fragments present in 70-15, suggesting an amplification event in the *TLH* gene family. A deletion event was also observed in two other strains.

DISCUSSION

A novel helicase gene family is tightly linked to *M. grisea* telomeres. Certain chromosome ends of two distantly related fungi, *S. cerevisiae* (Louis 1995) and *U. maydis* (Sánchez-Alonso and Guzmán 1998), are also associated with a helicase gene family. Many chromosome ends of *S. cerevisiae* contain the *Y′* element, arranged in one to four tandem copies, adjacent to the telomeric repeat (Walmsley *et al.* 1984; Louis 1995). One of the two overlapping ORFs encoded by the *Y′* element is predicted to encode a putative helicase (Louis and Haber 1992). *UTASa*, a repetitive element that is closely associated with the telomeres of *U. maydis*, was also predicted to encode a helicase (Sánchez-Alonso and Guzmán 1998). Members of the gene family appear to be highly conserved within both *S. cerevisiae* and *M. grisea*. There is a 0.3–1.1% divergence in nucleotide sequence among the *Y′* elements within a strain of *S. cerevisiae*, and between strains the degree of divergence increases only slightly, to 1.2–1.9% (Louis and Haber 1992). Similarly, three members of the *TLH* gene family in O-137 showed 0.1–1.1% nucleotide divergence (Figure 5). In contrast, two copies of *UTASa* from a single strain exhibited 10% divergence (Sánchez-Alonso and Guzmán 1998).

Not all chromosome ends are associated with the helicase gene families in *S. cerevisiae*, *M. grisea*, and *U. maydis*, and the number of telomere-linked helicase genes varies from strain to strain in each species. In *S. cerevisiae*, different strains also differ with respect to the chromosomal locations of *Y′* (Louis and Haber 1990b; Louis *et al.* 1994), and the total number of *Y′* elements also varies, due to the presence of tandem arrays of *Y′* in certain strains (Louis and Haber 1990b). Strains lacking *Y′* have not yet been identified in *S. cerevisiae* (Louis 1995), but several strains in a sibling species, *S. bayanus*, lack the homolog of *Y′* (Naumov *et al.* 1992). A karyotype analysis of two *U. maydis* strains, FB2 and I2, using *UTASa* as a probe, showed that while at least 15 chromosomes of FB2 hybridized to the probe, only two chromosomes of I2 were detected by the probe (Sánchez-Alonso and Guzmán 1998). It is not known if certain *U. maydis* strains lack *UTASa*. As shown in Figure 6, in *M. grisea* the number of helicase genes ranged from 0 (almost all isolates from hosts other than rice) to ~10. At least 7 and 4 of the 14 chromosome ends of Guy11 and O-137, respectively, carry a member of the *TLH* gene family within 15 kb of the telomeric repeat (Figure 4). Considering the number of restriction fragments that hybridized to *TLH1* in both strains (Figure 3), additional telomeric regions of Guy11 and O-137 might also be associated with this gene family. Some members of this family (such as the one linked to *Avr-Pita*) might be located farther down from the telomeric repeat (>30 kb). Telomeric fragments of this size, released by RecA-AC, would not be resolved from the undigested genomic DNA in the gel and thus would not have been detected in our assay. Alternatively, mutations in some members (*e.g.*, deletion of the targeted *Mbo*I site) might have prevented the release of linked telomeric fragments by RecA-AC.

The conserved physical association in phylogenetically diverse fungi between a helicase gene family and the telomere raised an intriguing question about the evolutionary origin of these helicase gene families. It has been estimated that the ascomycetes split from the basidiomycetes ~400 million years ago (Berbee and Taylor 1993). A recent report predicted that the timing of this split could be >1 billion years ago (Heckman *et al.* 2001). Considering the presence of a telomere-linked helicase gene family in both ascomycetes (*M. grisea* and Saccharomyces spp.) and basidiomycetes (*U. maydis*), the initial association of the ancestral helicase gene family with fungal telomeres could therefore date back to at least 400 million years ago. Of course, considering the small number of fungi that have been surveyed to date, it is formally possible that the association of helicase gene families with telomeres might be a trait that has been independently acquired multiple times after the ascomycetes split from the basidiomycetes. Assuming that the ancestral gene family appeared in fungi before the split, it is quite intriguing that gene families that can be missing in certain strains of at least two fungal species have remained at their current locations for several hundred million years.

Considering the highly dynamic nature of the *TLH* gene family, as demonstrated by the current study, in the absence of positive selection pressure this gene family would have quickly deleted. The ubiquitous presence of this gene family and the sequence conservation among members of this family therefore suggest that the gene family may confer an important function in rice pathogens. However, its absence in many isolates from other hosts suggests that its function is unlikely to be essential. The *Y′* element is also widely distributed among Saccharomyces species. However, certain strains of *S. bayanus* lack *Y′* (Naumov *et al.* 1992), suggesting that *Y′* may also confer strain or species-specific function. In *S. cerevisiae*, the second ORF of *Y′* encoding a putative helicase remained intact and well conserved, while flanking X regions are highly diverged (Louis *et al.* 1994). Such a pattern suggests that similar to the *TLH* gene family, the *Y′* elements have also avoided the accumulation of mutations due to unknown selection pressure against such mutations (Louis and Haber 1992; Louis *et al.* 1994).

Considering putative functions of related RecQ helicases, including DNA repair and recombination (Shen

and Loeb 2000), maintaining genome stability (Chakraverty and Hickson 1999), and post-transcriptional gene silencing (Cogoni and Macino 1999), the gene products encoded by the *TLH* gene family might also perform one or more of these functions. In *S. cerevisiae* the expression of *Y′* is minimal during vegetative growth (Louis 1995) and is induced during meiosis, suggesting that its gene product might have a role during sexual development and/or meiosis. However, as summarized in results, we failed to detect transcripts from members of the *TLH* gene family in two different strains under various growth and developmental conditions, suggesting that the role of these sequences in *M. grisea* may therefore be structural.

One potential function of members of the *TLH* gene family at chromosome ends is protection of the telomere from accidental shortening and other forms of damage via various recombination-mediated mechanisms. The *Y′* element has been shown to play such a role in *S. cerevisiae*. When yeast *EST1*, a gene essential for proper telomere replication, is mutated, the telomeric repeat progressively shortens, leading to chromosome loss and subsequent cell death (Lundbald and Szostak 1989). Amplification and acquisition of *Y′* along with the internal telomeric repeat associated with *Y′* at the shortening chromosome ends rescued the *est1*$^{-}$ mutants (Lundbald and Blackburn 1993). The sequence comparison of *TLH*-linked chromosome ends in O-137 (Figure 5) supports the frequent occurrence of recombination events involving the gene family. Such events could potentially repair a shortened or damaged chromosome end and could also drive the concerted evolution at the *TLH*-associated subtelomeric regions. The highly conserved sequence (only two polymorphic sites) in the 4-kb region flanking the *TLH* gene family between *TLH1* and pSK529 (Figure 5) suggests a recent gene conversion event that created these two nearly identical chromosome ends in O-137. All the nucleotides of the *TLH1* gene polymorphic to the one in pSK529 were located in the first 700 bp of the 1.4-kb region, suggesting that the gene conversion probably initiated in the middle of this region.

Our data suggest that recombination events at the chromosome ends may not have been limited to those chromosome ends associated with the *TLH* gene family. The high degree of copy number variation in the *TLH* gene family among field isolates (Figure 6) and in culture (Figure 7) suggests that the gene family has undergone amplification and/or deletion in nature. Since a reciprocal recombination between members of the gene family should conserve gene copy number, we hypothesize that different recombination mechanisms are responsible for the changes in copy number. Potential mechanisms include gene conversion between two chromosome ends (one with a member of the gene family and the other without it), leading to a deletion or an addition in the gene family depending on the direction of recombination. Such a gene conversion could be mediated by various repetitive elements present in the chromosome ends. At the *TLH1* locus, three such elements, Pot3, MGR619, and Mg-SINE, were identified (Figure 1). Two different chromosome ends of O-137 also carry repetitive elements, some of which are present in more than one chromosome end (Orbach *et al.* 2000; Kang *et al.* 2001). Unequal sister chromatid exchanges mediated by some of these repetitive elements could be another mechanism underpinning the changes in copy number. These types of recombination events appear to occur at the chromosome ends of *S. cerevisiae* (Louis and Haber 1990a, 1992).

Given the presence of several host specificity genes at telomeric locations, certain recombination events involving the *TLH* gene family may in part account for genetic changes affecting these genes. As summarized in the Introduction, a diverse array of spontaneous mutations occurs at the *AVR-Pita* locus (Orbach *et al.* 2000; Kang *et al.* 2001). The close linkage between a member of the *TLH* gene family and *AVR-Pita* suggests that certain recombination events mediated by the *TLH* gene family or its flanking repetitive elements might be responsible for some of the deletion mutations. Testing this possibility is currently underway. Considering the close association of the *TLH* gene family with the telomere of rice pathogens and a number of possible roles played by the family, it would be interesting to investigate the structure and organization of chromosome ends in those strains lacking the gene family.

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LITERATURE CITED

Berbee, M. L., and J. W. Taylor, 1993 Dating the evolutionary radiations of the true fungi. Can. J. Bot. **71:** 1114–1127.

Bonman, J. M., T. I. Vergel De Dios, J. M. Bandong and E. J. Lee, 1987 Pathogenic variability of monoconidial isolates of *Pyricularia oryzae* in Korea and in the Philippines. Plant Dis. **71:** 127–130.

Chakraverty, R. K., and I. D. Hickson, 1999 Defending genome integrity during DNA replication: a proposed role for RecQ family helicases. Bioessays **21:** 286–294.

Chao, C. T., and A. H. Ellingboe, 1991 Selection for mating competence in *Magnaporthe grisea* pathogenic to rice. Can. J. Bot. **69:** 2130–2134.

Cogoni, C., and G. Macino, 1999 Posttranscriptional gene silencing in *Neurospora* by a RecQ DNA helicase. Science **286:** 2342–2344.

Dioh, W., D. Tharreau, J. L. Notteghem, M. J. Orbach and M. H. Lebrun, 2000 Mapping of avirulence genes in the rice blast fungus, *Magnaporthe grisea*, with RFLP and RAPD markers. Mol. Plant-Microbe Interact. **13:** 217–227.

Ellis, N. A., J. Groden, T. Z. Ye, J. Straughen, D. J. Lennon *et al.*, 1995 The Bloom's syndrome gene product is homologous to RecQ helicases. Cell **83:** 655–666.

Farman, M. L., and S. A. Leong, 1995 Genetic and physical mapping

of telomeres in the rice blast fungus, *Magnaporthe grisea*. Genetics **140:** 479–492.

Farman, M. L., and S. A. Leong, 1998 Chromosome walking to the *AVR1–CO39* avirulence gene of *Magnaporthe grisea*: discrepancy between the physical and genetic maps. Genetics **150:** 1049–1058.

Farman, M. L., S. Taura and S. A. Leong, 1996 The *Magnaporthe grisea* DNA fingerprinting probe MGR586 contains the 3′ end of an inverted repeat transposon. Mol. Gen. Genet. **251:** 675–681.

Farman, M. L., Y. Eto, T. Nakao, Y. Tosa, H. Nakayashiki *et al.*, 2002 Analysis of the structure of the *AVR1–CO39* avirulence locus in virulent rice-infecting isolates of *Magnaporthe grisea*. Mol. Plant-Microbe Interact. **15:** 6–16.

Freitas-Junior, L. H., E. Bottius, L. A. Pirrit, K. W. Deitsch, C. Scheidig *et al.*, 2000 Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of *P. falciparum*. Nature **407:** 1018–1022.

Gorbalenya, A. E., E. V. Koonin, A. P. Donchenko and V. W. Blinov, 1989 Two related superfamilies of helicases involved in replication, recombination, and repair and expression of DNA and RNA genomes. Nucleic Acids Res. **17:** 4713–4729.

Heckman, D. S., D. M. Geiser, B. R. Eidell, R. L. Stauffer, N. L. Kardos *et al.*, 2001 Molecular evidence for the early colonization of land by fungi and plants. Science **293:** 1129–1133.

Henandez-Rivas, R., D. Mattei, Y. Sterkers, D. S. Peterson, T. E. Wellems *et al.*, 1997 Expressed *var* genes are found in *Plasmodium falciparum* subtelomeric regions. Mol. Cell. Biol. **17:** 604–611.

Kachroo, P., S. A. Leong and B. B. Chattoo, 1995 Mg-SINE: a short interspersed nuclear element from the rice blast fungus, *Magnaporthe grisea*. Proc. Natl. Acad. Sci. USA **92:** 11125–11129.

Kang, S., 2001 Organization and distribution pattern of MGLR-3, a novel retrotransposon in the rice blast fungus *Magnaporthe grisea*. Fungal Genet. Biol. **32:** 11–19.

Kang, S., J. A. Sweigard and B. Valent, 1995 The *PWL* host specificity gene family in the rice blast fungus *Magnaporthe grisea*. Mol. Plant-Microbe Interact. **8:** 939–948.

Kang, S., E. D. Mullins, T. M. DeZwann and M. J. Orbach, 2000 Pathogenesis and genome organization of the rice blast fungus, pp. 195–235 in *Fungal Pathology*, edited by J. W. Kronstad. Kluwer Academic Press, Boston.

Kang, S., M. H. Lebrun, L. Farrall and B. Valent, 2001 Gain of virulence caused by insertion of a Pot3 transposon in a *Magnaporthe grisea* avirulence gene. Mol. Plant-Microbe Interact. **14:** 671–674.

Kitao, S., A. Shimamoto, M. Goto, R. W. Miller, W. A. Smithson *et al.*, 1999 Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. Nat. Genet. **22:** 82–84.

Koob, M., A. Burkiewicz, J. Kur and W. Szybalski, 1992 RecA-AC: single-site cleavage of plasmid and chromosomes at any predetermined restriction site. Nucleic Acids Res. **20:** 5831–5836.

Levy, M., J. Romao, M. A. Marchetti and J. E. Hamer, 1991 DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell **3:** 95–102.

Louis, E. J., 1995 The chromosome ends of *Saccharomyces cerevisiae*. Yeast **11:** 1553–1573.

Louis, E. J., and J. E. Haber, 1990a Mitotic recombination among subtelomeric *Y′* repeats in *Saccharomyces cerevisiae*. Genetics **124:** 547–559.

Louis, E. J., and J. E. Haber, 1990b The subtelomeric *Y′* repeat family in *Saccharomyces cerevisiae*: an experimental system for repeated sequence evolution. Genetics **124:** 533–545.

Louis, E. J., and J. E. Haber, 1992 The structure and evolution of subtelomeric *Y′* repeats in *Saccharomyces cerevisiae*. Genetics **131:** 559–574.

Louis, E. J., E. S. Naumova, A. Lee, G. Naumov and J. E. Haber, 1994 The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics. Genetics **136:** 789–802.

Lundbald, V., and E. H. Blackburn, 1993 An alternative pathway for yeast telomere maintenance rescues *est1*$^{-}$ senescence. Cell **73:** 347–360.

Lundbald, V., and J. W. Szostak, 1989 A mutant with a defect in telomere elongation leads to senescence in yeast. Cell **57:** 633–643.

Nakayama, H., K. Nakayama, R. Nakayama, N. Irino, Y. Nakayama *et al.*, 1984 Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (*recQ1*) that blocks the RecF recombination pathway. Mol. Gen. Genet. **195:** 474–480.

Naumov, G. I., E. S. Naumova, R. A. Lantto, E. J. Louis and M. Korhola, 1992 Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus*: electrophoretic karyotypes. Yeast **8:** 599–612.

Orbach, M. J., L. Farrall, J. A. Sweigard, F. G. Chumley and B. Valent, 2000 A telomeric avirulence gene determines efficacy for the rice blast resistance gene *Pi-ta*. Plant Cell **12:** 2019–2032.

Ou, S. H., 1980 Pathogen variability and host resistance in rice blast disease. Annu. Rev. Phytopathol. **18:** 167–187.

Sánchez-Alonso, P., and P. Guzmán, 1998 Organization of chromosome ends in *Ustilago maydis*. RecQ-like helicase motifs at telomeric regions. Genetics **148:** 1043–1054.

Shen, J., and L. A. Loeb, 2000 The Werner syndrome gene: the molecular basis of RecQ helicase deficiency diseases. Trends Genet. **16:** 213–220.

Stewart, E., C. R. Chapman, F. Al-Khodairy, A. M. Carr and T. Enoch, 1997 *rqh1*$^{+}$, a fission yeast gene related to the Bloom's and Werner's syndrome genes, is required for reversible S phase arrest. EMBO J. **16:** 2682–2692.

Sweigard, J. A., B. Valent, M. J. Orbach, A. M. Walter, A. Rafalski *et al.*, 1993 Genetic map of the rice blast fungus *Magnaporthe grisea* (n = 7), pp. 3.112–113.114 in *Genetic Maps*, edited by O. Brien. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sweigard, J. A., A. M. Carroll, S. Kang, L. Farrall, F. G. Chumley *et al.*, 1995 Identification, cloning, characterization of *PWL2*, a gene for host-species specificity in the rice blast fungus. Plant Cell **7:** 1221–1233.

Valent, B., and F. G. Chumley, 1991 Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. Annu. Rev. Phytopathol. **29:** 443–467.

Valent, B., and F. G. Chumley, 1994 Avirulence genes and mechanisms of genetic instability in the rice blast fungus, pp. 111–153 in *Rice Blast Disease*, edited by R. S. Zeigler, P. S. Teng and S. A. Leong. CAB International, Wallingford, UK.

van der Lee, T., A. Robold, A. Testa, J. W. van 't Klooster and F. Govers, 2001 Mapping of avirulence genes in *Phytophthora infestans* with amplified fragment length polymorphism markers selected by bulked segregant analysis. Genetics **157:** 949–956.

Wada, M., and Y. Nakamura, 1994 MSG gene cluster encoding major cell surface glycoproteins of rat *Pneumocystis carinii*. DNA Res. **1:** 163–168.

Walmsley, R. M., C. S. M. Chan, B. K. Tye and T. D. Petes, 1984 Unusual DNA sequences associated with the ends of yeast chromosomes. Nature **310:** 157–160.

Watt, P. M., E. J. Louis, R. H. Borts and I. D. Hickson, 1995 Sgs1: a eukaryotic homolog of *E.coli* recQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. Cell **81:** 253–260.

Yu, C., J. Oshima, Y. Fu, E. M. Wijsman, F. Hisama *et al.*, 1996 Positional cloning of the Werner's syndrome gene. Science **272:** 258–262.

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