Research Note

Insertion of the LINE Retrotransposon MGL Causes a Conidiophore Pattern Mutation in *Magnaporthe grisea*

Marie Nishimura,¹ Nagao Hayashi,² Nam-Soo Jwa,¹ Gee W. Lau,³ John E. Hamer,³ and Akira Hasebe¹

¹National Institute of Agrobiological Resources (NIAR), 2-1-2, Kan-non dai, Tsukuba, Ibaraki, 305-8602, Japan; ²National Agriculture Research Center (NARC), 3-1-1, Kan-non dai, Tsukuba, Ibaraki, 305-8666, Japan; ³Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.
Accepted 13 April 2000.

We obtained three *Magnaporthe grisea* morphological mutants that had the LINE transposon MGL inserted into the *ACRI* locus. Sequence analysis revealed that *ACRI* is homologous to *medA*, a developmental regulator of *Aspergillus nidulans* conidiation. These results demonstrated that MGL elements could transpose and cause insertional mutagenesis in *M. grisea*.

Transposable elements are agents of mutagenesis that mediate many changes in gene structures and functions in all organisms (Berg and Howe 1989). Insertion of a transposon within a gene may create a defective phenotype because transcription or translation of the gene is inhibited or altered. In *Magnaporthe grisea*, seven transposons have been reported (Daboussi 1996). Although they are generally thought to be responsible for the genetic variability of this fungus, there is little evidence that these transposons are active and cause mutations.

In the process of mapping an avirulence gene of *M. grisea*, we isolated three morphological mutants among a collection of 113 random ascospores from the cross between rice-pathogenic field isolates CHNOS 60-2-3 and CHNOS 59-9-1 (Table 1). We could not obtain full tetrads because the viability of the ascospores was very low (<50%). The mutants, 4000-R-67, 4000-R-69, and 4000-R-102, produce conidia in head-to-tail arrays (Fig. 1A). In the wild type, conidia were generated in a sympodial array (Fig. 1B). The mutants formed fewer appressorium and showed decreased pathogenicity on rice, compared with their parents (data not shown). These phenotypes were similar to that of the *acr1* (acropetal) mutants identified by Lau and Hamer (1998).

We performed co-segregation analysis to determine if this mutant phenotype was due to a single gene mutation. The mutant strain 4016-R-29 was derived by the first backcross

Table 1. Characteristics and origins of *Magnaporthe grisea* strains

Strain	Characteristics	Origin
CHNOS 60-2-3	Wild type, <i>MAT1-1</i> (MAFF101280, MAFF gene bank, Japan)	Field isolate from rice
CHNOS 59-9-1	Wild type, <i>MAT1-2</i> (MAFF101393)	Field isolate from rice
4000-R-67	Acropetal mutant, <i>MAT 2</i>	Progeny of CHNOS 60-2-3 × CHNOS 59-9-1
4000-R-69	Acropetal mutant, <i>MAT 1</i>	Progeny of CHNOS 60-2-3 × CHNOS 59-9-1
4000-R-102	Acropetal mutant, <i>MAT 2</i>	Progeny of CHNOS 60-2-3 × CHNOS 59-9-1
4016-R-29	Acropetal mutant, <i>MAT 2</i>	Progeny of CHNOS 60-2-3 × 4000-R-67

Fig. 1. Conidial morphology in the wild-type strain and the acropetal mutant. **A**, Catenated conidia were produced in the acropetal mutant. **B**, Pyriform conidia were produced sympodially on a conidiophore in the wild-type strain. Scale bars represent 10 μ m.

Corresponding author: Marie Nishimura; E-mail: marie@abr.affrc.go.jp

Current address of Marie Nishimura; Department of Botany, Purdue University, West Lafayette, IN 47907, U.S.A.; Telephone: 1-765-496-7496; E-mail: marien@bilbo.bto.purdue.edu

Fig. 2. Southern hybridization analysis of the wild-type strain and the acropetal mutants. **A**, Parental strain CHNOS59-9-1. **B**, Parental strain CHNOS60-2-3. **C**, Acropetal mutant 4000-R-67. **D**, Acropetal 4000-R-69. **E**, Acropetal mutant 4000-R-102. Genomic DNA from these strains was digested by *SacI*. pACR1-3, which covered the *ACR1* open reading frame, was used as a probe. A single 2.2-kb band was detected in the parental strains by Southern hybridization analysis. Band shifts (3.0 and 5.1 kb) were detected in these acropetal mutants, which indicated the insertion events occurred at the *ACR1* gene.

between acropetal mutant 4000-R-67 and its parental strain CHNOS 60-2-3. A segregation analysis was performed on random ascospores obtained by a backcross of 4016-R-29 to CHNOS60-2-3. A segregation ratio of 34 wild type to 35 mutant phenotype was recovered from the 69 random ascospore progeny, fitting 1:1 segregation. The result indicates that the mutant phenotype was likely to be caused by a single gene mutation. Appressorium formation ability was greatly decreased in all of the 35 mutant progeny. The 34 wild-type progeny formed appressoria normally (data not shown).

Since these morphological mutants exhibit a phenotype similar to that of the *acr1* mutant, we investigated whether the corresponding *ACR1* gene in these mutants was altered. Southern hybridization was conducted with the *SacI*-digested

genomic DNA of the three mutants, 4000-R-67, 4000-R-69, and 4000-R-102, and their parental strains, CHNOS60-2-3 and CHNOS59-9-1. The clone pACR1-3, which carries the partial open reading frame (ORF) of *ACR1*, was used as a probe. A single 2.2-kb band was observed in the parental strains. In contrast, the 2.2-kb band was missing in all mutants. Instead, two bands, 5.1-kb and 3.0-kb, respectively, were observed (Fig. 2). These observations strongly suggest that various phenotypic changes in these mutant strains might be caused by the insertion of an unknown, approximately 5.9-kb sequence into the *ACR1* gene.

We performed polymerase chain reaction (PCR) amplification on the genomic DNA of the mutants with the DNA primers that flank the *ACR1* gene (5'-ACGAGGACACCACTCTCAAG-3' and 5'-CAGAGATGGGAGTTGTTGAC-3'). A 7.0-kb PCR product was obtained in all three mutants. We sequenced the 7.0-kb PCR products containing the insertion and its flanking *ACR1* sequences of these mutants, and searched the available data bases for the presence of homologous DNA sequences. The search results indicated that 6.0 kb of the PCR product is identical to the *M. grisea* LINE retrotransposon (MGL; Hamer et al. 1989; Kachroo et al. 1997; Meyn et al. 1998). In all these *acr1* morphological mutants, MGL inserted at the same site in the *ACR1* gene. A 19-bp sequence was duplicated at the insertion (Figs. 3 and 4). These data confirmed that the conidial morphological changes in these mutants were the direct result of the MGL insertion into the *ACR1* gene. Interestingly it appears that the same transposition event generated all three mutants. To test whether these strains are clonally related, Southern analysis was performed on *EcoRI* digested genomic DNA with an MGL 3' fragment amplified by PCR (970 bp, primers: 5'-cccaccttggtagcgattc-3', 5'-cgtatgtg tgccaggtgtgc-3') as a probe. The mutants showed different restriction fragment length polymorphism patterns, demonstrating they were individual progeny (data not shown). Taken together, the data suggest that it is most likely that these mutants are siblings and that the MGL insertion event occurred premeiotically.

The longest ORF of *ACR1* was reported to be 593 amino acids with glutamine-rich domains that shared a weak similarity to several transcriptional factors (Lau and Hamer 1998). In this study, more recent sequence analysis showed that the Acr1 amino acid sequence is very similar to a presumed transcriptional regulator of *Aspergillus nidulans* called MedA (medusa) (GenBank AF080599). As shown in Figure 4, amino acids 290 to 593 in Acr1 showed 42% identity and 57% similarity to MedA. Most strikingly, amino acids 471 to 593 were almost identical to MedA, suggesting that this region may be highly conserved in this class of presumed transcription factors. Our sequence analysis also revealed that MGL inserted into amino acid 425 in the C terminus of Acr1, in the middle of the conserved region between MedA and Acr1. The *medA* gene is necessary for conidial differentiation in *A. nidulans* (Miller 1990). In the wild-type *A. nidulans*, the conidiophore vesicle bears two types of sterigmata, metulae and phialides, and chains of conidia are produced from phialides. A mutation in the *medA* gene results in aberrant conidiophores with branching chains of metulae, delayed conidial differentiation, and frequent reinitiation of the secondary conidiophores (Busby et al. 1996). *medA* and *acr1* mutants thus appear to share a similar mutant phenotype, in which the conidiogenesis

```

Acr1: 290 SAAGVTAPEAQSVPSQVQHQLNDLAAAGYNQQGGNDMLSSYRTRSSYLDHSYRPPSSLRQ 349
      | | : | | : | | : : : | | : | | | :
MedA: 231 SYAAPYSPYLQPLPSM-----NGFAASYHTDSSPRMGATQYTTVSTNSQPSIRAPSPM-- 283

Acr1: 350 SAGSTWAPAYGTQDGTERTYDYGRTATNALARPAMMTASPRMSQHGHQARMSSGLVRTSHL 409
      | | || : : : : | | : : | : | : : : | : | |
MedA: 284 -APSPMAPSWNSSFLSVNHDQ-RGSGYAVGRGV---CQPKPSSPASFSNPT--LIRTSTL 336

      MGL insertion
      ▼
Acr1: 410 SASAQQLGMGYSGSWGVS--QKAQLHIRGGTAALDSMMTDRWTSEEWTNQRRIVLFTKRHN 467
      | | : : : | | : | : | | | | | | : | | : :
MedA: 337 QQSAGVVQTQSFNPYAMYPSKAVLKLNGDL-----NTMTHNWTREEQVAQRRLVQFTRMQS 392

Acr1: 468 GPNLHIDFRPVSVSERPSNSICISCIYWARGPNERGECYVTSVDTIYLLERLIEPLATMG 527
      | : | | : | | | | | | | | : | | : | | | | | :
MedA: 393 GSTIHADFKPVSPEERAPNSICISCIYW----EGKDECFITSVDTIYLLESLVGV----- 443

Acr1: 528 KFGVEEKNRIRRNLEGFKPITVSKGKPESEEFFKLIMGFPPNPKPRNIEKDVKVFPWKYWG 587
      : | | | | | | | | : : | | | : | | : | | | | | |
MedA: 444 RFTVEEKNRIRRNLEGFRPLTVSKSKADSEEFFKVIMGFPAKPRNIEKDVKVFPWKILG 503

Acr1: 558 QHSRRL 593
      : : :
MedA: 504 HALKKI 509

```

cells are reiterated in chains. Thus, MedA and Acr1 appear to represent founding members of a novel class of conserved regulators controlling conidium pattern formation.

We thank Jin-Rong Xu of Purdue University for helpful discussion and reading the manuscript.

- Berg, D. E., and Howe, M. M. 1989. In *Mobile DNA*. American Society for Microbiology, Washington, D.C.
- Busby, T. M., Miller, K. Y., and Miller, B. L. 1996. Suppression and enhancement of the *Aspergillus nidulans medusa* mutation by altered