**Hop, an Active Mutator-like Element in the Genome of the Fungus**

*Fusarium oxysporum*

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A new type of active DNA transposon has been identified in the genome of *Fusarium oxysporum* by its transposition into the *niaD* target gene. Two insertions within the final exon, in opposite orientations at the same nucleotide site, have been characterized. These elements, called *Hop*, are 3,299 bp long, with perfect terminal inverted repeats (TIRs) of 99 bp. The sequencing of genomic copies reveals a 9-bp target site duplication and no apparent sequence specificity at the insertion sites. The sequencing of a cDNA indicates that *Hop* does not contain an intron and encodes a putative transposase of 836 amino acids. The structural features (length, TIRs size, and 9-bp duplication) together with the presence of conserved domains in the transposase, strongly suggest that *Hop* is a Mutator-like element (MULE). *Hop* is thus the first active member of this family found beyond plants. The high rate of excision observed indicates that *Hop* is very active and thus represents a promising efficient tagging system for the isolation of fungal genes. The distribution of *Hop* elements within the *Fusarium* genus revealed that they are present in different species, suggesting that related elements could be present in other fungal genomes. In fact, *Hop*-related sequences have been identified in the survey of the entire genome sequence of three other ascomycetes, *Magnaporthe grisea*, *Neospora crassa*, and *Aspergillus fumigatus*.

**Introduction**

Transposable elements (TEs) are ubiquitous components of prokaryotic and eukaryotic genomes. They are classified in two major classes according to their structure and mechanism of transposition (Finnegan 1992). Class I elements are retroelements using a reverse transcriptase for transposition via an RNA intermediate; class II elements, or DNA transposons, transpose directly from DNA to DNA by means of a transposase. Some families of TEs have been found in all phyla, whereas others appear to have a restricted host range (e.g., the *P* element of insects and the *Mutator* system in plants).

The *Mutator* (*Mu*) system of transposable elements has been originally identified by Robertson (1978) in a line of maize exhibiting an extremely high mutation frequency. This system is composed of diverse families of class II elements sharing similar approximately 220-bp terminal inverted repeats (TIRs), but each containing unrelated internal sequences (for review, Chandler and Hardeman 1992; Bennetzen 1996; Walbot and Rudenko 2002). At least 15 different nonautonomous *Mu* elements have been described (Walbot and Rudenko 2002). Their transposition requires the presence and expression of members of the *MuDR* class of elements (Chomet et al. 1991; Hershberger, Warren, and Walbot 1991; James et al. 1993), and creates an 9-bp host sequence duplication at the insertion site. *MuDR* is a 4.9-kb autonomous element containing two genes: *mudrA* and *mudrB*. The *mudrA* gene product, the MURA protein of 823 amino acids, is probably the transposase, because (1) its expression is sufficient to catalyze somatic excision of *Mu* elements (Lisch et al. 1999; Raizada and Walbot 2000), (2) it binds to *Mu* elements TIRs within a 32-bp motif known as the MURA binding site or MBS (Benito and Walbot 1997), and (3) *MURA* transposase shares an amino acid sequence motif with putative transposases of a group of bacterial insertion sequences (IS) (Eisen, Benito, and Walbot 1994). *mudrB* encodes the MURB protein, whose function is not clearly established, but which seems to be necessary for a completely active *Mu* system in maize (Lisch et al. 1999; Raizada and Walbot 2000). The *Mutator* system, well studied both at the genetical and molecular levels, is the most active transposable element system described in any organism and has been used to generate and tag thousands of new mutants in maize (reviewed in Chomet 1996; Martienssen 1998; Walbot 2000). Recently, wide-ranging surveys of exotic maize strains, complete genomic sequencing of some plants, and PCR strategies have permitted the discovery of elements called *Mutator*-like elements (MULEs) that encode a putative MURA-related protein. They have been identified in plant species as diverse as *Arabidopsis* (Le et al. 2000; Yu, Wright, and Bureau 2000; Singer, Yordan, and Martienssen 2001), rice (Eisen, Benito, and Walbot 1994; Yoshida et al. 1998; Mao et al. 2000; Turcotte, Srinivasan, and Bureau 2001), and different grasses (Lisch et al. 2001). These studies demonstrated that MULEs are widely dispersed in plants. As in maize, *Arabidopsis* and rice MULEs are heterogeneous in structure, sequence, and size (Yu, Wright, and Bureau 2000; Singer, Yordan, and Martienssen 2001; Turcotte, Srinivasan, and Bureau 2001).

To date, no nonplant species has been reported to harbor *Mu*-related sequences. The fact that a group of bacterial insertion sequences shared a sequence motif with the MURA transposase raised questions about the evolutionary history of the *Mutator* family. Horizontal transmission is a possible explanation for their presence across such an evolutionary distance as reported for other transposable elements, such as *P* and *mariner* elements in Drosophila, and insertion sequences in bacteria (Daniels et al. 1990; Maruyama and Hartl 1991; Lawrence, Ochman, and Hartl 1992). An alternative hypothesis is that other
transposons of this group exist in other eukaryotes but have not yet been characterized (Eisen, Benito, and Walbot 1994).

In filamentous fungi, many different types of transposable elements have been identified by several methods. Representatives of DNA transposons and retroelements have been characterized in these species, indicating that fungal transposons reflect the overall spectrum of eukaryotic transposable elements (for a review see Daboussi 1996; Daboussi 1997; Kempken and Kück 1998). Plant pathogenic fungi, such as Magnaporthe grisea, the main pathogen of rice, and Fusarium oxysporum, pathogenic on more than a hundred plant species, appeared to be very rich niches for TEs. Different families of DNA transposons belonging to the Tc1-mariner and hAT superfamilies, including active elements, have been characterized. Interestingly, active members of the pogo family are widely dispersed in ascomycetes, whereas no transposing elements have been found in other organisms (Daboussi 1997). Since some families of transposons appeared to be prevalent in fungi, these organisms seem to be of great interest in the search for transposons up to now identified in fewer host species.

During our study of active transposons trapped after their insertion into the niaD gene encoding nitrate reductase used as a target, we identified inserted sequences from the three transposable elements impala, Fot1, and Fot2 previously characterized (Daboussi, Langin, and Brygoo 1992; Daboussi and Langin 1994; Langin, Capy, and Daboussi 1995). In this report, we present the results of the molecular characterization of a novel insertion corresponding to a DNA transposon that we call Hop. We provide evidence that Hop shares common structural features with MULEs. It appears to be a very active transposon in F. oxysporum, thus representing a promising tool for the development of efficient tagging systems in fungi. The discovery of an active MULE element in a fungus provides an exceptional opportunity to identify related elements in other fungal species. The detection of Hop-related sequences in different species of ascomycetes suggests that this type of element is more widespread than normally thought.

Materials and Methods
Fungal Strains and Media

The niaD12 and niaD78 mutants are derived from the TR7 strain, in which the niaD21 mutation in strain FOM24 was complemented by one copy of the Aspergillus nidulans niaD gene introduced by genetic transformation (Daboussi, Langin, and Brygoo 1992). Both mutants were identified among nia mutants selected by chlorate resistance, as described in Daboussi, Langin, and Brygoo (1992). For the genome-wide survey of the presence of Hop, strains belonging to different formae speciales (based on their pathogenicity towards particular host) of the F. oxysporum species complex and strains belonging to different species within the Fusarium genus have been described previously (Hua-Van, Langin, and Daboussi 2001; Daboussi et al. 2002) (see table 1). For the transposon display technique, three mutants affected in pathogenicity and selected by impala transposition (Migheli et al. 2000) were used. For each mutant, three cultures corresponding to the laboratory stock (ls), isolation from root (r), or stem (s) were analyzed. The minimal and complete media and culture conditions were previously described (Daboussi-Bareyre 1980).

Screening of Revertants

Somatic revertants of niaD12 and niaD78 mutants were selected on solid mineral medium with nitrate as sole nitrogen source (MMN). They are easily detected by the appearance of patches of aerial mycelium with a wild-type phenotype on a background of sparse mycelium corresponding to a niaD mutant. Their frequencies were estimated by counting the number of aerial colonies after plating 10³ mutant conidia per Petri dish onto MMN after a month of incubation at 26°C.

Southern and Northern Blot Analysis

Preparation of genomic DNA, Southern blotting and hybridization were performed as described by Daboussi, Langin, and Brygoo (1992). Total RNA was isolated from mycelia as described by Lockington et al. (1987). Northern blots were performed on positive membranes (Appligene) using standard procedures.

Cloning of Hop Copies

Cloning of Hop78: basic protocols and methods of DNA extraction were as described (Maniatis, Fritsch, and Sambrook 1982). The 6.5-kb EcoRI DNA fragment from the niaD78 strain, including the Hop78 3.3-kb insertion, was cloned using a partial library. This library was obtained by digesting niaD78 genomic DNA with EcoRI; DNA fragments between 6 and 7 kb were isolated by electrophoresis and inserted in the EcoRI site of pUC19. The ligation products were used to transform the E. coli strain DH5α. The library was screened using the 32P-labeled 3.2-kb EcoRI fragment of the niaD gene as a probe. One of the clones, including the Hop78 element, pHop78, was then used as a probe in Southern blotting experiments and sequenced. Cloning of Hop12 was performed as described for Hop78.

Hop PCR products were cloned by purification using the Jetsorb kit (Bioprobe) and the pGEMT-easy kit (Promega). For each strain, one or two individual clones were sequenced using the reverse primer.

Polymerase Chain Reaction

PCR experiments were conducted in a PTC-100 thermocycler (MJ Research, Watertown, Mass.). One hundred to three hundred nanograms of genomic DNA were used as a template. Amplifications of the full Hop element were performed using a primer specific of the TIRs, TIR1 (5′-GGGAAGCCATACCTGCACG-3′), or TIR2 (5′-GGTCTGGCGGATTAGATCCG-3′) at 94°C for 4 min, followed by 30 cycles of 1 min at 94°C, 1 min at 60°C, and 3 min 30 s at 72°C, then a final extension of 10 min at 72°C. Hop-related elements in the F.
oxysporum strains were amplified using the TIR1 (see above) and the L8 (5'-CTGACTGAAATGAGGTCCG-3') primers and using the same program except for the elongation time (1 min). The primers U1 (5'-GTGCGCA- CATCCAACACCTGG-3') and U2 (5'-CAATACAGAA-GGTCCGTTG-GC-3') were used to amplify an internal region of Hop-like sequences in Fusarium javanicum var. radicicola (using the same protocol except for the elongation time of 1 min). The localization of the primers relative to the Hop-78 sequence is shown in figure 1C.

Sequencing and Sequence Analysis

Sequencing was performed using the ABI PRISM Dye-Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) on an ABI373 Automated DNA sequencer (Applied Biosystems). Primers used for the sequencing of *Hop* genomic copies in the FOM24 strain were L8 (see above) and U4 (5'-GTGGCAACATCCAACACCTGG-3'). Sequences were analyzed using the DNA Strider 1.3 package. Similarities to known sequences were sought using the Blast programs (Altschul et al. 1997). Preliminary sequence data were obtained from the following ongoing genome projects: for *Aspergillus fumigatus*, The Institute for Genomic Research (with support from the National Institute of Allergy and Infectious Diseases, NIH and Wellcome Trust), the Web site at http://www.tigr.org; for *Neurospora crassa*, Data Version 3, Whitehead Institute/MIT Center for Genome Research (www-genome.wi.mit.edu); for *Magnaporthe grisea*, Data Version 10/4/2002, Ralph Dean, Fungal Genomics Laboratory at North Carolina State University (www.fungalgenomics.ncsu.edu), and the Whitehead Institute/MIT Center for Genome Research (www.genome.wi.mit.edu); for *Cryptococcus neoformans*, Stanford Genome Technology Center, funded by the NIAID/NIH under the cooperative agreement U01 AI47087, and The Institute for Genomic Research, funded by the NIAID/NIH under the cooperative agreement U01 AI48594. Multiple alignments of sequences were obtained using the ClustalX program (Thompson et al. 1997) and manually adjusted. The nucleotide sequence data reported in this paper are available in the GenBank database under the following accession numbers: AY267761 (*Hop*-78 complete sequence), AY267762 to AY267780 (partial sequence of *Hop*-related elements from a range of *F. oxysporum* strains), and AY267760 (*F. oxysporum* putative O-methyltransferase gene). Maximum-parsimony phylogenetic
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Fig. 1.—(A) Hop insertion sites in the niaD12 and niaD78 mutants. The niaD gene contains seven exons (black boxes). Structure of the integrated pAN301 plasmid carrying the niaD gene (gray box). E: EcoRI restriction site. Sizes are in kb. (B) Southern blot of EcoRI-digested genomic DNA isolated from the TR7 strain containing the wild-type niaD transgene, the niaD78 strain, and a [Nia+] revertant selected from niaD78. The blot was probed with the pAN301 plasmid carrying the niaD gene of A. nidulans (see Materials and Methods). (C) Structure of the Hop-78 element. Terminal inverted repeats (TIRs) are denoted by the black arrowheads at each end of the element. Gray box represents internal noncoding sequences. The virtual translation of the 836-amino acid ORF begins at ATG193 and ends at TAG2701. Small arrowheads indicate the presence of a DNA transposable element. To determine the nature of this element, the 6.5-kb EcoRI fragment of the niaD78 and niaD12 alleles containing the 3.3-kb insertion and the 1.9-kb insertion of the Hop-78 insertion were obtained after drying the gels and X-ray film exposure for 48 h.

Results

Characterization of Hop, a Novel F. oxysporum Transposon

The Hop element was isolated by its transposition within the niaD target gene. Southern hybridizations of genomic DNA of niaD mutants and wild-type strains digested with EcoRI and probed with the niaD gene revealed in two mutants, niaD78 and niaD12, structural modifications of the niaD gene consistent with the insertion of a 3.3-kb fragment, either in the final exon or in the 3’ untranslated region (fig. 1A and B). These inserted sequences are larger than the 1.9-kb insertion of the niaD37 allele, which corresponds to the Fot1 element (Daboussi, Langin, and Brygoo 1992) and to the 1.3-kb insertion of the niaD160 allele due to an insertion of the impala element (Langin, Capy, and Daboussi 1995).

The niaD12 and niaD78 mutants are unstable, giving rise on MMN to patches of aerial mycelium corresponding to [Nia+] revertants (see Materials and Methods). Southern analysis of a sample of revertants reveals that the restoration of nitrate reductase activity is due to the excision of the 3.3-kb inserted DNA, as indicated by the presence of the fragment, which has the wild-type size. (fig. 1B). Such spontaneous insertions and excisions indicate the presence of a DNA transposable element. To determine the nature of this element, the 6.5-kb EcoRI fragment of the niaD78 and niaD12 alleles containing the 3.3-kb insertion (fig. 1B) were cloned into the pUC19 vector. The resultant clones, pHop78 and pHop12, were respectively entirely or partially sequenced.
The borders of the Hop-78 element were defined by comparing the wild-type sequence of niaD with that of the mutant (fig. 1D). The nine flanking nucleotides (GGGACACCCG) observed at both extremities of Hop-78 probably correspond to a direct duplication of the target site of the element upon its insertion, since this sequence is present in one copy in the wild-type gene. The analysis of flanking sequences of the insertion indicates that Hop-78 is inserted in the final exon of the niaD gene, within the region corresponding to the flavin domain (Campbell and Kinghorn 1990). Sequence of the flanking DNA of Hop-12 revealed that this copy is inserted at the same nucleotide site as Hop-78, but in the opposite orientation. This insertion is also flanked by a 9-bp direct duplication.

The Hop-78 sequence corresponds to an element of 3,299 bp. It contains perfect TIRs of 99 bp, suggesting that Hop-78 corresponds to a DNA transposon. An uninterrupted ORF of 2,507 bp (nucleotides 193 to 2700), potentially encoding a polypeptide of 836 amino acids, can be deduced from this sequence (fig. 1C). The orientation of this ORF indicates that the Hop element has inserted in the orientation opposite to that of niaD in the niaD78 mutant. A pyrimidine-rich tract (CT block) found upstream of the translational initiation site in strongly expressed fungal genes is present in the 5′-flanking sequence (Gurr, Ungles, and Kinghorn 1987).

A cDNA library constructed using mRNAs from the strain TR7 was probed with a specific Hop-78 probe, and one positive excised recombinant pBK-CMV phagemid vector was further characterized. This Hop cDNA is 2.6 kb long and matches perfectly the Hop-78 sequence (from nucleotide 152 to nucleotide 2782). This result indicates that Hop does not contain an intron. A motif TAAAT, located −35 bp upstream of the presumed transcriptional start site, could correspond to a TATA-box, whereas the only potential polyadenylation signal located within the transcribed sequence corresponds to AATATA at position 2762.

Hop Putative Transposase Shows Similarity with Transposases of MULEs

The Hop-78 sequence was used to screen the DNA and protein NCBI data banks using Blast programs (Altschul et al. 1997). A BlastN search did not yield any significant alignment. Using the BlastX program, the putative translation product of Hop displays similarities with different putative proteins and far-red protein-like impaired responses in Oryza sativa. The Hop putative product is also similar to Arabidopsis putative proteins noted as Mutator-like transposases and putative transposases from rice (Oryza sativa) and maize (Zea mays). A region of approximately 100 amino acids, corresponding to the previously identified domain conserved among transposases of plant MULEs and some bacterial IS sequences (Eisen, Benito, and Walbot 1994; Lisch et al. 2001; Singer, Yordan, and Martienssen 2001), is seen in the Hop putative transposase (fig. 2A). For some of the significant alignments (e values < 10⁻⁶), this region of similarity can be extended in the C-terminal part of the proteins revealing a second conserved domain, which includes a CX₂CX₄HX₄/C₆C motif (fig. 2B). This motif is present in MURA transposase of maize and in many MURA-related proteins in Arabidopsis (Yu, Wright, and Bureau 2000). However, this domain is not systematically present in plant MURA-like proteins, and it is absent in bacterial transposases. This signature also exists in many nucleic acid binding proteins and has been demonstrated to interact with RNA and single stranded DNA. In MURA-related proteins, the CCHC motif might play a role in MULE transposition or regulation as suggested by Yu, Wright, and Bureau (2000). This domain might interact with transposon DNA or RNA, and/or host genome insertion site.

The nucleotidic sequence of TIRs has been more precisely analyzed. No significant similarity to any MULE TIR, or to the maize MURA transposase-binding site (Benito and Walbot 1997) could be identified in the Hop-78 sequence. It is noteworthy that the first three nucleotides in Hop are G, as in Arabidopsis TIR-MULEs, which all start or end with one to four G nucleotides (Singer, Yordan, and Martienssen 2001).

Hop Related Elements Are Also Present in Other Fungal Species

Blast searches were also performed using fungal genome databases. No significant similarity was seen when the ascomycete Candida albicans (http://www.sanger.ac.uk/) and the basidiomycete Phanerochaete chrysosporium (http://www.genome.wi.mit.edu) genome database were tested; nor with the Fusarium sporotrichioides (http://www.genome.ou.edu/) cDNA database. However, TBlastN searches revealed that the Hop putative transposase shows a very strong similarity (e value = 2.10⁻⁷⁸) with a virtual translation product from the rice blast Magnaporthe grisea (http://www-genome.wi.mit.edu) and a weaker but significant similarity (e values between 10⁻¹⁴ and 10⁻⁶) with a set of translated Neurospora crassa sequences (http://www-genome.wi.mit.edu) and one from Aspergillus fumigatus (http://www.tigr.org/). The homologous region of the Magnaporthe grisea virtual translation product extends throughout the Hop putative transposase, whereas in Neurospora crassa and Aspergillus fumigatus, the sequences similar to Hop transposase correspond to the conserved domain 1 (fig. 2A). In all three species, the translation products contain many stop codons, suggesting that these Hop-related sequences are inactive.

A multiple alignment of the amino acid sequences corresponding to the conserved domain 1 shared by Hop putative transposase, the three identified fungal virtual translation products, the MuDR MURA transposase, diverse plant putative transposases, and a bacterial IS transposase is shown in figure 2A. The unrooted phylogenetic tree derived from parsimony analysis of this domain is shown on figure 2C. This tree is incongruent with the phylogeny of plant species. This difference can be explained by horizontal transfers, faster evolution in certain lineages, and/or ancestral polymorphism (Capy, Anxolabéhère, and Langin 1994). Recent studies clearly show that MULEs form a broadly diversified group with
multiple classes within individual genomes, leading to discrepancies between MULE sequences and their hosts (Lisch et al. 2001). In this phylogenetic tree, fungal sequences group but with a very low statistical support (bootstrap value close to 20).

Copy Number and Features of Hop Elements in Strain FOM24

To determine the representation of Hop elements within the genome of the FOM24 strain from which they have been trapped, genomic DNA was digested with EcoRI, which has no restriction site within Hop-78.

Southern blot hybridization using the pHop78 probe revealed an array of Hop-homologous fragments, probably representing single elements, which we estimated at approximately 50 copies (fig. 3B). When the genomic DNA of this strain is digested with restriction enzymes that cut twice within Hop-78, one observes a major band corresponding to the predicted internal fragment and faint bands of different size corresponding to fragments flanking different elements. These results indicated that the

FIG. 2.—Phylogeny of several MULEs. (A) and (B) Multiple alignments of the conserved domain 1 and 2, respectively. This alignment includes the transposase of a bacterial IS element (IS6120: accession no. M69182.1); the MURA transposase from the Zea mays MuDR element (accession no. M76978.1); and putative MURA-related transposases from the plants Zea mays (Zm-Jittery: accession no. AF247646.1 and Zn-3015: accession no. AF466931.1), Arabidopsis thaliana (AtMut1: accession no. AC029938.1 and At-189666: accession no. NM_113946.1), Oryza sativa (Os-74278: accession no. AC083943.8 and Os-16510: accession no. AP005198), and Lotus japonicus (Lj-4927: accession no. AP004927.1), and from the fungi Fusarium oxysporum (Hop-78), Magnaporthe grisea (Mg-2.2154: contig no. 2.2154), Neurospora crassa (Nc-3.344: contig no. 3.344), and Aspergillus fumigatus (Af-930: contig no. 930). Identical amino acids are shaded in black, and similar amino acids are shaded in gray. (C) Unrooted phylogenetic tree of several MULEs and a bacterial IS element derived from parsimony analysis of amino acid sequences corresponding to the conserved domain 1. Bootstrap values based on 1,000 replicates are indicated on the nodes.
many copies of the Hop element are fairly homogeneous in size and structure. To test whether some nonautonomous Hop elements could share the same TIRs as full-length elements, we performed PCR experiments on FOM24 genomic DNA using a primer located in the TIR region (either the TIR1 or the TIR2 primer, see Materials and Methods and fig. 1C)). A single, wide band of the predicted size (3.3 kb with TIR1 and 3.2 kb with TIR2) was amplified in each case (fig. 3C). This result suggests that the FOM24 strain does not contain internally deleted elements sharing TIRs identical to those of Hop-78, as reported for MuDR in maize.

To determine whether Hop elements from the FOM24 strain are also homogeneous in sequence, we investigated Hop polymorphism, sequencing the 5′ and 3′ sections of several genomic copies, identified by screening half of a cosmid library probed using a specific Hop-78 sequence (Diolez et al. 1993). Fifteen of 48 positive cosmids were sequenced using the Hop internal L8 or U4 primer (see Materials and Methods and fig. 1C). The L8 primer was chosen as containing the sequence of the first approximately 450 nucleotides of Hop (including the 5′ TIR, the 5′ noncoding region, and the first approximately 250 nucleotides of the ORF), whereas the U4 primer yielded the final 250-nucleotide sequence (including part of the 3′ noncoding region and the 3′ TIR). Since three different flanking regions were recovered twice among the cosmid samples, we compared the nucleotide sequence of the 12 cosmids containing different Hop insertions. Except for one copy, whose 3′ portion contains 10 nucleotides different from Hop-78, we observed no nucleotide polymorphism (over 455 nucleotides in the 5′ end and 250 nucleotides in the 3′ end), indicating that at least many Hop copies present in the FOM24 strain are extremely similar.

Transposition Properties of Hop

To investigate Hop transposition properties such as insertion site specificity and size of the duplicated sequences after insertion, we sequenced the 12 Hop flanking sequences using the L8 and U4 Hop-specific primers (see Materials and Methods and fig. 1C). The results, presented in figure 4, show that for seven insertions, a perfect 9-bp direct repeat of host sequences is detected, as observed in the niaD78 mutant. For two insertions, this repeat is 10 bp long. For the three other insertions, no direct duplication could be identified adjacent to intact TIRs. The comparison between the overall data concerning the target site duplication sequences and that of adjacent sequences reveals no clear consensus.
**Materials and Methods**

The PCR was performed as described by Melayah et al. (2001), with 35 ng of the Hop-specific primer L6 previously 3P-labeled, and 50 ng of T00, a selective primer homologous to the TaqI adaptor sequence (see Materials and Methods). The S-SAP autoradiography obtained after TaqI restriction displays flanking insertion sequences upstream of the 5’ end of the Hop element. Polymorphism is characterized by appearance of new bands or by missing bands, both marked with black arrows. 1. 473Ls; 2. 473r; 3. 473s; 4. 493Ls; 5. 493r; 6. 493s; 7. 468Ls; 8. 468r; 9. 468s; 10. Fom24.

**Fig. 5.—Polymorphism of Hop insertions into the mutants 473, 493, and 468** affected in pathogenicity and issued from FOM24 strain (see Materials and Methods). For each mutant, three cultures corresponding to the laboratory stock (ls), isolation from root (r), or stem (s) were analyzed.

Hop elements appeared to be very active in the FOM24 strain, as indicated by the two independent insertion events within the **niaD** gene and the unexpectedly high frequency of excision of Hop-78 and Hop-12 when inserted within an exon. Indeed, we observed, typically, one to 20 reversion events per plate, thus a frequency of wild-type revertants ranging from 10⁻³ to 2.10⁻² with respect to the number of spores plated. The sequencing of the excision site in two revertants revealed a wild-type sequence.

Another indication of the high activity of Hop in the FOM24 genetic background was derived from the analysis of the transpositional behavior of Hop in different mutant strains using the Transposon Display technique. This high-resolution method developed for the simultaneous visualization of **dPh1** elements in *Petunia hybrida* (Van den Broek et al. 1998) was successfully used to monitor the occurrence of transposition events of transposable elements present in high copy number (De Keukeleire et al. 2001; Melayah et al. 2001). Comparison of the patterns obtained with different *F. oxysporum* cultures originating from the same clone revealed a high level of polymorphism, due mainly to the appearance of new bands of different size (fig. 5). The profiles differ, indicating that insertions at new positions in the genome are frequent. In addition, signal intensity differences and loss of some bands suggest somatic excisions. The analyses demonstrate the efficacy of this technique as a tool in the study of the behavior of the entire Hop family and suggest that Hop is actively transposing in the strains.

After analysis of the flanking regions of the 12 genomic copies identified by screening half of the cosmids library of the FOM24 strain, we found that one Hop element had inserted into a gene. This copy is located in an interrupted ORF of 951 bp capable of coding for a putative protein of 317 amino acids, which presents clear homologies to a set of **O-methyltransferases** from very diverse organisms, such as prokaryotes (Archaea and Bacteria) and eukaryotes (plants, animals, and fungi). A high score was obtained with a fungal (*Podospora anserina*) putative SAM-dependent **O-methyltransferase**, which accumulates during the senescent stage (Averbeck et al. 2000). The fact that Hop insertion does not determine a visible mutant phenotype suggests that this gene is not essential in *F. oxysporum* and/or is present in more than one copy. Additionally, a second Hop element is located 184 bp downstream of an ORF that we have partially sequenced, which encodes a putative protein strongly similar to transposases from fungal Ac-like transposons such as *Tfo1* of *F. oxysporum* (Okuda et al. 1998) and **restless** from *Tolypocladium inflatum* (Kempken and Kück 1996). This ORF is 99% identical to the putative product of a repetitive sequence (GenBank accession number X82490.1) reported to be responsible for the disruption of an *F. oxysporum* cytochrome **P450** gene (Mouyna 1994).

**Distribution and Polymorphism of Hop-Related Elements Within the F. oxysporum Species and the Fusarium Genus**

To test for the presence of Hop-related sequences, we carried out Southern blot analysis on *F. oxysporum* strains belonging to different formae speciales (table 1) and a more specific PCR assay using a primer deduced from the TIR (TIR1) and a second (L8) within the ORF (see Materials and Methods and fig. 1C). The salient points of the data presented in table 1 and figure 6A are as follows: (1) Hop-related sequences are present in most of the *F. oxysporum* strains tested, with a variable copy number ranging from 0 to about 50, (2) all strains showing Hop-related sequences by Southern analysis yielded a unique PCR product of the expected size (526 bp);
conversely, strains devoid of hybridizing fragments gave no amplification.

To investigate Hop polymorphism, PCR products obtained with 10 strains belonging to six formae speciales were cloned and, generally, two different clones were sequenced for each strain. The sequenced region spans nucleotide positions 20 to 506, which include the 5' noncoding region and approximately 300 nucleotides of the ORF. These sequences, as compared with the Hop-78 sequence copy, yielded a polymorphism lower than 2% (0 to 9 nt differ out of 487 bp). Of the 72 differences detected, the majority (75%) were in noncoding regions. Among the 17 differences in the ORF, two resulted in the probable inactivation of the transposase, and half the others corresponded to synonymous changes.

The presence of Hop in different Fusarium species was also investigated by Southern blot analysis using high stringency conditions (fig. 6B). Of the 10 species surveyed, genomic DNAs from F. javanicum var. radicicola and F. caucasicum species showed a clear Hop-78 hybridization with, respectively, two and more than 20 hybridizing bands. DNAs from four other species, namely Fusarium solani f. sp. pisi, F. solani var. minus, Neocosmospora sp., and F. solani sp. exhibited very faint signals. All these strains belong to the Martiella section, which are distantly related to F. oxysporum. In contrast, Hop-related elements

![Image of figure 6](https://academic.oup.com/mbe/article-abstract/20/8/1362/1081651/Hop-an-Active-Mutator-like-Element-in-the-Genome/7)

**Fig. 6.—** Hop-related sequences in the Fusarium genus. (A) Genomic distribution of Hop-related elements within the F. oxysporum complex and (B) genomic distribution of Hop-related elements within species of the Fusarium genus. Strains are listed in table 1. F. ver = Fusarium verticillioides; G. fuj = Gibberella fujikuroi; F. sol mar = Fusarium solani var. martii; F. sol pisi = Fusarium solani f. sp. pisi; F. jav rad = Fusarium javanicum var. radicicola; F. sol min = Fusarium solani var. minus; F. cau = Fusarium caucasicum; Neo sp. = Neocosmospora sp.; F. sol sp. = Fusarium solani. Genomic DNA was digested with EcoRI and the filters were probed with a full-length Hop-78 PCR product. The upper portion is the ethidium-stained DNA of each sample before blotting. (C)Aligned DNA sequences corresponding to part of the ORF of Hop-78 and a Hop element from F. javanicum var. radicicola (Hop-Fj).
were not found in the most closely related species of the *Liseola* section. To determine if the hybridizing signals observed corresponded to *Hop*-related elements, we chose to clone and sequence one PCR product from *F. javanicum* var. *radicicola*, which showed a clear hybridization signal but less intense than that of *F. caucasicum*. Among 380 bp corresponding to a part of the ORF, 60 nucleotide changes were detected, for a level of nucleotide divergence of 15%. The polymorphism was distributed along the *Hop* sequence and essentially corresponded to transitions (fig. 6C). Over the 60 nucleotide differences between *Hop*-78 and the *F. javanicum*-related sequence, 29 were synonymous substitutions and 31 were nonsynonymous substitutions. Using the method of Nei and Gojobori (1986) with the Jukes and Cantor correction for multiple hits, the ratio of synonymous to nonsynonymous substitutions ($K_s/K_a$) found was 3.88. This result suggests that *Hop* sequences have been under selective pressure. Mutations that may destroy the transposase have not been detected, suggesting that *Hop* elements in *F. solani* var. *radicicola* may encode an active transposase.

**Discussion**

Structural and Functional Features of *Hop*, the First *Mutator*-like Element Identified in the Fungal Kingdom

A new DNA transposon, *Hop*, active in the genome of the fungal plant pathogen *Fusarium oxysporum*, has been characterized. This element is 3,299 bp long and has long, perfect TIRs (99 bp) flanking a single uninterrupted ORF, potentially encoding an 836-amino acid protein. *Hop* displays several features typical of *Mutator*-like elements. Initially, upon insertion, *Hop* elements recently transposed in the *niaD* gene typically generate a 9-bp target site duplication. In addition, most *Hop* genomic copies are flanked by a 9-bp or 10-bp target site duplication (9/12 among the surveyed elements). This target site duplication is characteristic of MULEs (Chandler and Hardeman 1992; Bennetzen 1996; Le et al. 2000; Yu, Wright, and Bureau 2000; Singer, Yordan, and Martienssen 2001; Turcotte, Srinivasan, and Bureau 2001). Surprisingly, we also observed that 3/12 *Hop* elements have complete TIRS but are not flanked by target site duplications. This may arise from ectopic recombination between *Hop* copies located at different loci. Indeed, it has been proposed that the high number of dispersed repeated sequences of the FOM24 strain could serve as substrate for ectopic recombination leading to a high level of chromosomal rearrangements (Davière, Langin, and Daboussi 2001). Alternatively, *Hop* might have the capacity to insert within preexisting double-strand breaks; such insertion events would not lead to TSD. Secondly, *Hop*78 contains a MURA-related ORF with two regions that can possibly be aligned with transposases of MULEs. The initial conserved region corresponds to the major part of the approximately 130 amino acids domain identified by Eisen, Benito, and Walbot (1994), containing the 25-amino acid signature sequence [D-x(3)-G-(LIVMF)-x(6)-(ESTAV)-(LIVMFYW)-(PT)-x-(STAV)-x(2)-(QR)-x-C-x(2)-H]. This domain is shared by the maize MURA transposase, plant MURA-related proteins, and some bacterial transposases, including two MULEs of *Arabidopsis* (*AtMu1* and *AtMu2*), which probably encode a functional transposase, Singer et al. (2001) proposed the extension of the signature consensus sequence [D-x(3)-G-(LIVMF)-x(6)-(ESTAV)-(LIVMFYW)-(PT)-x-(STAV)-x(2)-(QR)-x-C-x(2)-H]. The putative *Hop* transposase does not perfectly match this consensus: it differs from the signature at four residues (positions 5, 6, 13, and 20 of the motif). Assuming that bacterial, plant, and *Hop* MURA-related transposases are encoded by TEs belonging to the same superfamily, we propose a new extended consensus motif [D-x(3)-(AG)-(CLIVMF)-x(6)-(CESTAV)-(LIVMFYW)-(PT)-x-(STAV)-x(2)-(QR)-x-C-x(2)-H]. To date, no function has been assigned to this domain. The second conserved domain includes the motif CX$_2$CXX$_{14-16}$C at the C-terminal region present in the maize MURA transposase, in many *Arabidopsis* MURA-related proteins and some MURA-related proteins from rice (Yu, Wright, and Bureau 2000 and this study). This motif, mainly found in the nucleocapsid protein of retroviruses, is also present in some retrotransposons and in several known nucleic acid binding proteins. It has been previously proposed by Yu, Wright, and Bureau (2000) as a role player in a transposase/transposon (DNA or RNA) interaction involved in transposition mechanism and/or regulation. However, this CCHC zinc finger is absent in bacterial transposases and many eukaryotic MURA-related putative transposases. Since DNA-binding ability is certainly an essential feature for protein function in these situations, these proteins probably contain another DNA-binding domain instead of the CCHC zinc finger. Finally, the *Hop* element has no *mudB* encoding product, as observed in many plant species. Indeed, genome survey of MULEs from the *Arabidopsis* and rice sequencing projects indicated that none of the elements encode a protein resembling MURB (Yu, Wright, and Bureau 2000; Singer, Yordan, and Martienssen 2001; Turcotte, Srinivasan, and Bureau 2001). A recent analysis of the distribution of MULEs revealed that *mudA* sequences are widespread in the grasses, whereas *mudB* sequences appeared to be restricted to the Zeas (Lisch et al. 2001).

The *Hop* Family, Unlike Other MULEs, Is Very Homogeneous

Within the FOM24 strain, the 50 *Hop* copies were to be found homogeneous in structure and in size. Even by a sensitive PCR amplification method, we did not detect any nonautonomous elements that could be internally deleted and/or containing host DNA sequences. Indeed, one of the characteristics of the maize *Mu* transposons and the *Arabidopsis* and rice MULEs is their composition of diverse elements. Beyond the common similarity between TIRs, MULEs of a given family differ by their size and their internal sequences, which have no similarities with the transposase but sometimes correspond to host DNA segments. Such a situation of acquired DNA fragments has been observed in different plant species (Le et al. 2000; Yu, Wright, and Bureau 2000; Turcotte, Srinivasan, and Bureau 2001).

Beyond the structural homogeneity of *Hop* elements, we were unable to detect nucleotide polymorphism among
11 of the 12 sequenced copies, over 700 bp corresponding, for two thirds, to noncoding regions. This strongly suggests that *Hop* has amplified very recently in the FOM24 strain. Moreover, in regard to its high copy number in this genetic context, one can conclude that the amplification has been remarkably efficient. However, we cannot exclude that such an amplification results from duplications of genomic regions carrying *Hop* copies, since the FOM24 genome has been shown to harbor duplicated or triplicated portions of chromosomes (Davière, Langin, and Daboussi 2001). However, the data we present suggest that the high copy number of *Hop* in the FOM24 context may rather result from a burst of transposition, since most *Hop* insertions lie in different genomic sites.

*Hop* Elements Are Widespread Constituents of Some Fusarium Genomes

To understand the evolutionary origin of *Hop*, we analyzed its distribution and polymorphism within the *F. oxysporum* species and in a range of species belonging to the *Fusarium* genus. The presence of *Hop* in most of the *formae specialae* analyzed indicates that this transposon is an ancient component of the *F. oxysporum* complex, which has been vertically transmitted during the establishment of pathogenic specialization. The existence of rare strains devoid of *Hop* copies may be interpreted as the result of stochastic losses. The fact that some strains contain a low number of copies can accelerate such a process. *Hop* elements from strains with various host specificities and originating from worldwide areas exhibit a very low level (<2%) of polymorphism, suggesting that they have been amplified in the recent past or are subjected to selective constraints, limiting the occurrence of divergent copies.

*Hop* elements are not restricted to the *F. oxysporum* complex, since they have been recognized in some strains of distant species belonging to the section *Martella*, whereas they appeared to be absent in the species most closely related to *F. oxysporum*. More strikingly, their distribution in the *Fusarium* genus is very similar to that reported for another transposable element, *Fot1*, which belongs to the *pogo* family (Daboussi et al. 2002), with the notable exception of *F. solani var. minus*. Genomic DNA from various strains was probed at lower stringency (55°C). Using this condition, some bands are revealed with DNA from *F. oxysporum* strains FOMP2 and FOVr1, and from distantly related species *Fusarium solani var. minus* and *Neocosmospora* *sp.* (data not shown). This result suggests that diverged members of the *Hop* family are present in *F. oxysporum* and distantly related species. To understand the evolutionary history of these elements, a more thorough analysis of *Hop*-related elements in *Fusarium* species is necessary.

The discovery that *Hop* elements are present in multiple copies in different species of the *Fusarium* genus raises questions as to the occurrence of MULEs in other fungal genomes. We performed sequence similarity searches in the available databases from ongoing genome projects. We detected *Hop*-related sequences in three other ascomycetes, namely *Magnaporthe grisea*, *Neurospora crassa*, and *Aspergillus fumigatus*. Interestingly, the MULE putative product identified in *Magnaporthe* shows similarity to the main part of the *Hop* transposase. However, the presence of many stop codons indicates that this element does not encode a functional transposase. The sequence found in *Aspergillus fumigatus* and the set of sequences present in *Neurospora crassa* contain the conserved domain 1 but are also nonfunctional. In *Neurospora*, this likely results from an inactivation process of repeated sequences called RIP (repeat-induced point mutation) that occur during the sexual cycle between fertilization and karyogamy and which leads to C-to-T and G-to-A transitions (Selker et al. 1987). Indeed, different types of transposable elements found in this species show hallmarks of RIP (reviewed in Singer and Selker 1995). The *Neurospora* nucleotide sequence contained in the contig no. 3,344 that encodes a region similar to the domain 1 of *Hop* transposase has a A + T content of 76%, which is normally high for *Neurospora* DNA and is consistent with RIP (Margolin et al. 1998). Finally, TBLASTN searches on the basidiomycete *Cryptococcus neoformans* preliminary databases (http://www.sequence.stanford.edu/ and http://rcweb.bcgsc.bc.ca/cgi-bin/cryptococcus/cn.pl) using *Hop* putative transposase gives significant hits (e values between 10^{-20} and 10^{-4}) for different traces and BAC ends. Thus, *Hop* may be an ancient component of fungal genomes, since it is present in three different ascomycetes and possibly in a basidiomycete. However, this latter point, relying on preliminary sequence data from *Cryptococcus neoformans* remains to be confirmed.

*Hop* As a Promising Tool for Gene Tagging

The Mutator system in maize is an extremely active agent in promoting mutations and has been developed for gene insertion mutagenesis. This system, called “the Mutator gene machine,” is very efficient for several reasons, including a very high germlinal insertion frequency (Alleman and Freeling 1986; Walbot and Warren 1988), preferential insertions in single-copy DNA (Cresse et al. 1995), more specifically within and around genes (Hanley et al. 2000; Raizada, Nan, and Walbot 2001; Edwards et al. 2002), and germinal insertions in both linked and unlinked sites (Lisch, Chomet, and Freeling 1995). However, technical problems of plasmid stability carrying *mudrA* sequences have delayed the transfer of this system to heterologous hosts (Benetzen 1996; Raizada and Walbot 2000). The Mutator-like structural features of the *Hop* element offer the opportunity to test if it presents a high activity as *Mu* in maize.

The data presented in this paper strongly suggest that *Hop* displays several features pertinent for the development of a gene-tagging system. First, it appears to be very active as demonstrated by the high level of polymorphism in the genomic position of copies and its high rate of excision, permitting the functionality of the niaD gene. Indeed, the *Hop*-78 and *Hop*-12 copies are inserted in an exon, within one of the three important regions encoding a functional domain of the nitrate reductase, the flavin domain (Campbell and Kinghorn 1990). Both copies
excise with a frequency in the same order of magnitude as that observed for the pogo-like Fot1 transposon inserted within the third intron of the niaD gene (Daboussi, Langin, and Brygoo 1992 and unpublished results). Whereas excisions of Fot1 that generally leave a footprint of few nucleotides at the donor site do not affect the functionality of niaD, one would expect, in the case of Hop, to detect only precise excisions or, contingently, excisions leaving/deleting multiples of 3 bp, which maintain the translational reading frame and thus the functionality of the niaD gene. The two revertants we sequenced correspond to precise excisions. Mu elements of maize have been shown to leave very diverse footprints when they excise (Britt and Walbot 1991; Doseff, Martienssen, and Sundaresan 1991; Raizada, Nan, and Walbot 2001). Thus, if Hop also frequently generates large deletions or fillers as Mu elements do, one can assume that restoration of the functionality of the niaD gene probably represents a small part of all excision events and that the real excision frequency may be much higher than that observed. Analysis of the actual array of possible excision products will require a more exhaustive assay. Second, Hop was found inserted into one gene, a putative O-methyltransferase, on the small sample of insertion sites (12) we analyzed. Third, we did not detect any consensus sequence, suggesting that Hop can insert elsewhere in the genome. However, the fact that we obtained two Hop copies at the same nucleotide site in the niaD gene indicates the existence of hot spot regions. Such features have been already described with the “RescueMu” derivative of Mu1 (Raizada, Nan, and Walbot 2001). To account for these observations, it is probable that a structural feature of the target DNA is recognized during insertion, rather than a specific sequence of nucleotides.

An interesting subject for future investigations will be the demonstration of the autonomy of Hop copies inserted in niaD through the phenotypic assay we developed using Fusarium strains free of Hop elements and an extensive analysis of random insertion sites. Further studies should also determine the ability of Hop to transpose in heterologous species.

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Literature Cited


Hua-Van, A., T. Langin, and M. J. Daboussi. 2001. Potential
Diolez, A., T. Langin, C. Gerlinger, Y. Brygoo, and M. J.
De Keukeleire, P., T. Maes, M. Sauer, J. Zethof, M. Van
Daboussi, M. J. 1980. Heterokaryosis in
Nectria
1374 Chalvet et al.
Daboussi-Bareyre, M. J. 1980. Heterokaryosis in
Nectria
1374 Chalvet et al.
16:563–6572.
mosaic virus 35S-driven MURA cDNA in transgenic maize.
Plant Cell 12:5–21.
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