Nht1, a Transposable Element Cloned from a Dispensable Chromosome in *Nectria haematococca*

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Certain isolates of the plant-pathogenic fungus *Nectria haematococca* mating population VI (MPVI) contain dispensable chromosomes that are unstable during sexual reproduction. Several of these chromosomes carry genes for phytoalexin detoxification and thus contribute to the pathogenic potential of this organism. A repeated DNA sequence, *Nht1*, was cloned from one of these dispensable chromosomes in *N. haematococca* MPVI. One copy of the repeated element (*Nht1A*) was completely sequenced. It is 2,198 bp long and it possesses incomplete inverted terminal repeats (ITRs) at each end. *Nht1B*, a partially sequenced copy of *Nht1*, has complete ITRs. *Nht1A* appears to contain 2 introns and encodes a protein of 550 amino acids that is highly similar to the protein encoded by the *Fusarium oxysporum* transposon, *Fot1*. Due to the presence of ITRs, its repeated nature, and its similarity to *Fot1*, we conclude that *Nht1* is a transposable element. Within North American *N. haematococca* MPVI populations, *Nht1* is distributed discontinuously. Its copy number in different field isolates varies from zero to approximately 100 copies per genome. The *Nht1A* source isolate is estimated to contain nine to 11 copies of *Nht1*; at least six are on the chromosome from which *Nht1A* was cloned.

Additional keywords: chromosome-length polymorphisms, *Fusarium solani*.

Nectria haematococca mating population VI Berk. & Broome (*Fusarium solani* f. sp. *pisi*) is a plant-pathogenic, filamentous ascomycete with a wide host range. It is able to infect monocot species such as maize and barley, as well as dicot species such as chickpea, garden pea, and red clover. Its interaction with chickpea (*Cicer arietinum*) and garden pea (*Pisum sativum*) has been studied intensely with respect to the importance of phytoalexin detoxification as a virulence determinant (VanEtten et al. 1989a; Miao and VanEtten 1992b). One gene (*MAK1*) encoding detoxification of maackiain, a chickpea phytoalexin, and six genes (*PDA1* to *PDA6*) encoding detoxification of pisatin, a pea phytoalexin, have been cloned from *N. haematococca* mating population VI (MPVI) (Weltring et al. 1988; VanEtten et al. 1989b; Reimann and

VanEtten 1994; Covert et al. 1996). Transformation-mediated addition of *PDA* to Pda⁻ strains (Ciuffetti and VanEtten 1996) and disruption of *PDA1* (Wasmann and VanEtten 1996) both indicate that highly expressed *PDA* genes contribute to the virulence of *N. haematococca* MPVI on garden pea.

All of the known phytoalexin detoxification genes in *N. haematococca* MPVI are located on dispensable chromosomes (VanEtten et al. 1994). These chromosomes can be lost from the genome without affecting saprophytic growth, and do not hybridize to the essential portion of the genome when used to probe Southern blots of pulsed field gels (Miao et al. 1991a). Evidence for the presence of additional pathogenicity genes on the *PDA1* chromosome was recently published. Loss of this particular chromosome (Wasmann and VanEtten 1996) or deletion of 100 kb from this chromosome (Kistler et al. 1996) leads to nearly complete loss of virulence on garden pea. It appears, therefore, that dispensable chromosomes are reservoirs for pathogenicity/host specificity genes in this organism. Dispensable chromosomes have also been reported in at least three other fungal species but their role in pathogenicity, if any, remains uncertain (Tzeng et al. 1992; Masel et al. 1993b; Orbach et al. 1996). Knowledge of dispensable chromosome structure and organization is very limited. In addition to *MAK1* and the *PDA* genes, the only fungal gene to be cloned from a dispensable chromosome is a cyclin homologue in *Colletotrichum gloeosporioides* (Masel et al. 1993c). A few molecular markers specific to dispensable chromosomes have also been reported (Tzeng et al. 1992; Masel et al. 1993a; Orbach et al. 1996).

N. haematococca MPVI isolate 156-30-6 has a 1.6-Mb dispensable chromosome that carries the *MAK1* and *PDA6-1* genes (Miao et al. 1991a). Genetic evidence suggests that *MAK1* contributes to the virulence of this isolate on chickpea (Miao and VanEtten 1992b). *PDA6-1*, however, does not appear to contribute to pathogenicity on pea (VanEtten et al. 1989b). In addition to being dispensable, the *MAK1 PDA6-1* chromosome is unstable during sexual reproduction. In progeny analyzed from two crosses, this chromosome was frequently lost or converted into nonparental-sized chromosomes (Miao et al. 1991a). The generation of nonparental chromosomes during sexual reproduction has also been reported in other fungi, including *Coprinus cinereus* (Zolan et al. 1994) and *Leptosphaeria maculans* (Plummer and Howlett 1993). Although the mechanisms underlying these chromosomal rearrangements have not been elucidated, it has been proposed that dispersed repeated sequences play a critical role by serv-

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ing as sites for recombination within or between chromosomes (Zolan 1995).

Transposable elements are a common type of dispersed repeated sequence in fungal genomes. They are classified according to their mechanism of transposition (Finnegan 1989). Class I elements transpose via RNA intermediates and may or may not be flanked by direct terminal repeats. Class II elements transpose via a DNA-DNA mediated mechanism and are defined by inverted terminal repeats (ITRs). In recent years, members of both classes of transposable elements have been found in several different filamentous fungi (Kinsey and Helber 1989; Dobinson et al. 1993; Daboussi and Langin 1994; Kachroo et al. 1994; Glayzer et al. 1995). Transposable elements have been shown to play an important role in genome evolution in many types of organisms (see McDonald 1993 for review). This is likely to be especially true in asexual fungi, as sexual recombination does not contribute to genetic variation in these species. Although most fungal transposons have only been recently described, there already is evidence for the contribution of a transposable element (*Pot3*) to pathogenic variation in the clonally propagated pathogen *Magnaporthe grisea* (Farman et al. 1996).

In this study we report the molecular characterization of a putative transposable element, *Nht1*, that was cloned from the 1.6-Mb *MAK1 PDA6-1* chromosome in *N. haematococca* MPVI isolate 156-30-6. This repeated element is highly concentrated on the chromosome from which it was cloned. Because transposable elements are associated with chromosomal aberrations in other organisms we hypothesize that *Nht1* contributes to the instability of the 1.6-Mb dispensable chromosome in isolate 156-30-6 during sexual reproduction.

RESULTS

Cloning and sequence analysis of *Nht1*.

The apparent transposable element described herein was discovered during the cloning of *MAK1* from the 1.6-Mb dispensable chromosome of *N. haematococca* MPVI isolate 156-30-6 (Covert et al. 1996). *MAK1* was cloned from a cosmid (1F7) that hybridized to approximately 22% of the clones in a chromosome-specific cosmid library. Such a large number of positive clones suggested the presence of repeated DNA sequences on the 1F7 cosmid. In order to identify a clonable DNA fragment containing such sequences, a collection of clones that hybridized to, but did not overlap with, cosmid 1F7 was used in detailed cross-hybridization studies. Southern blots of the restriction enzyme-digested clone collection were probed independently with several representatives of the group. These experiments led to identification of a 1.3-kb *EcoRI* fragment in cosmid 2D11 that hybridized to 50% of the 1F7-homologous clones in the chromosome-specific library. This fragment was cloned and sequenced. A BLAST data base search revealed that the deduced amino acid sequence of the *N. haematococca* MPVI clone was similar to portions of two fungal transposons: *Fot1* of *Fusarium oxysporum* (Daboussi et al. 1992) and *Pot2* of *Magnaporthe grisea* (Kachroo et al. 1994). These sequence comparisons also revealed that the 1.3-kb *EcoRI* fragment from *N. haematococca* MPVI did not appear to contain the entire transposon. Additional subclones from 2D11, therefore, were sequenced to identify the remainder. This copy of the repeated element is named *Nht1A* and its

complete sequence is shown in Figure 1. The 5' and 3' termini of *Nht1* were defined by sequencing the corresponding regions of two additional copies of *Nht1*, designated *Nht1B* and *Nht1C*, on two other 1F7-homologous cosmid clones (cosmids 1A8 and 2H8, respectively). The homology between the three *Nht1* copies terminates after a TA dinucleotide at each end of the common sequences (data not shown).

Nht1A is 2,198 bp long and has incomplete ITRs of 102 and 84 bp (Fig. 1). Its 5' ITR contains a direct repeat of 14 bp; its 3' ITR contains only one, imperfect copy of this repeat (Figs. 1 and 2). The ITRs of *Nht1C* are organized similarly (data not shown), but those of *Nht1B* differ in that its 3' ITR contains two copies of the 14 bp repeat (Fig. 2). The identity between *Nht1A* and *Nht1B* at their 5' and 3' ITRs is 83 and 82%, respectively, not including the difference caused by the missing copy of the 14-bp repeat in *Nht1A* (Fig. 2). Within their coding regions these two copies of *Nht1* are almost identical. For example, the last 1,500 bp of *Nht1A* are 91% identical to the corresponding bases in *Nht1B* (data not shown).

When the complete *Nht1A* amino acid sequence was compared with that of *Fot1* and *Pot2* the presence of two short introns in *Nht1* became apparent (Fig. 1). If the introns are not spliced out prior to translation, the *Nht1A* sequence homologous to the transposons shifts frame twice. However, if the putative introns are spliced out, a single open reading frame homologous to *Fot1* results (Fig. 3). The sequences of the 5' and 3' splice junctions of the *Nht1A* introns match known fungal splice junctions (Gurr et al. 1987). Introns are also present in the corresponding regions of *Nht1B*. *Nht1A* and *Nht1B* share 75% identity in the first intron and 100% identity in the second intron (data not shown).

There are two potential translation start sites in *Nht1A* (Fig. 1, nucleotides 298 and 340). Assuming the first start site is correct, the putative transposase encoded by *Nht1A* consists of 550 amino acids. *Nht1A*, *Fot1*, and *Pot2* are most similar over the first two-thirds of their proteins. The region defined by amino acids 10 to 384 of *Nht1A* is 48% identical to *Fot1* (Fig. 3) and 42% identical to *Pot2*. *Nht1A* is less similar to *Pot3* and *Fcc1*, transposable elements of the same structural family, isolated from *Magnaporthe grisea* and *Cochliobolus carbonum*, respectively (Farman et al. 1996; Panaccione et al. 1996). In the region defined by amino acids 10 to 384 of *Nht1A*, *Pot3* shares 30% identity and *Fcc1* shares 32% identity with *Nht1A*.

Copy number and geographic distribution of *Nht1*.

Southern blots of isolate 156-30-6 and several *N. haematococca* MPVI field isolates were used to investigate the copy number and geographic distribution of *Nht1*. Genomic DNA from these isolates was digested with two restriction enzymes that do not cut within *Nht1A*, and probed with an internal fragment of *Nht1A* (Fig. 4A, probe A). The number of *Nht1* copies in *N. haematococca* MPVI varies widely. Isolates T-69, T-95, and T-110 are devoid of the transposon (Fig. 4B). These three isolates were collected in the southeastern United States (Table 1). In contrast, isolates T-30 and T-126 are estimated to have up to 100 copies (Fig. 4B). These isolates were both collected in the northwestern United States (Table 1). The remaining isolates are estimated to contain approximately 10 copies (156-30-6, T-77, T-161, and T-200) or 20 copies (T-25, T-27, and T-33) of *Nht1* and do not fall into clear geographical categories (Fig. 4B and Table 1).

CCATCCACACCCCTCTTCGCAATACCCCTCTTTCGGCAAGCAAAATAAAAAAGCTT 60
 CACCAAACTCACCACTTACCTTATACCAAGATTGGCCAAAGTAATAGCTACTTAGAG 120
 GACAAATGCTCTATCGATTATAGAAATGATTGGAGTTAACGACTTATAGCTCTTAGGCT 180
 GTGCGGGGTACGCGATCATGCTGCTGTGCTATGGAAATAGTGTGGTTTAAATCATCAA 240
 ACTCTCACCGCTCTGTTCCGTGTTTTTGACCTTTGTCTGCGACAACGACAAGAAATG 300
 M
 CCTAGGTGAGACCTCGATGGAATATACAGAGGAAACATGGCAGGGCTATCTTGGAT 360
 P R S R P R W K Y T E E N M A E A I L D
 GTTACTGATGATGGCGTTTACCTCCTCAAGCGCTCAGAGACGGGAGTGCCTCGAAGC 420
 V T D D G V S P P Q A A Q R R G V P R S
 ACTCTAGTCGACAGACTTAACGGCCAGAAAGCGGTAAGAGCAGATTACGCTCTCGCGA 480
 T L V D R L N G Q K A V K E Q I Q P R R
 CGTTTGTCCAAGAATCAAGAGGACAGATTGGCTTCTGGATCCTCGCTCAGGAATCTCTG 540
 R L S K N Q E D R L A F W I L R O E S L
 GGCTATGCTCCGTCACCAATCAGATCCGCGCTTGCCTCAGGGCTTGTGAGACAGCAG 600
 G Y A P S H N Q I R A C V T G L L R Q O
 GGCGAATCTCCGAGTTCAGGACCAATGGGTGACTAGATTATCAAGCGCCGACAGAC 660
 G E H P E L G R N W V T R F I K R R T D
 CTGACGACCAAGATGGGTAGACGCAAGAAGCCAAAGGTCTGACTCTTTCACGCTAAG 720
 L T T K M G R R Q E A K R S D S F T P K
 GCAGTTCATTGGTACTTTGATATCAGGAGGGCGAGTATGGCTGGATCAAGCTGAAAC 780
 A V H W Y F D I R E G Q Y G W I K P E N
 ACCGTCAACGTTGACGAGGGGTATTATGCTGGTTTCGGTAAGCATCTATCTGTATAC 840
 T V N V D E G G I M T G F
 CTTGTCAGTCTGAAATTTGACCGACTAATCTTTTATTTAGGCTTAGATAGCTGGT 900
 G L D S L V
 TGTGGAAAGCGGACCCAAAGGCGAAGGCATTCTCAAGGACCAACAACTCGAACTG 960
 V G S A D P R W K Y T E E N M A E A I L D
 GACTTCATTATCGAAGCTGCTACTGCTGACGCGCGGCTTAATCCCTGGCATAATCTT 1020
 T S F I E A V T A D G R A L I P G I I F
 CAAGGGGAAAGAACTCGAAGAACAGTGGTTGTTGAGGAATTCAGGAGATAGCAGACTG 1080
 K G K E L O K Q W F V E E F K E I A D W
 GTATTACATAACTTCGCTTAACGGGTGAGTACGACGACCATTTGGCTTGAATGGCTTGA 1140
 Y Y I T S P N G R V T G N W P I S R A K A
 AAGAGTCTATCTGCCAGACAAATCCGCGCCGACGACTCTGATGCGGCTCTGATCATATT 1200
 R V Y L P Q T M P A D D S D A R L I I L
 AGATGGCCATGGAAGTATGCAACGGTATGTTCTTCTTCTCAGCTCAAGGTCACCTA 1260
 D G H G S H A T
 CTAAGTAAGGAAGGATGAATGGATGGCCAGCTGCTTTTGAATAACGTTTATGTTGCT 1320
 D E W M A T C F L N N V Y C C
 ATCTGCCAGCACACTGCTCTCATGGGCTCCAGCGTGGACAAAGGATTCATAGCTCT 1380
 Y L P A H C S H G L Q P L D N G V F N A
 CCAAGCGCATATCGACGAGATGGAGAAATTCGCTTCTACTGACTGATTCCACTCAA 1440
 S K A A Y R R E L E N F A S L T D S T P
 TGGACAAGGTCAATTTCAAGGGCTCAGCTAAGGCGGCTGAGTTGGAATGACTGAGA 1500
 M D K V N F I R A Y A K A R R V G M T E
 AGAACAATCTCTCCGGCTGAGGGTTACTGGGAATTTGCCGATCTCAGTGGCCAAAGCGC 1560
 K N I L S G W R V T G N W P I S R A K A
 TGCCACACCCAGAAATCCAAGATAGGCAATGGCAGCCCAAGAGCGACTCCCGAAC 1620
 L P H P E I D O D R P N G S P R A T P E
 CCAGGCGGATTTTGAATCGGACGATACACCAAGAGCGGCTGCAATTCGATCTTG 1680
 P R P Y F D S D D T P K T S R Q I R D L
 GGCTGAACAAACACCAAGACACGAAGCGGTATAACGTAATAGCAAGGGCTTTGAGG 1740
 G L N K T P K T R R Y N V I A K G F E
 CTCACAGCAGACGGTAGCGGCGCTACTGCGAGGATTCGCTAGCTAGAGGAGGAATTGG 1800
 A Q Q Q T V A A H T A R I A S L E E E L
 CTCGCTGAAGAGAGGGAAGAGAGGAGGCGGTGCCGAATCTTAACAAACGTTTATGA 1860
 A R L K R G K K R K A V P N P N K R F M
 CTCCTGGTGAGACTCTAGCTGCTGGGGAAGCCATATCCGAAGAGGAGACTCAAAATATGC 1920
 T L G E T L A A G A I S E E E T O N M
 CTGTTGCGGTGGAATGTGGCGCTTCAGGGGAGCGGGGTCAGAATCGGAGGCTGCGTCGG 1980
 P V A V E C G R S G E P G S E A A S
 TCATTGAAGTCAGAGAGGAACCATACCCATCACTAACCAACACGCGGTGAGGCGGCTTA 2040
 V I E V R E E T I P H O L T T R S G R L
 TCAAAAGACCCAGGATTCATAGAGAGATGCAACTATTATTCACAAGACGCAAAATCTTG 2100
 I K R P R I Q
 CACGCGCTATCAATTTGGCCCAAGTTCTGTGTAAGTAACATGTTGATTTTGGTGGTGC 2160
 TTTTITTTTTTGCCTGGCGAAAGGGTGGTTGCGATGG 2198

Fig. 1. Nucleotide sequence of *Nht1A*. Deduced amino acid sequence is shown below the DNA sequence in one letter code. Putative introns are indicated by a solid line. Terminal inverted repeats are underlined with a dashed line and tandem repeats are overlined with arrows.

Distribution of *Nht1* within the genome.

To investigate the genomic distribution of *Nht1*, the chromosomal DNA of isolate 156-30-6 was separated by contour-clamped homogeneous electric field (CHEF) electrophoresis (Fig. 5, lane 1) and probed with an internal fragment of *Nht1A* (Fig. 4A, probe A). Four chromosomal bands hybridized to this probe (Fig. 5, lane 2). Each band was quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) to compare the relative intensities of their hybridization signals. The most intense hybridization signal corresponds to the 1.6-Mb chromosome from which *Nht1A* was cloned. The hybridization signals from the 2.5- and 3.4-Mb chromosomal bands are 5 to 6 times less intense than that of the 1.6-Mb band. The weakest hybridization signal corresponds to a 5.0-Mb chromosomal band; it is 19 times less intense than that of the 1.6-Mb band and is not clearly visible in the exposure displayed in Figure 5. This weak signal appears to be partially due to the fact that the 5.0-Mb band contains a lower concentration of chromosomal molecules than the smaller bands in the gel (Fig. 5, lane 1). An ongoing physical mapping project in our laboratory suggests that the 1.6-Mb chromosome in isolate 156-30-6 contains at least six copies of *Nht1* (data not shown). Based on this information and the relative intensity of the hybridization signals shown in Figure 5, the 2.5- and 3.4-Mb hybridization signals are estimated to indicate the presence of one to two copies of *Nht1* per chromosomal band. One copy of *Nht1* is also estimated to be present on the 5.0-Mb band. Therefore, in total, isolate 156-30-6 contains nine to 11 copies of *Nht1* in its genome, a number that corresponds well to the copy number estimated from the Southern blot in Figure 4. Out of these nine to 11 copies, most are concentrated on the 1.6-Mb dispensable chromosome.

CHEF-separated DNA of isolate 156-30-6 was also probed with a flanking fragment of *Nht1A* to confirm that *Nht1A* was cloned from the 1.6-Mb dispensable chromosome. The 1.6-Mb chromosome was the only positive band with this probe (Fig. 5A, lane 3).

To examine the genomic distribution of *Nht1* in the parents of 156-30-6, a Southern blot of T-126 and T-161 CHEF-separated DNA was probed with an internal fragment of *Nht1A* (Fig. 5B). The distribution of *Nht1* in the T-161 genome resembles its distribution in 156-30-6; multiple *Nht1* copies appear to be on a 1.6-Mb chromosome and a few copies are on larger chromosomes. T-126 is very different in that it has several chromosomes in the range of 1.0 to 3.5 Mb that contain multiple copies of *Nht1*. On long exposures *Nht1* also appears to be present on some of the large chromosomes (>5 Mb) of isolate T-126 (data not shown). As in 156-30-6, interpretation of hybridization intensity in T-126 and T-161 is confounded by the fact that the largest chromosomes (>4.5 Mb) are under-represented relative to the smaller chromosomes on CHEF gels.

DISCUSSION

We conclude *Nht1* is a transposon because of its repeated nature and structural similarity to previously described fungal transposons. This particular family of transposable elements possesses two short direct repeats embedded within longer ITRs. The ITRs flank a coding sequence for a putative transposase. The presence of TA dinucleotides on either end of

The structure of the three cloned copies of *Nht1* is not uniform. The differences are most evident in the ITRs. *Nht1B* contains two complete sets of a 14-bp direct repeat in its ITRs. *Nht1A* and *Nht1C*, however, both lack a copy of the direct repeat in their 3' ITRs. This raises the possibility that *Nht1* is able to transpose without having complete ITRs. Alternatively, multiple copies of the element with the truncated 3'

[illegible]

Fig. 2. Alignments of 5' inverted terminal repeats (ITRs) and 3' reverse-complement ITRs from *Nht1A* and *Nht1B*. Identical nucleotides are indicated with vertical lines and tandem repeats are over-lined or under-lined. Dashes represent gaps introduced to improve alignments.

Nht1A MPRSRPRWKYTEENMAEAAILDVTDDGVSPQAAQRGRVPRSTLVDRLNGQKAVKEIQPRRRLSKNQEDR 70
:
Fot1 -----MPVYSADDLENAIADFKN-GVSLKTAACKNGLPPSTLRGRLTGAQSRQVARQEQLRLTTDQEDD 63

Nht1A LAFWILRQESLGYPASHNQIRACVTGLLRQQGEHPELGRNWVTRFIKRRTDLTTKMGRRQAEAKRSDSFTP 140
|
Fot1 LERWILRQEKLGHAPTHAQVRTIVRSVLARHGHDHAPLGRKWTRFVERHPALKTKLGRRTDWERVNAATP 133

Nht1A KAVHWYFDIREGGYGWIKPENTVNVDDEGGIMTGFLDSLVLVGSAADPRRKAF-LKGPQTRNWTSFIEAVTA 209
:
Fot1 ANIKRLFVDVYETV-DWIPPERRYNADEGGIMEGGQVGNLVIGSSQESPNAVVPKTATVRTWTSIIECISA 202

Nht1A DGRALIPGIIFKGKELQKQWFVEEF-KEIADWYYITSPNGWTDHIGVEWLERVYLPTMPADDSDARLI 278
|
Fot1 VGVVLHPLVIFKAktiEQEWFRRREFLQKHLGWQVTFSKNGWTSNSIALEWLEKVFLPQTAPADPADARLL 272

Nht1A ILDGHGSHATDEWMATCFLNNVYCCYLPAHCSHGLQPLDNGVFNAASKAAYRRELENFASLTDPMDKVN 348
|
Fot1 IVDGHGSHATEQFMACYLNNVYLLFLPAHCShVLQPLDLGCFSslKAAYRTLvGEHTALTDSrVGKQR 342

Nht1A FIRAYAKARRVGMTEKNILSGWRVTGNWPISRakALPHPEIQQDRPNgsPRATPEPRPYfDsDDTPktsR 418
|
Fot1 FLDFYARAREIGFRKVNIRSGWRAAGLWPVNINKPL-----ASRWVMVlTKSalppSETldIA-TPKRGg 406

Nht1A QIRDGLNKTPKTRRRYNV--IAKGFEAQQTVAaHTARIASLEEELARLKrgKKrKAvPNPNkrfMTlg 486
:
Fot1 DVVKLFsAKSSSPSSRLSIrkAAAAlDKVAIELAMKDREIERLraqlEaaqPKKKrKiRQDPNECFISla 476

Nht1A ETLAAGEAISeee-tQNMPVAVEc-grSGEPGseSEaAsViEVREETIPHQLtTRSgrLiKrPRIq 550
:
Fot1 QILAeANREPdQRVIQSqKgDLDCIvVDGKSsseEdPaPVrrSTRVRRatKmYiRQdlsSeEsD 542

Fig. 3. Amino acid alignment of putative transposases from *Nht1A* and *Fot1*. Bars represent identical amino acids and dots indicate a frequently observed amino acid change, according to the PAM 250 matrix (Dayhoff et al. 1978).

ITR might have arisen from recombination events. The single cloned copy of *Fcc1* also lacks the second direct repeat in its 3' ITR (Panaccione et al. 1996). This copy of *Fcc1*, however, is thought to be inactive because of the incomplete 3' ITR and because its putative transposase open reading frame is interrupted by a stop codon.

Whether or not *Nht1* is an active transposon in any of the *N. haematococca* MPVI isolates that contain it remains an open question. Processes (named RIP and MIP) that inactivate repeated sequences during sexual reproduction have been well characterized in *Neurospora crassa* and *Ascobolus immersus* (Cambareri et al. 1989; Goyon and Faugeron 1989). In addition,

inactivation of multiple copy transgenes has been observed in sexual progeny of *Gibberella fujikuroi* (Leslie and Dickman 1991). Consequently, repeated transposable elements in any of these three ascomycetes would probably be rendered immobile if put through a cross. Although it is not known if similar inactivation processes exist in *N. haematococca* MPVI, these data from related fungi raise the possibility that all of the *Nht1* copies in isolate 156-30-6 were inactivated during the cross that produced 156-30-6. Future work on the mobility of *Nht1* will need to be carried out with this possibility in mind.

The number of *Nht1*-homologous bands in *N. haematococca* MPVI field isolates from different locations in North America is highly variable. For example, of the 11 isolates examined here, three completely lack *Nht1* while two others each appear to have about 100 copies. Our survey did not include enough isolates to determine if there is a correlation

Table 1. Strains of *Nectria haematococca* mating population VI used in this study

Isolate	Origin	Habitat	Reference
T-25	New York, USA	Chickpea	VanEtten 1978
T-27	California, USA	Chickpea	VanEtten 1978
T-30	Washington, USA	Pea	VanEtten 1978
T-33	Canada	Cottonwood	VanEtten 1978
T-69	South Carolina, USA	Tuliptree	VanEtten 1978
T-77	Pennsylvania, USA	Alfalfa	VanEtten 1978
T-95	South Carolina, USA	Tuliptree	VanEtten 1978
T-110	Louisiana, USA	Cottonwood	VanEtten 1978
T-126	Montana, USA	Sainfoin	VanEtten 1978
T-161	Unknown	Unknown	Lucy et al. 1988
T-200	Unknown	Red clover	Lucy et al. 1988
156-30-6	Progeny isolate of cross T-126 × T-161	...	Miao and VanEtten 1992a

Fig. 4. Presence of *Nht1* in various *Nectria haematococca* mating population VI isolates. **A**, Restriction map of *Nht1A* and flanking DNA. *Nht1A* is indicated as a thick black bar. **B**, Equal amounts of genomic DNA (3 µg) were double digested with *KpnI* and *XbaI*, separated on a 0.8% agarose gel, and Southern blotted. The corresponding Southern blot was probed with an internal fragment of *Nht1A* (**A**, probe A). Molecular weight standards are indicated on the left in kilobases (kb).

Fig. 5. Intragenomic distribution of *Nht1* and chromosomal location of *Nht1A*. **A**, Lane 1: Ethidium bromide-stained contour-clamped homogeneous electric field (CHEF) gel of isolate 156-30-6. Lane 2: Southern blot of lane 1 hybridized to an internal fragment of *Nht1A* (**A**, probe A). Lane 3: Southern blot of 156-30-6 DNA, separated as in lane 1, hybridized to DNA flanking *Nht1A* (**A**, probe B). **B**, Ethidium bromide-stained CHEF gel of field isolates. Lane 1: T-126; Lane 2: T-161; Lanes 3 and 4: Southern blot corresponding to lanes 1 and 2 hybridized to an internal fragment of *Nht1A* (**A**, probe A). **A** and **B**, Molecular weight markers (*Schizosaccharomyces pombe* chromosomes) and the 1.6-Mb dispensable chromosome are indicated on the left of each panel in Mb.

between habitat (host plant) and presence or absence of *Nht1*; however, our data does suggest there is an association between geographical location and *Nht1* distribution. All three *N. haematococca* MPVI isolates lacking the transposon were collected in the southeastern United States, and both isolates containing the highest *Nht1* copy number were collected in the northwestern United States. Maruyama and Hartl (1991a) proposed two hypotheses to explain a discontinuous distribution of transposable elements within one species. One model proposes that the transposon was present in a common ancestor, but has been lost over time by individual lineages. The second model proposes a recent introduction of the element by interspecific transfer. To test the applicability of these models to *Nht1*, a more detailed phylogenetic study of the *N. haematococca* MPVI field isolate collection is required.

The distribution of *Fot1*, *Pot2*, *Pot3*, and *Fcc1* has also been investigated in the fungal species from which they were isolated. *Fot1* is present in most *F. oxysporum* isolates as well as a few isolates of different *Fusarium* spp., such as *Fusarium solani* (Daboussi and Langin 1994). The *Pot3* element in *M. grisea* appears to be distributed in a host-specific manner; it is present in high copy number in rice pathogens and rare or absent in non-rice pathogens (Hamer et al. 1989; Farman et al. 1996). In contrast, the *Pot2* element of *M. grisea* appears to be distributed rather homogeneously between isolates from different host plants and also between different geographical regions (Kachroo et al. 1994). *Fcc1* is present at least in three *C. carbonum* races and two other *Cochliobolus* spp. (Panaccione et al. 1996).

In field isolate T-161, most copies of *Nht1* are present on a single chromosome. This distribution of *Nht1* contrasts with the even distribution of *Pot2* and *Pot3* across all of the chromosomes in *M. grisea* (Talbot et al. 1993; Kachroo et al. 1994; Orbach et al. 1996). The uneven distribution of *Nht1* in T-161 could be the result of more than one evolutionary process. It is possible that when *Nht1* was first introduced in T-161 it inserted by chance into the 1.6-Mb chromosome and since then has transposed primarily within this chromosome. Such an idea is not without precedent, as a preference for short distance transposition has been demonstrated for the *Ac* element in *Zea mays* (Van Schaik and Brink 1959; Dooner and Belachew 1989) and the *P* element in *Drosophila* (Tower et al. 1993; Zhang and Spradling 1993). Another possibility is that the chromosome containing multiple copies of *Nht1* was added intact to the T-161 genome, perhaps by crossing with an isolate containing *Nht1* throughout its genome, or via horizontal transfer from another species. The horizontal transfer of genetic information between different fungal species, or between genetically isolated lines of the same species, has been proposed to occur in *C. carbonum* (Nikolskaya et al. 1995) and *Colletotrichum gloeosporioides* (Masel et al. 1996). Horizontal transfer of transposable elements has also been documented to occur between species of the *Drosophilidae* (Daniels et al. 1990; Maruyama and Hartl 1991b). However, considering the potential for sexual reproduction in *N. haematococca* MPVI and the existence of field isolates containing many copies of *Nht1*, transfer from another species does not appear to be the most likely explanation for the introduction of *Nht1* into T-161. Regardless of how *Nht1* was introduced into T-161, its limited intragenomic distribution suggests either that it was introduced relatively recently, or that it

became inactive shortly after its introduction, or that it transposes only infrequently.

Our current understanding of how chromosome-length polymorphisms arise and are maintained in fungi is limited (Zolan 1995). The rearrangements of chromosomes observed in *C. cinereus* and *L. maculans* have been explained as the result of recombination between homologous chromosomes of unequal length (Zolan et al. 1994; Plummer and Howlett 1995), but direct evidence in support of this hypothesis in either case has not yet been reported. In *N. haematococca* MPVI, chromosomal instability has been observed even when homologues of equal size are paired (Miao et al. 1991a), and the resulting chromosome sizes suggest that intrachromosomal recombination is at work. A better understanding of chromosomal rearrangements in fungi will contribute not only to our general knowledge of fungal genome evolution, but also is likely to contribute to our understanding of pathogenic variation in organisms capable of causing disease. Transposable elements are likely agents in these processes, as they stimulate chromosomal aberrations such as translocations, deletions, and inversions in other eukaryotes (Roeder and Fink 1980; Engels and Preston 1984; Rothstein et al. 1987; Lim 1988; Döring et al. 1990). Therefore, the discovery of *Nht1* in many copies on an unstable chromosome in *N. haematococca* MPVI may be an important step toward elucidation of the mechanisms underlying chromosomal instability in this organism.

MATERIALS AND METHODS

Fungal isolates and plasmids.

The *Nectria haematococca* MPVI isolates used in this study are listed in Table 1. Field isolates are prefaced by the letter "T" and were provided by Hans VanEtten, University of Arizona. All isolates were grown and maintained on V8 agar medium (Miller 1955) at 28°C in the dark.

DNA manipulations.

Standard molecular biology techniques were used throughout (Sambrook et al. 1989). The cosmid clones used in this study are from a genomic library of 156-30-6 (Covert et al. 1996) in the cosmid vector pMOcosX (Orbach 1994). In the version of the library used previously, cosmid 1F7 was named 1G8 (Covert et al. 1996). All subclones from cosmid 2D11 were made in Bluescript II SK⁺ (Stratagene, La Jolla, CA). Fungal DNA was isolated as previously described (Covert et al. 1996). Gels were blotted onto Hybond N⁺ membranes (Amersham, Arlington Heights, IL). All hybridization reactions were carried out according to the aqueous solution protocol described in Ausubel et al. (1987) at 65°C. Probes were synthesized with the DECAprime II DNA labeling Kit (Ambion, Austin, TX). Blots were washed at 65°C twice with 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M Na₃citrate, pH 7.0 with HCl), 0.1% sodium dodecyl sulfate (SDS) and once with 0.5× SSC, 0.1% SDS. Agarose gels were scanned with an Eagle Eye II gel scanner (Stratagene) to measure band intensity, and hybridization signals were quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

To prepare chromosomal DNA for pulsed-field gel electrophoresis the protoplasting protocol described by Powell and Kistler (1990) was used with a few modifications. Glucanex (Novo Nordisk Ferment AG, Dittingen, Switzerland) was used

as the protoplasting enzyme at a concentration of 3.75 mg/ml in 1.2 M MgSO₄, 50 mM sodium citrate pH 5.8. Protoplasts were embedded and lysed as described by (Miao et al. 1991b). Chromosomal sized DNA was separated in 0.8% agarose gels (low EEO grade, Fisher Scientific, Pittsburgh, PA) in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA) on a CHEF DR III unit (BioRad, Hercules, CA). The separation was carried out at 14°C for 72 h at 2 V/cm with ramped switch intervals from 1,200 to 2,400 s. *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (FMC Bioproducts, Rockland, ME) were loaded on each gel as size markers.

DNA sequence analysis.

All nucleotide sequencing was carried out on an PE Applied Biosystems sequencer (Foster City, CA) at the Molecular Genetics Instrumentation Facility at the University of Georgia, Athens. A mixed strategy of sequencing subclones with T3 and T7 primers, or with *Nht1*-specific, synthesized primers, was applied to sequence both strands of *Nht1A*. The same synthesized primers were used to sequence the ends and internal portions of *Nht1B* and *Nht1C*. BLAST (Altschul et al. 1990) and the DNASTAR software package (DNASTAR, Madison, WI) were used to analyze, translate, and compare DNA sequences.

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