ISSN 0026-8933, Molecular Biology, 2007, Vol. 41, No. 5, pp. 756–763. © Pleiades Publishing, Inc., 2007.
Original Russian Text © O.S. Novikova, V. Fet, A.G. Blinov, 2007, published in Molekulyarnaya Biologiya, 2007, Vol. 41, No. 5, pp. 830–838.

GENOMICS. TRANSCRIPTOMICS. PROTEOMICS

UDC 577.21

LTR Retrotransposons in the *Aspergillus fumigatus* and *A. nidulans* Genomes

O. S. Novikova[a], V. Fet[b], and A. G. Blinov[a]

[a] *Institute of Cytology and Genetics, Siberian Division, Russian Academy of Sciences, Novosibirsk, 630090 Russia; e-mail: novikova@bionet.nsc.ru*

[b] *Marshall University, Huntington, West Virginia 25755-2510, United States*

Received December 8, 2006

Accepted for publication February 5, 2007

Abstract—Fungi of the genus *Aspergillus* can infect all tissues and organs, causing invasive mycosis (aspergillosis). This disease can be fatal, especially in immunocompromised patients. Microbiological monitoring of these infectious agents is obligatory in modern medical facilities. Mobile elements can be used as markers to identify the *Aspergillus* species and strains found indoors as well as to diagnose aspergillosis. Genomic sequences of two *Aspergillus* species, *A. fumigatus* and *A. nidulans*, were analyzed in silico in order to detect LTR retrotransposons. These species were found to considerably differ in the composition of retrotransposon families. One of the families, present in both *Aspergillus* species, was phylogenetically quite different from all known fungal retrotransposons. The majority of its elements were damaged copies. Nevertheless, allegedly undamaged LTR retrotransposon copies were described that contained intact ORFs and might be active.

DOI: 10.1134/S0026893307050081

Key words: *Aspergillus*, LTR retrotransposons, Metaviridae, in silico analysis, phylogeny

INTRODUCTION

Eukaryotic mobile elements belong to moderately repetitive DNA sequences able to migrate within the genome. They occur in the genomes of virtually all present-day eukaryotes, forming almost 45% of the genome in man, 15% in *Drosophila melanogaster*, and 50 to 80% in some plants [1–4].

Mobile elements whose transposition is mediated by reverse transcription are called retrotransposons [5, 6]. A distinguishing feature of LTR retrotransposons is the presence of long terminal repeats (LTRs) at their ends and the transposition mechanism similar to that of retroviruses. Two major groups are recognized in the class of LTR retrotransposons: Pseudoviridae, or *Ty1*/*copia*, and Metaviridae, or *Ty3*/*gypsy.* Two minor groups, studied to a lesser extent, are *DIRS* and *Bel* [7]. The *Bel* group is sometimes assigned to Metaviridae [8].

The structure of LTR retrotransposons resembles that of retroviruses. In most cases, the only difference is the absence of sequences coding for the virus envelope protein (the *env* gene of retroviruses). Most LTR retrotransposons have two open reading frames (ORFs) in their middle regions. ORF1 codes for the Gag protein. The processing of the *gag* product yields numerous proteins, including short nucleocapsid proteins, which cover the mRNA of the retroelement [9]. The other reading frame, ORF2, codes for the Pol polyprotein. The 5'-region of *pol* contains protease-encoding sequences. Alignment of internal portions of the Pol domains in many elements has revealed conserved blocks of amino acid residues [10–12]. The blocks are functional domains of reverse transcriptase (RT), ribonuclease H (RNase H), and integrase (Int).

Phylogenetic analysis of the common RT domain and some structural features make it possible to divide Metaviridae into two subgroups (genera), *Metavirus* and *Errantivirus.* They differ in the presence (*Errantivirus*) or absence (*Metavirus*) of an additional *env* reading frame. Another group sometimes referred to metaviruses, *Semotivirus*, was previously classified as the independent *Bel* group and not included in Metaviridae [8]. Several so-called phylogenetic groups are recognized by phylogenetic analysis of Metaviridae members. By now, they include ten groups: *Chromovirus, CsRn1*, mdg3, *Osvaldo, Athila, Mag, gypsy*, mdg1, *Cer*, and *Tor* [13–15].

Most phylogenetic groups of Metaviridae occur in genomes of specific taxa. For example, mdg1, mdg3, and *gypsy* occur only in arthropods [13], *Cer* elements are detected only in nematodes [16], and members of the *Athila* group have been described only for plant

genomes [17]. Two phylogenetic groups, *Cigr* and *Tor*, are found in the *Ciona intestinalis* genome (Urochordata) [15, 18]. Only one phylogenetic group of metaviruses, *Chromovirus*, is present in virtually all eukaryotic species [15]. Its members occur in most fungi, including Ascomycota and Basidiomycota. They are also present in Oomycetes, Zygomycota, and plants. Chromoviruses have been found in vertebrates. They probably occur in all deuterostome animals [15].

The genus *Aspergillus* includes about 200 mold species. Only a few of them are pathogenic for humans [19]; nevertheless, they frequently cause invasive mycoses in immunocompromised patients. *Aspergillus* species attack any tissue or organ, causing so-called aspergilloses: bronchopulmonary aspergillosis, generalized aspergillosis, *Aspergillus* endophthalmitis, skin aspergillosis, etc. Colonization by *Aspergillus* is facilitated by immunodeficiency caused by medicines, infections, or neoplasms. The severest form of the disease is caused by *Aspergillus fumigatus*, found in more than 90% of aspergillosis patients [20]. Other major infectious agents are *A. niger* and *A. flavus* [21].

Microbiological monitoring is necessary in modern medical facilities. The probability of hospital infections, including aspergillosis, depends on the hospital conditions [21, 22]. Mobile elements can be helpful for identification of various *Aspergillus* species and lineages present indoors and for aspergillosis diagnosis [22].

The genome sequences of two lines of *A. fumigatus* and *A. nidulans* are available in databases [23, 24]. We compared and phylogenetically analyzed the LTR retrotransposons of these related species. It was demonstrated that the LTR retrotransposons of these species included only members of the metavirus group (Metaviridae). The *Aspergillus* species showed significant differences in the range of various retrotransposon families and in their copy numbers. *Aspergillus nidulans* had full-size intact LTR retrotransposon copies, whereas *A. fumigatus* contained only damaged (degraded) copies. In addition, both species contain a new LTR retrotransposon family that has not been described before. These data can be applied to developing diagnostic kits not only for the species studied but also for other *Aspergillus*.

EXPERIMENTAL

The search for LTR retrotransposons of the Metaviridae group. Genome sequences were retrieved from databases: *A. fumigatus* from the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/), with the assistance of D. Denning and A. Brass (University of Manchester, http://www.man.ac.uk/), and *A. nidulans* from the Broad Institute of MIT and Harvard University (http://www.broad.mit.edu/).

A search for LTR retrotransposons with specified parameters was performed with the UniPro GenomeBrowser (http://genome.unipro.ru/), implementing an algorithm based on hidden Markov models. We applied multiple sequence alignment of amino acid sequences of the polyproteins from known LTR retrotransposons to the construction of the search profile. The names and accession numbers in GenBank and Repbase are shown in Fig. 3. A multiple sequence alignment was constructed with CLUSTAL W [25] and then manually edited. The sequences of about 700 residues in length contained three domains: RT, RNase H, and Int. The corresponding nucleotide sequences were obtained with the UniPro GenomeBrowser.

Sequence analysis. Tentative analysis of retrotransposon sequences was performed with various programs of the BLAST server, available at http://www.ncbi.nlm.nih.gov/BLAST/. Multiple sequence alignments of amino acid sequences were constructed with CLUSTAL W [25] and then manually edited. The sequences of known LTR retrotransposons were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/entrez/) and Repbase (http://www.girinst.org/repbase/index.html; Genetic Information Research Institute). The accession numbers of the sequences in GenBank or locus names according to Repbase are shown on the phylogenetic tree.

The phylogeny of the sequences was analyzed with MEGA2 [26]. First, sequences containing all of the three domains (RT, RNase H, and Int) were selected with A Conserved Domain Database and Search Service (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). Sequences lacking at least one of the domains were excluded from consideration. Evolutionary trees were constructed by the neighbor-joining method [27]. The reliability of the resulting trees was estimated by bootstrap [28] with 1000 replications.

RESULTS AND DISCUSSION

LTR Retrotransposons from *A. fumigatus*

It has been demonstrated that the *A. fumigatus* genome contains at least two different LTR retroelements, *Afut1* and *Afut2* [29, 30].

A search for LTR retrotransposons in the *A. fumigatus* genome revealed 60 mobile element sequences. Their BLAST comparison with NCBI data showed that all amino acid sequence fragments were typical of the metavirus group (Metaviridae). For phylogenetic analysis, we selected 27 sequences possessing all enzyme domains typical of metaviruses in the proper order (RT, RNase H, and Int). Their comparison and phylogenetic analysis showed that the retrotransposons found in *Aspergillus* could be divided into four distinct clusters (Fig. 1, table).

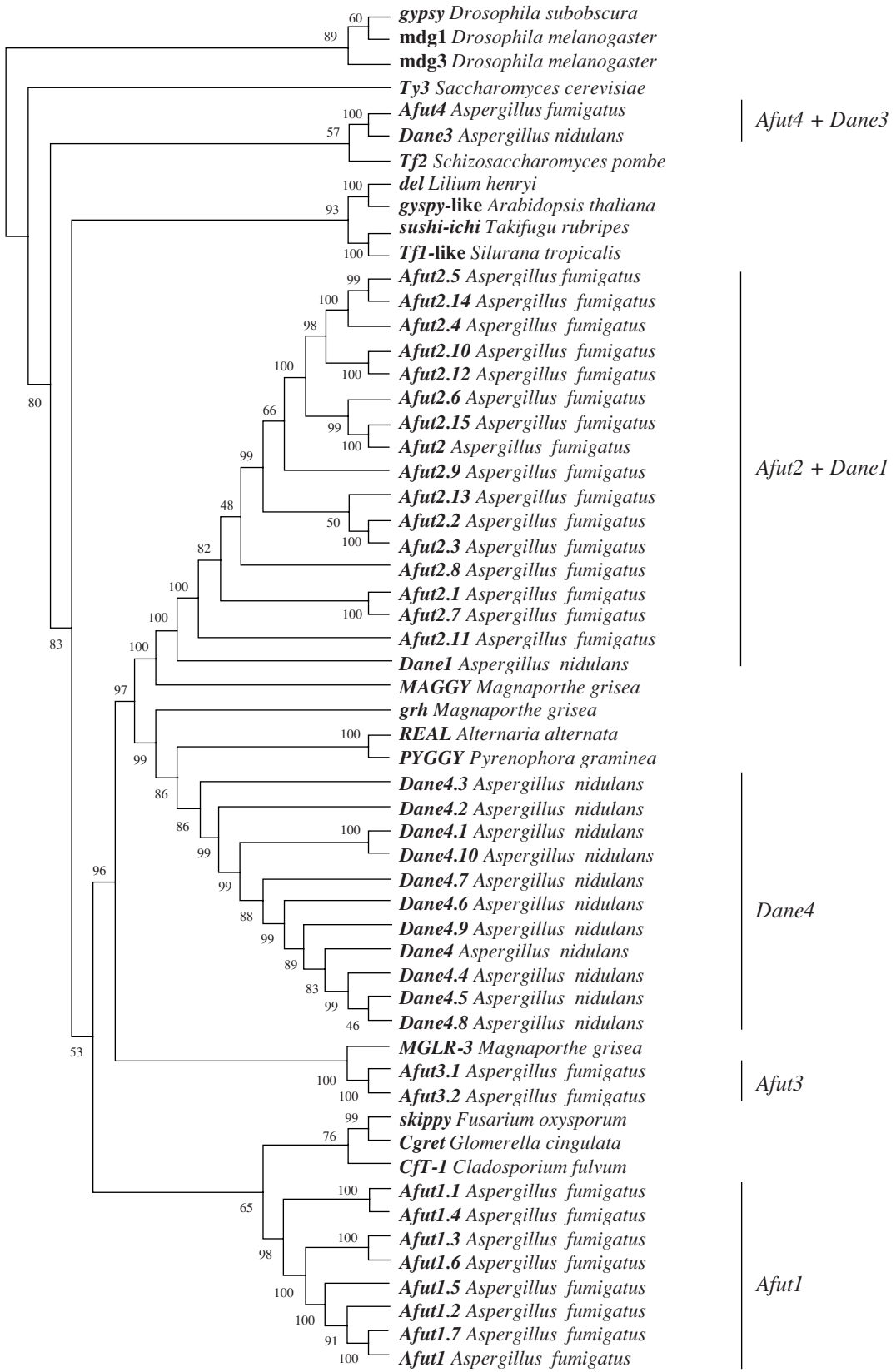

Fig. 1. Phylogenetic tree based on an alignment of the amino acid sequences encoded by fragments of *A. fumigatus* and *A. nidulans* LTR retrotransposons and Metaviridae elements retrieved from databases. Bootstrap indices below 50% are omitted.

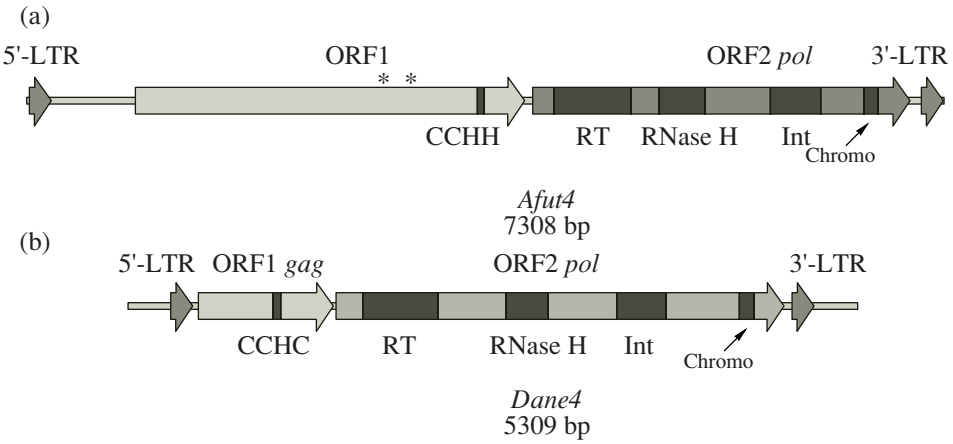

Fig. 2. Structure of (a) *Afut4* and (b) *Dane4.* 5'- and 3'-LTRs are the 5' and 3' long terminal repeats; ORF, open reading frame; RT, reverse transcriptase domain; RNase H, ribonuclease H domain; Int, integrase domain; Chromo, chromodomain; CCHH and CCHC, cysteine motifs. Asterisks indicate stop codons in *Afut4* coding sequences (pseudo-ORF).

The first cluster includes 16 sequences close to *Afut2* [30] and similar to *Maggy* from *Magnaporthe grisea* [31]. A comparison of the amino acid sequences encoded by these elements showed that the similarity between the *Afut2* copies reached 96.1% on average and the similarity to *Maggy* varied from 47.7 to 56.9%.

The second group includes eight sequences forming a common branch with *Afut1* [29] and *CfT1* from *Cladosporium fulvum* [32]. However, the similarity between the amino acid sequences encoded by the detected elements and *CfT1* was very low, from 30 to 34.5%. All amino acid sequence fragments encoded by *Afut1* and *Afut2* were translation products read from templates with multiple distortions, such as stop codons and frameshifts. Thus, the *A. fumigatus* genome lacks intact copies of *Afut1* or *Afut2*, which would code for enzymes required for transposition. A comparison of LTRs allows the estimation of the relative time of insertion of an element into the genome [33]. Pairwise comparisons of LTRs for several *Afut1* copies revealed significant differences between 5'-LTR and 3'-LTR within individual copies: from 9.9 to 21.7%. The repeats of the *Afut2* copies had 90% similarity on average. Therefore, we suggest that the *Afut1* and *Afut2* copies were inserted into the genome long ago.

Two elements, named *Afut3.1* and *Afut3.2*, are new LTR retrotransposons, not previously described in *A. fumigatus*. Both of these elements belong to the same branch of the phylogenetic tree as *MGLR-3* from *M. grisea* [34]. The similarity of *Afut3.1* and *Afut3.2* to this retrotransposon is 43.6 and 47%, respectively.

The total nucleotide sequences of the new elements isolated from *A. fumigatus* were 6255 bp for *Afut3.1* and 6256 bp for *Afut3.2*. Both have 256-bp LTRs and are flanked by target duplications, AAGAT…AAGAT and AATAT…AATAT, resulting from the element insertion. The nucleotide sequences of *Afut3.1* and *Afut3.2* have 94% similarity. The similarity between

LTR retrotransposons from *A. fumigatus* and *A. nidulans*

Aspergillus	Element	GenBank accession no., fragment no.	Length, bp	LTR length/similarity, %	*DIR*	TSD	Solo LTR	Reference
fumigatus	*Afut1*	L76086	6924	282/90	tg/ca	tcctt/tcctt	+	[29]
	Afut2	AF202956	5698	150/96.7	gc/ta	–	+	[30]
	Afut3	a_*fumigatus*|*chr*_0|TIGR.5132|52	6258	265/97.4	gt/ta	aagat/aagat	–	
	Afut4	a_*fumigatus*|*chr*_0|Sanger.4960.fas.00001|	7308	184/100	tg/ca	ctcag/ctcag	+	
nidulans	*Dane1*	AF295689	5665	223/97.3	tg/ca	accc/accc	+	[38]
	Dane2	AF295688	5664	223/98.2	tg/ca	ccc/ccc	+	[38]
	Dane3	*Aspergillus nidulans* contig 1.44	3718 (one ORF)	–	–	–	–	
	Dane4	*Aspergillus nidulans* contig 1.89	5309	202/98	tac/cat	–	+	

Note: DIR, dinucleotide inverted repeats; TSD, terminal site duplications.

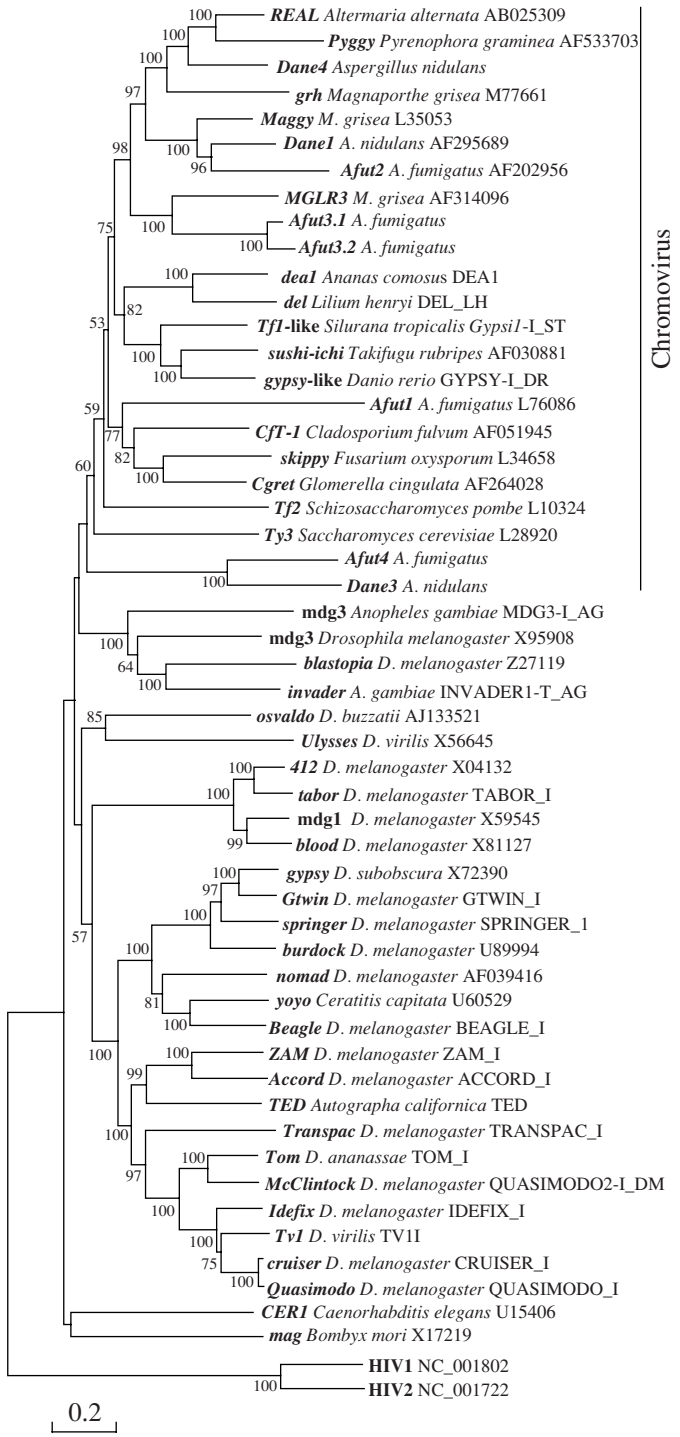

Fig. 3. Phylogenetic tree of Metaviridae LTR retrotransposons as based on an alignment of the amino acid sequences of their RT, RNase H, and Int fragments of about 700 residues in length. Pol fragments of the human immunodeficiency viruses (HIV-1 and HIV-2) are used as an outgroup. The *Chromovirus* phylogenetic group, which includes the elements discovered in *A. fumigatus* and *A. nidulans*, is shown on the right.

LTRs of one element was 97.8% in *Afut3.1* and 97% in *Afut3.2*. This close similarity between LTRs suggests their recent insertion into the genome [33]. Both elements are damaged copies since they do not have intact ORFs. Nevertheless, we managed to restore in part the 2247-bp *Afut3.1* ORF, which coded for a polyprotein containing the RT and Int domains.

The only intact amino acid sequence belongs to an element that is far from all other *A. fumigatus* elements on the phylogenetic tree. Moreover, the element groups on the same branch as yeast *Tf2* [35] (Fig. 1). This element is named *Afut4*. The complete *Afut4* sequence was chosen for further analysis.

Structural Organization of *Afut4* from the *A. fumigatus* Genome

The full-size *Afut4* element is 7308 bp and includes two identical 184-bp LTRs (Fig. 2a). The element is flanked by two short inverted repeats TG…CA, as typical of all LTR retrotransposons and retroviruses. In addition, *Afut4* is surrounded by short target duplications CTCAG…CTCAG. All of these facts are indicative of recent *Afut4* insertion into the host genome.

The interior of this element contains four ORFs of different lengths. The two first ORFs are in frame and, most likely, constituted one ORF in the past. The reconstructed ORF1 is 2639 bp. Its translation product does not resemble any known protein, but the C end of the sequence has a cysteine motif Cys-X_3-Cys-X_4-His-X-His (CCHH). One or more cysteine motifs are typical of DNA-binding proteins, including proteins encoded by ORF1 in known retrotransposons and retroviruses [36].

The two other ORFs also seem to have been a single ORF, because they are separated by a single stop codon. They were combined into the second reading frame (ORF2). The restored ORF2 codes for the polyprotein that is typical of LTR retrotransposons and includes the RT, RNase H, and Int domains, as well as the so-called chromodomains (Chromo), known in elements of the phylogenetic group *Chromovirus* [15].

The relative integrity of *Afut4* in comparison with other *A. fumigatus* LTR retroelements points to its recent integration. However, the mechanism of retrotransposon migration implies an increase in the copy number of the elements. Hence, either the ancestor element of this single *Afut4* copy has been lost or the copy was acquired by *A. fumigatus* from outside and has as yet not propagated over the genome. The latter suggestion is supported by the fact that the *Afut4* branch on the phylogenetic tree is distant from other LTR retroelements from the genomes of fungi of the subphylum Pezizomycotina (Ascomycota). To find the *Afut4* ancestor or its parts, we screened the *A. fumigatus* genome for the presence of so-called solo

elements (solo LTRs), resulting from recombination between LTRs of one or two elements with excision of the retrotransposon body [37]. The search for solo LTRs was also performed with GenomeBrowser (see Experimental).

Four solo *Afut4* were found. The nucleotide sequences of these solo elements are identical but have only 75.1% similarity to the LTR of the full-size *Afut4*. The solo elements have a 5-bp deletion in comparison with the *Afut4* LTR.

LTR Retrotransposons in *A. nidulans*

Analysis of the *A. nidulans* genome revealed 25 potential retrotransposons, belonging to the metavirus group according to BLAST. However, only 11 had all three essential domains (RT, RNase H, and Int) and could be used in analysis of the amino acid sequences (Fig. 1).

One of these 11 elements, named *Dane3* by analogy with *Dane1* and *Dane2*, previously described in *A. nidulans* [38], is more similar (51%) to *Afut4* than to the other *A. nidulans* elements (22.3% on average). Moreover, *Afut4* and *Dane3* form a common branch on the phylogenetic tree. The nucleotide sequence of *Dane3* was extracted from databases and examined. *Dane3* contains no LTRs or sequences that could represent LTR remains. The reconstructed *Dane3* pseudo-ORF has one stop codon, but the sequences of RNase H and Int domains preserve their integrity.

The other ten sequences are closely similar to *Real* from *Alternaria alternata* [39], *Pyggy* from *Pyrenophora graminea* [40], and *grh* from *M. grisea* [41]. These elements form a common branch on the phylogenetic tree (Fig. 1). The similarity between their amino acids is 99% on average and that between the nucleotide sequences is 88 to 91%. Four out of the ten copies are very similar to each other. They encode intact protein sequences. The complete nucleotide sequence of one of these elements was obtained. This element was named *Dane4*.

Dane4 contains two 202-bp LTRs similar to each other by 98% (Fig 2b, table). Some regulatory domains and transcription factor-binding sites were found in the LTRs: a TATA box and a binding site for the CCAAT/enhancer-binding protein (C/EBP). Short inverted repeats (TAC…CAT) are present at the ends of the LTRs.

The central region of the element contains two ORFs. ORF1 is 1131 bp (377 amino acid residues). BLAST analysis of the ORF1 translation product showed its close similarity (41% on average) to Gag of the *Real* [39] and *Pyggy* [40] elements, confirming their relationship to *Dane4*. ORF2 is 3690 bp (1230 residues). The corresponding Pol polyprotein has the RT, RNase H, and Int domains, as well as chromodomains. Thus, *Dane4* is an LTR retroelement with two intact ORFs. Moreover, four copies of this element are similar to each other in the Pol-encoding domain by 99.9%, indicating recent migrations of the elements. It is likely that *Dane4* is active.

As mentioned above, the *A. nidulans* genome has two LTR retrotransposons, *Dane1* and *Dane2* [38]. We did not find these elements in *A. nidulans*. It has been shown that few copies of *Dane1* and *Dane2* are present in various *A. nidulans* strains and are absent from some isolates [38]. Therefore, *Dane1* and *Dane2* may be absent from the strain under study, FGSC A4. It is thought that *Dane1* and *Dane2* occur in pericentric and/or subtelomeric genome regions [38], whose sequences are often omitted during genome sequencing and assembly because of the difficulty of analyzing repetitive sequences, which abound in these regions.

Thus, the in silico search for LTR retrotransposons in the two *Aspergillus* genomes (*A. fumigatus* and *A. nidulans*) revealed significant differences in the range of retrotransposon families between the species studied (Fig. 3). The two species had only one common retrotransposon family, which includes *Dane3* and *Afut4*; the other families were species-specific. However, *Dane1* and *Dane2*, not found in the *A. nidulans* strain FGSC A4, belong to the same phylogenetic branch as *Afut2* from *A. fumigatus*.

The *A. fumigatus* genome contains four LTR retrotransposon families. Two of them were detected before, while *Afut3* and *Afut4* are described here for the first time. The families differ in copy number. There are a few *Afut3* and *Afut4* copies, while *Afut1* and *Afut2* are present in dozens of copies. The overwhelming majority of the elements are represented by damaged copies.

Two element families were found in *A. nidulans* in addition to *Dane1* and *Dane2* [38]. In contrast to *A. fumigatus*, whose genome lacks retrotransposon copies with intact coding sequences, *A. nidulans* has several full copies of *Dane4*.

The abundance of damaged copies suggests directional inactivation of retroelements in both *Aspergillus* species. Some fungi are known to possess mechanisms inactivating repetitive sequences. For example, repetitive sequences of *Ascobulus immersus* are recognized and inactivated by methylation induced premeiotically (MIP) [42]. Another mechanism, which also includes methylation followed by cytosine deamination, has been discovered in *Neurospora crassa*. This mechanism is named repeat-induced point mutations (RIP) [43]. Comparison of the nucleotide sequences of some *Afut1, Afut2*, and *Dane4* copies shows that point mutations accumulated in these elements are distributed nonrandomly. Most of them are C⟶T transitions in dinucleotides CpG and CpA

(data not shown). It is reasonable to suggest that the species under study possess inactivation mechanisms similar to RIP.

The *Afut4* and *Dane3* elements form a common branch on the phylogenetic tree (Fig. 3), distant from clusters formed by LTR retrotransposons from other fungi belonging to the subphylum Pezizomycotina (Ascomycota). Probably, *Afut4* and *Dane3* belong to a metavirid family that is most ancient among the elements of nonyeast fungi. The presence of this family in other fungus species is questionable. A preliminary search with various BLAST programs and *Afut4* Pol as a query showed that elements of this family or their remains could be present in many sac fungi of the subphylum Pezizomycotina (Ascomycota), whose sequences were available in databases, but were absent from yeasts (Ascomycota, Saccharomycotina) and basidiomycetes (Basidiomycota) (unpublished data).

The LTR retrotransposons described can be used as markers to monitor the *Aspergillus* species and strains [22].

ACKNOWLEDGMENTS

This study was supported by the program Biosphere Origin and Evolution of the Russian Academy of Sciences (project no. 10104-34/P-18/155-270/1105-06-001/28/2006-1). Sequencing of the *Aspergillus fumigatus* genome was supported by the National Institute of Allergy and Infectious Diseases (United States; grant no. U01 AI 48830 to D. Denning and W. Nierman), the Wellcome Trust Foundation (United Kingdom), and Fondo de Investigaciones Sanitarias (Spain).

REFERENCES

1. Biemont C., Cizeron G. 1999. Distribution of transposable elements in *Drosophila* species. *Genetica*. **105**, 43–62.
2. Lander E.S., Linton L.M., Birren B., et al. 2001. Initial sequencing and analysis of the human genome. *Nature*. **409**, 860–921.
3. SanMiguel P., Tikhonov A., Jin Y.K., Motchoulskaia N., Zakharov D., Melake-Berhan A., Springer P.S., Edwards K.J., Lee M., Avramova Z., Bennetzen J.L. 1996. Nested retrotransposons in the intergenic regions of the maize genome. *Science*. **274**, 765–768.
4. Bannert N., Kurth R. 2004. Retroelements and the human genome: New perspectives on an old relation. *Proc. Natl. Acad. Sci. USA*. **101**, Suppl. 2, 14,572–14,579.
5. Finnegan D.J. 1992. Transposable elements. *Curr. Opin. Genet. Dev.* **2**, 861–867.
6. Christensen S.M., Bibillo A., Eickbush T.H. 2005. Role of the *Bombyx mori* R2 element N-terminal domain in the target-primed reverse transcription (TPRT) reaction. *Nucleic Acids Res*. **33**, 6461–6468.
7. Poulter R.T., Goodwin T.J. 2005. DIRS-1 and the other tyrosine recombinase retrotransposons. *Cytogenet. Genome Res*. **110**, 575–588.
8. Eickbush T., Boeke J.D., Sandmeyer S.B., Voytas D.F. 2004. Metaviridae. In: *The Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*. Eds. Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A. London: Elsevier/Academic Press, pp. 409–420.
9. Havecker E.R., Gao X., Voytas D.F. 2004. The diversity of LTR retrotransposons. *Genome Biol*. **5**, 225.
10. Xiong Y., Eickbush T.H. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. *EMBO J*. **9**, 3353–3362.
11. Langdon T., Seago C., Mende M., Leggett M., Thomas H., Forster J.W., Jones R.N., Jenkins G. 2000. Retrotransposon evolution in diverse plant genomes. *Genetics*. **156**, 313–325.
12. Marin I., Llorens C. 2000. *Ty3/gypsy* retrotransposons: Description of new *Arabidopsis thaliana* elements and evolutionary perspectives derived from comparative genomic data. *Mol. Biol. Evol*. **17**, 1040–1049.
13. Malik H.S., Eickbush T.H. 1999. Modular evolution of the integrase domain in the *Ty3/gypsy* class of LTR retrotransposons. *J. Virol*. **73**, 5186–5190.
14. Tubio J.M., Naveira H., Costas J. 2005. Structural and evolutionary analyses of the *Ty3/gypsy* group of LTR retrotransposons in the genome of *Anopheles gambiae*. *Mol. Biol. Evol*. **22**, 29–39.
15. Kordis D. 2005. A genomic perspective on the chromodomain-containing retrotransposons: Chromoviruses. *Gene*. **347**, 161–173.
16. Ganko E.W., Fielman K.T., McDonald J.F. 2001. Evolutionary history of *Cer* elements and their impact on the *C. elegans* genome. *Genome Res*. **11**, 2066–2074.
17. Pelissier T., Tutois S., Deragon J.M., Tourmente S., Genestier S., Picard G. 1995. *Athila*, a new retroelement from *Arabidopsis thaliana*. *Plant Mol. Biol*. **29**, 441–452.
18. Simmen M.W., Bird A. 2000. Sequence analysis of transposable elements in the sea squirt, *Ciona intestinalis*. *Mol. Biol. Evol*. **17**, 1685–1694.
19. Brakhage A.A., Jahn B., Schmidt A. 1999. *Aspergillus fumigatus*. *Biology, Clinical Aspects and Molecular Approaches to Pathogenicity*. Basel: Karger.
20. Latge J.P. 1999. *Aspergillus fumigatus* and aspergillosis. *Clin. Microbiol. Rev.* **12**, 310–350.
21. Marr K.A., Patterson T., Denning D. 2002. Aspegillosis. Pathogenesis, clinical manifestation, and therapy. *Inf. Dis. Clin. North. Am*. **16**, 857–894.
22. Varga J. 2004. Molecular typing of aspergilli: Recent developments and outcomes. *Med. Mycol*. **44**, S149–S161.
23. Nierman W.C., Pain A., Anderson M.J., Wortman J.R., Kim H.S., Arroyo J., Berriman M., Abe K., Archer D.B., Bermejo C., et al. 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature*. **438**, 1151–1156.
24. Galagan J.E., Calvo S.E., Cuomo C., Ma L.J., Wortman J.R., Batzoglou S., Lee S.I., Basturkmen M., Spevak C.C., Clutterbuck J., et al. 2005. Sequencing of

Aspergillus nidulans and comparative analysis with *A. fumigatus* and *A. oryzae. Nature*. **438**, 1105–1115.

25. Thompson J.D., Higgins D.G., Gibson T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. **22**, 4673–4680.
26. Kumar S., Tamura K., Jakobsen I.B., Nei M. 2001. MEGA2: Molecular evolutionary genetic analysis software. *Bioinformatics*. **17**, 1244–1245.
27. Saitou N., Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **4**, 406–425.
28. Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. **39**, 783–791.
29. Neuveglise C., Sarfati J., Latge J.-P., Paris S. 1996. *Afut1*, a retrotransposon-like element from *Aspergillus fumigatus. Nucleic Acids Res*. **24**, 1428–1434.
30. Paris S., Latge J.P. 2001. *Afut2*, a new family of degenerate *gypsy*-like retrotransposon from *Aspergillus fumigatus. Med. Mycol*. **39**, 195–198.
31. Farman M.L., Tosa Y., Nitta N., Leong S.A. 1996. *MAGGY*, a retrotransposon in the genome of the rice blast fungus *Magnaporthe grisea. Mol. Gen. Genet*. **251**, 665–674.
32. McHale M.T., Roberts I.N., Noble S.M., Beaumont C., Whitehead M.P., Seth D., Oliver R.P. 1992. *CfT-I*: An LTR-retrotransposon in *Cladosporium fulvum*, a fungal pathogen of tomato. *Mol. Gen. Genet*. **233**, 337–347.
33. Jordan I.K., McDonald J.F. 1998. Evidence for the role of recombination in the regulatory evolution of *Saccharomyces cerevisiae Ty* elements. *J. Mol. Evol*. **47**, 14–20.
34. Kang S. 2001. Organization and distribution pattern of *MGLR-3*, a novel retrotransposon in the rice blast fungus *Magnaporthe grisea. Fungal Genet. Biol*. **32**, 11–19.
35. Weaver D.C., Shpakovski G.V., Caputo E., Levin H.L., Boeke J.D. 1993. Sequence analysis of closely related retrotransposon families from fission yeast. *Gene*. **131**, 135–139.
36. Orlinsky K.J., Sandmeyer S.B. 1994. The Cys–His motif of Ty3 NC can be contributed by Gag3 or Gag3–Pol3 polyproteins. *J. Virol*. **68**, 4152–4166.
37. Schmid-Berger N., Schmid B., Barth G. 1994. *Ylt1*, a highly repetitive retrotransposon in the genome of the dimorphic fungus *Yarrowia lipolytica. J. Bacteriol*. **176**, 2477–2482.
38. Nielsen M.L., Hermansen T.D., Aleksenko A. 2001. A family of DNA repeats in *Aspergillus nidulans* has assimilated degenerated retrotransposons. *Mol. Genet. Genomics*. **265**, 883–887.
39. Kaneko I., Tanaka A., Tsuge T. 2000. *REAL*, an LTR retrotransposon from the plant pathogenic fungus *Alternaria alternata. Mol. Gen. Genet*. **263**, 625–634.
40. Taylor E.J., Konstantinova P., Leigh F., Bates J.A., Lee D. 2004. *Gypsy*-like retrotransposons in *Pyrenophora*: An abundant and informative class of molecular markers. *Genome*. **47**, 519–525.
41. Dobinson K.F., Harris R.E., Hamer J.E. 1993. *Grasshopper*, a long terminal repeat (LTR) retroelement in the phytopathogenic fungus *Magnaporthe grisea. Mol. Plant Microbe Interact*. **6**, 114–126.
42. Faugeron G. 2000. Diversity of homology-dependent gene silencing strategies in fungi. *Curr. Opin. Microbiol*. **3**, 144–148.
43. Galagan J.E., Selker E.U. 2004. RIP: The evolutionary cost of genome defense. *Trends Genet*. **20**, 417–423.