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Research Article

The biology and potential for genetic research of transposable elements in filamentous fungi

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Abstract

Recently many transposable elements have been identified and characterized in filamentous fungi, especially in species of agricultural, biotechnological and medical interest. Similar to the elements found in other eukaryotes, fungal transposons can be classified as class I elements (retrotransposons) that use RNA and reverse transcriptase and class II elements (DNA transposons) that use DNA. The changes (transposition and recombination) caused by transposons can supply wide-ranging genetic variation, especially for species that do not have a sexual phase. The application of transposable elements to gene isolation and population analysis is an important tool for molecular biology and studies of fungal evolution.

Key words: transposable elements, filamentous fungi, genetic application.

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Introduction

Transposons are mobile genetic transposable elements that can multiply in the genome of eubacteria, archaea and eukaryotes using a variety of mechanisms and were first discovered in maize in the 1940s by Barbara McClintock. Since their initial discovery a growing number of transposons have been detected in bacteria, plants and animals (Finnegan, 1989). Transposons were first identified in fungi in the yeast *Saccharomyces cerevisiae* (Boeke, 1989), with the first evidence for their presence in filamentous fungi coming from conventional genetic studies with *Ascobolus immersus* mutants unstable for spore-staining (Decaris *et al*., 1978).

Advances in genome molecular analysis of the species used as models for fungal genetics (*e.g.* the ascomycetes *Neurospora crassa* and *Aspergillus nidulans*) showed that they contain silenced transposons, the loss of activity of which may be the consequence of continuous selection for phenotypic stability and the action of several mechanisms of genetic silencing which inactivate repeated sequences, including transposable elements (Selker, 1999; Faugeron, 2000; Cogoni, 2001). More detailed descriptions on the biology of transposons in filamentous fungi have been published especially for species of agricultural, biotechnological and medical interest (see reviews by Oliver, 1992; Kistler and Miao, 1992), although the sexual stage has not been described for most of these species which generally show a high level of genetic variability (Daboussi, 1997; Kempken and Kück, 1998). The study of the transposons in these technologically useful species led to the discovery of many types of elements, covering practically the whole spectrum of transposable eukaryotic elements (Daboussi, 1997; Kempken and Kück, 1998).

Several types of DNA retroelements and transposons are active and induce a variety of modifications and have the potential to influence many aspects of fungal genome evolution. These mutagenic properties have also been explored to develop a gene isolation strategy, known as transposon tagging. The dynamic of these elements includes different mechanisms, such as transposition, ectopic recombination and horizontal transmission. Further, the study of the distribution of transposons in natural populations can provide important ecological and epidemiological data (Daboussi and Capy, 2003). This article will review

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some aspects related to the study of transposons in fungi especially the distribution and classification of these elements, transposition mechanism and consequences for the fungal genome, and the main strategies used to identify new elements and their potential for genetic research.

Transposable Element Structure and Distribution in Fungi

The transposable elements of fungi are similar to those of eukaryotes in general and can be divided into two main classes according to the mode of transposition and their structural organization (Figure 1). Class I elements (retroelements) which transpose by reverse transcription of an RNA intermediate, this class being subdivided into retrotransposons flanked by long terminal repeats (LTR) and non-LTR retroelements with long dispersed nuclear element structures (LINEs) and short dispersed nuclear element structures (SINEs). Class II elements (DNA transposons) are flanked by two inverted terminal repetitions (TIRs) and transpose directly using the enzyme transposase. Both classes are subdivided into different superfamilies based on the structure, internal organization, size of the duplication of the target site generated after insertion and similarity in DNA and protein sequences (Finnegan, 1989). The International Committee on Taxonomy of Viruses recently proposed a classification for LTR retrotransposons based on the relationships between the amino acid sequences of reverse transcriptase, the most highly conserved of the retrotransposon proteins (Havecker *et al.*, 2004). This classification separates the retrotransposons of animals, fungi, plants and protozoa into two great families, the Pseudoviridae and Metaviridae which are distinguished by the order of the coding regions of structural (*gag*) and enzymatic (*pol*) proteins. In the Metaviridae the *pol* genes are ordered in the sequence protease/reverse transcriptase/RnaseH/integrase while in the Pseudoviridae the order of the *pol* genes is protease/integrase/reverse transcriptase/RnaseH (Figure 2). In previous revisions (Daboussi, 1996; Kempken and Kück, 1998; Daboussi and Capy, 2003), the classification of the LTR retroelements

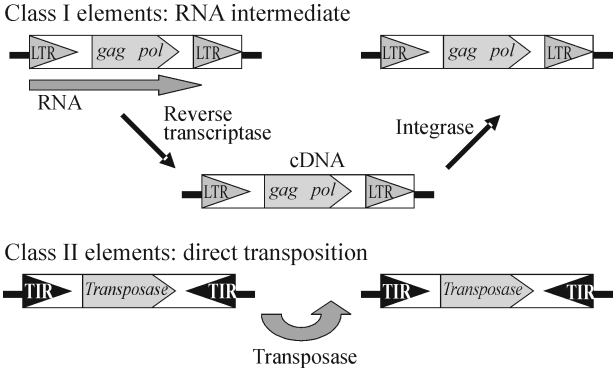

Figure 1 - General structure of transposable elements of eukaryotes (based in Finnegan, 1989).

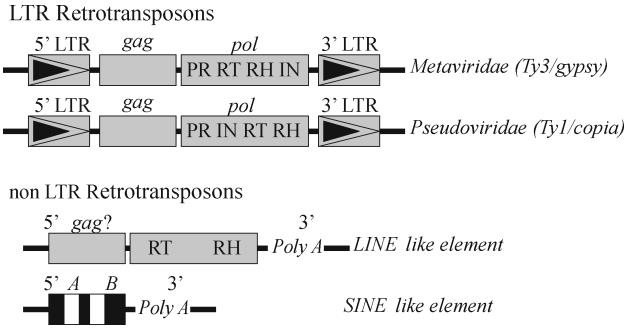

Figure 2 - Schematic representations of the structural features of class I transposable elements (based on Daboussi, 1996 and Havecker *et al.*, 2004). Long terminal direct repeat (LTR) retrotransposons resemble retroviruses in having LTRs flanking an internal domain encoding proteins analogous to the *gag* and *pol* retroviral gene products. The non LTR-retrotransposons lack terminal repeats and carry a poly (A) tail at their 3' ends. Elements with long dispersed nuclear element structures (LINEs) possess two long open reading frames (ORFs), with similarities to *gag* as well as the reverse transcriptase (RT) and RnaseH (RH) genes. Elements with short dispersed nuclear element structures (SINEs) are short elements which contain an internal RNA polymerase III promoter with bipartite structure (boxes A and B) and which rely on RT for mobilization but do not themselves encode the enzyme.

was based on their similarity to *gypsy* elements (equivalent to the Metaviridae) and *copia* elements (equivalent to the Pseudoviridae).

Thirty class I transposons (retroelements) have already been described including Metaviridae (*gypsy*) and Pseudoviridae (*copia*) LTR retrotransposons and retrotransposons without the LINEs and SINEs type LTRs. The *gypsy* retrotransposons identified to date are shown in Table 1, of which only *maggy* in *Magnaporthe grisea* showed activity (Talbot, 1998).

Few *copia* group retroelements have been characterized, these elements being inactive due to multiple deletions and mutations in conserved regions. Among the *copia* retrotransposons so far identified is the *tcen* element in found in the centromeric regions of the filamentous fungus *Neurospora crassa* (Cambareri *et al.*, 1998).

Several non-LTR retrotransposons have also been characterized. The *tad* element in *N. crassa* was the first transposon described in a fungus where it was found inserted in the glutamate dehydrogenase *am* gene (Kinsey and Helber, 1989), transposition of this element was having been demonstrated by transference between genetically marked nuclei in forced heterokaryons (Kinsey, 1993). Among the non-LTR retrotransposon of the *LINE* type (Table 1), only *tad* and *mgl* are active elements.

In *Colletotrichum gloeosporioides* (an anthracnose filamentous fungi pathogenic for *Stylosanthes* spp) the *cgt1* retrotransposon was isolated from the *Stylosanthes* B biotype but not from the A biotype and the same was observed for the *Colletotrichum* species *lindemuthianum*, *trifolii* and *destructivum* (He *et al.*, 1996). The *cgt1* element is considered an important tool for the study of population structure, genome dynamics and evolution in *C. gloeosporioides* (He

Table 1 - General classification of fungal class I transposable elements.

Element class	Element group	Element[1]	Host fungus	References
Class I transposons (retroelements)	LTR retrotransposon Metaviridae (*gypsy*)	*foret*	*Fusarium oxysporum*	Julien *et al.*, 1992
		skippy	*F. oxysporum*	Anaya and Roncero, 1995
		grh	*Magnaporthe grisea*	Dobinson *et al.*, 1993
		maggy	*M. grisea*	Farman *et al.*, 1996b
		pyret	*M. grisea*	Nakayashiki *et al.*, 2001
		mgl3	*M. grisea*	Kang, 2001
		cft-1	*Cladosporium fulvum*	McHale *et al.*, 1992
		cgret	*Colletotrichum gloeosporioides*	Zhu and Oudemans, 2000
		boty	*Botrytis cinerea*	Diolez *et al.*, 1995
		real	*Alternaria alternata*	Kaneko *et al.*, 2000
		dane 1, *2*	*Aspergillus nidulans*	Nielsen *et al.*, 2001
		afut	*A. fumigatus*	Neuvéglise *et al.*, 1996
		mars4	*Ascobolus immersus*	Goyon *et al.*, 1996
		dab1	*Neurospora crassa*	Bibbins *et al.*, 1998
		yeti	*Podospora anserina*	Hamann *et al.*, 2000b
		mary1	*Tricholoma matsutake*	Murata and Yamada, 2000
		prt1	*Phycomyces blakesleanus*	Ruiz-Pérez *et al.*, 1996
	LTR retrotransposon Pseudoviridae (*copia*)	*mars 2*, *3*	*A. immersus*	Goyon *et al.*, 1996
		tcen	*N. crassa*	Cambareri *et al.*, 1998
		nht2	*Nectria haematococa*	Shiflett *et al.*, 2002
	non LTR retrotransposon (*LINE*)	*tad*	*N. crassa*	Kinsey and Helber, 1989
		mgl	*M. grisea*	Nishimura *et al.*, 2000
		mgr583	*M. grisea*	Hamer *et al.*, 1989
		cgt1	*C. gloeosporioides*	He *et al.*, 1996
		mars1	*A. immersus*	Goyon *et al.*, 1996
		mary2	*T. matsutake*	Murata *et al.*, 2001
	non LTR retrotransposon (*SINE*)	*nrs1*	*N. haematococca*	Kim *et al.*, 1995
		foxy	*F. oxysporum*	Mes *et al.*, 2000
		mgsr1	*M. grisea*	Kachroo *et al.*, 1995
		egr1	*Erysiphe graminis*	Wei *et al.*, 1996
		egh1	*E. graminis*	Rasmussen *et al.*, 1993

[1]Classification based on reviews by Daboussi, 1996; Kempken and Kück, 1998 and Daboussi and Capy, 2003. *T. matsutake* is a basidiomycete and *P. blakesleeanus* a zigomycete, the remaining species are ascomycetes.

et al., 1996). Among the retrotransposons of the *SINE* type already isolated (Table 1) the *foxy* element showed activity after gamma radiation treatment and subsequent new insertions (Mes *et al.*, 2000).

The class II transposable elements (or DNA transposons) can be classified in four different superfamilies: *Tcl/mariner*, *hAT*, *Mutator* and *MITE*s (Daboussi and Capy, 2003). The *Tcl/mariner* superfamily is the most abundant, the most studied elements of this superfamily being the *fot1* and *impala* transposons of *Fusarium oxysporum* (Daboussi *et al.*, 1992; Daboussi and Langin, 1994; Langin *et al.*, 1995; Fernandez *et al.*, 1998; Hua-van *et al.*, 1998; Deschamps *et al.*, 1999; Migheli *et al.*, 1999; Chiocchetti *et al.*, 1999; Hua-van *et al.*, 2000; Rosevitch and Kistler, 2000; Hua-van *et al.*, 2001ab; Villalba *et al.*, 2001; Daviére *et al.*, 2001; Hua-van *et al.*, 2002; Daboussi *et al.*, 2002; Daboussi and Capy, 2003). Members of this superfamily have inverted terminal repetitions (TIRs) of variable size and a Thymine/adenine (TA) target site. This site is generally duplicated on excision of the transposon, leading to alteration in the DNA sequence of the donor site. It has been demonstrated that *fot1* is an autonomous element that codifies its own transposase that has a catalytic domain which cleaves the DNA strands. This transposon was active when introduced by transformation in *F. oxysporum* strains without the element (Daboussi *et al.*, 1992; Migheli *et al.*, 1999).

The activity of the *fot1* and *impala* transposons and of other elements has been shown by chromosome rearrangements detected by analysis of the electrophoretic karyotype, this analysis also showing a grouping of transposons in some regions and a correlation between the high level of chromosome polymorphisms and transposable element concentration (Davière *et al.*, 2001). In *F. oxysporum* chromosome duplications and gene rearrangements of the *skippy* LTR retrotransposon were also induced by growth under nutritional stress in the presence of potassium chlorate (Anaya and Roncero, 1996). The *fot1* and *impala* elements have also been used to asses the genetic diversity of *F. oxysporum* isolates from different French soils (Edel *et al.*, 2001).

Villalba *et al* (2001) introduced the *impala* element into *M. grisea* where transposition of the element was revealed by excision of the *niaD* gene promoter and molecular analysis of the revertents using hybridization and sequencing. One mycelial growth mutant and a non-

pathogenic mutant were isolated and it was shown that by insertion of the *impala* element a pathogenicity gene could be cloned and sequenced (ORP1) which is essential for the penetration of *M. grisea* into the host leaf tissue. This gene did not present homology with known genes, showing the potential of transposable elements for cloning of pathogenicity genes. In addition to the elements described above other transposons of the *Tc1/mariner* superfamily are described in Table 2.

The *hAT* superfamily was defined based on the similarity between the maize *Ac* elements and the *hobo* element of *Drosophila*. This superfamily is well represented in fungi, and has been identified in both the Ascomycota and Basidiomycota (square 2). Elements belonging to the *Mutator* superfamily were identified recently in fungi by Chalvet *et al* (2003), which is very interesting because these elements had previously been detected only in plants. This element, called *hop*, was identified in the *F. oxysporum* genome as being active and similar to the elements found in maize.

The category of small elements with terminal inverted repetitions, called miniature inverted-repeat transposable elements (*MITE*) includes the *F. oxysporum mimp* elements (Hua-Van *et al.*, 2000) and the *guest* element of on *N. crassa* (Yeadon and Catcheside, 1995). These elements are remainders of non-autonomous DNA transposons and their mobilization depends on the transposase produced by other class II elements (Feschottes *et al.*, 2002).

Identification Strategies for Transposable Elements

Different strategies can be used to identify transposable elements in fungi:

I) Identification and cloning of dispersed repeated sequences. Several transposons have been cloned and identified by comparison with elements described in other organisms but this method does not show whether these sequences remain active. This strategy is particularly appropriate to identify high copy number transposons regardless of their activity (Kempken and Kück, 1998). Some examples of elements identified by this strategy are the *foret1* and *palm* transposons of *F. oxysporum* (Julien *et al.*, 1992; Mouyna *et al.*, 1996) and the *cgt1* and *cgret* elements of *C. gloeosporioides* (He *et al.*, 1996; Zhu and Oudemans, 2000).

Table 2 - General classification of fungal class II transposable elements.

Element class	Element superfamily	Elements[1]	Host fungus	References
Class II transposons (DNA mediated elements)	*Tc1/Mariner*	*fot1*	*Fusarium oxysporum*	Daboussi *et al.*, 1992
		impala	*F. oxysporum*	Langin *et al.*, 1995
		fot2	*F. oxysporum*	Daboussi and Langin, 1994
		fot3;fot4	*F. oxysporum*	Hua-van *et al.*, 2000
		punt	*Neurospora crassa*	Margolin *et al.*, 1998
		flipper	*Botrytis cinerea*	Levis *et al.*, 1997
		tan1	*Aspergillus niger*	Nyyssonen *et al.*, 1996
		vader	*A. niger*	Amutan *et al.*, 1996
		ant1	*A. niger*	Glayzer *et al.*, 1995
		pot2	*Magnaporthe grisea*	Kachroo *et al.*, 1994
		mgr586	*M. grisea*	Farman *et al.*, 1996a
		fcc1	*Cochliobolus carbonum*	Panaccione *et al.*, 1996
		nht1	*Nectria haematococa*	Enkerli *et al.*, 1997
		pat	*Podospora anserina*	Hamann *et al.*, 2000a
		hupfer	*Beauveria bassiana*	Maurer *et al.*, 1997
		pce1	*Phanerochaete chrysosporium*	Gaskell *et al.*, 1995
	hAT	*restless*	*Tolypocladium inflatum*	Kempken and Kück, 1996
		folyt	*F. oxysporum*	Gómez-Gómez *et al.*, 1999
		tfo1	*F. oxysporum*	Okuda *et al.*, 1998
		hornet1,2,3	*F. oxysporum*	Hua-Van *et al.*, 2000
		palm	*F. oxysporum*	Mouyna *et al.*, 1996
		crypt1	*Cryphonectria parasitica*	Linder-Basso *et al.*, 2001
		ascot	*Ascobolus immersus*	Colot and Rossignol, 1995
		tasco	*A. immersus*	Goyon *et al.*, 1996
		scooter	*Schizophyllum comune*	Fowler and Mitton, 2000
		abr1	*Agaricus bisporus*	Sonnenberg *et al.*, 1999
	Mutator	*hop*	*F. oxysporum*	Chalvet *et al.*, 2003
	MITE	*mimp*	*F. oxysporum*	Hua-van *et al.*, 2000
		guest	*N. crassa*	Yeadon and Catcheside, 1995

[1]Classification based in previous reviews of Daboussi, 1996; Kempken and Kück, 1998 and Daboussi and Capy, 2003. *S. commune*, *A. bisporus* and *P. chrysosporium* are basidiomycetes, all other species are ascomycetes.

II) Spontaneous inactivation of cloned genes. This is the most satisfactory strategy for identifying active transposons and is generally applied to genes whose mutant phenotype can be positively selected. This is the case of mutations in the nitrate reductase structural gene, which can be selected for resistance to chlorate (Cove, 1976ab; Cove, 1979). This gene is particularly appropriate because it can be selected for transposon integration or excision. The transposable elements of such mutants can be identified by PCR. This method is suitable for identifying elements with high excision and insertion frequencies (Kempken and Kück, 1998). Examples of transposons cloned by spontaneous mutation selection in the nitrate reductase gene include *fot1* in *F. oxysporum* (Daboussi *et al.*, 1992); *impala* in *F. oxysporum* (Langin *et al.*, 1995); *ant1* in *Aspergillus niger* (Glayzer *et al.*, 1995); *vader* in *A. niger* var. *awamori* (Amutan *et al.*, 1996); *flipper* in *Botrytis cinerea* (Levis *et al.*, 1997); *hupfer* in *Beauveria bassiana* (Maurer *et al.*, 1997); and *folyt1* in *F. oxysporum* (Gómez-Gómez *et al.*, 1999).

III) Construction of degenerated oligonucleotides of conserved domains of reverse transcriptase and transposases. A particularly useful method for identifying class I elements (reverse transcriptase method) and class II elements (transposase method). The advantage of this strategy is that it permits the rapid analysis of a large number of organisms (Kempken and Kück, 1998) as described for the isolation of the *yeti* transposon in *Podospora anserina* (Hamman *et al.*, 2000b).

IV) Use of heterologous probes in hybridization experiments. This method requires appropriate probes and only detects known transposons (Kempken and Kück, 1998). An example is the isolation of the *skippy* element of *F. oxysporum* by hybridization with the *cft1* element of *Cladosporium fulvum* (Anaya and Roncero, 1995).

Effects of Transposable Elements on Genes and Genomes

The main alterations caused by these elements include changes in gene expression due to insertion in, or adjacent to, the genes, which can create a new phenotype due to blocked transcription of associated genes or alteration in the transcription pattern. Transposable elements can also change the gene sequence due to the 'footprints' generated in the donor site on excision of the transposable element and chromosome rearrangements such as deletions, inversions and translocations. These rearrangements can occur especially if the elements are present in more than one copy, because they can generate sites of reciprocal recombination leading to alterations in the chromosome structure (Daboussi, 1996). Karyotypic instability has been investigated in species carriers of many families of transposons such as *F. oxysporum* and *M. grisea*. The analysis of the karyotypic instability showed a high level of chromosome length polymorphism with a high density of transposons and that the occurrence of chromosome rearrangements is associated with the clustering of transposable elements on the chromosomes (Davière *et al.*, 2001; Hua-van *et al.*, 2000; Nitta and Farman, 1997). These changes are reported as being genetically neutral, but can also lead to genetic combinations important for adaptation to new environments. All these changes have potential for influencing many aspects of the evolution of the fungal genome and should supply the flexibility for the populations to adapt successfully to environmental conditions.

Control of Transposable Element Activity

In spite of the abundance of transposable elements in the genome, most eukaryotic elements only move sporadically (Fedoroff, 2002). Regulatory pathways controlled by the host and transposons act on the regulation of the transposition. In animals and plants, transposon control has been shown at different levels, revealing that these elements are generally quiescent during growth and development, but can be activated by stress (Capy *et al.*, 2000; Grandbastien, 1998; Wessler, 1996). Little is known about the mechanisms that control the activity of transposable elements in fungi, although recent evidence shows that they can be activated by stress and silenced by epigenetic processes.

Transposition as a response to environmental stress was proposed as an adaptive response of the genome (McClintock, 1984). Several transposons in plants, yeasts and *Drosophila* show activity under conditions of abiotic (irradiation, temperature, oxidative stress) or biotic (tissue culture, infection by pathogens or protoplast isolation) stress (Capy *et al.*, 2000; Grandbastien, 1998; Wessler, 1996). Some of the factors that stimulate transposition have been tested on fungi, *e.g.* heat shock, copper sulfate and oxidative stress act on *maggy* retrotransposons in *M. grisea* (Ikeda *et al.*, 2001); gamma radiation increased the number of copies of the *SINE* element *foxy* in *F. oxysporum* (Mes *et al.*, 2000); and exposure to chlorate activated rearrangement and induced *skippy* retrotransposon amplification in *Fusarium* (Anaya and Roncero, 1996).

Several inactivation mechanisms of repeated sequences have been revealed in some species such as *N. crassa*, *Ascobolus immersus* and *M. grisea* (Cogoni, 2001; Faugeron, 2000; Selker, 1999; Ikeda *et al.*, 2002). A repeat-induced point mutation (RIP) in *A. immersus* inactivated native or foreign linked or non-linked duplicate sequences during a specific period of the sex cycle between fertilization and kariogamy. This inactivation was associated with the cytosine methylation of duplicated sequences. The RIP process results in many base pair C-G for A-T changes and is irreversible. The methylation-induced point mutation (MIP) process inactivates genes reversibly by cytosine methylation.

These genetic silencing mechanisms can be considered as defense strategies which control invader trans-

posons. Transposable elements are natural targets for such mechanisms, and silencing may prevent invasion of the genome, methylation suppression of recombination and also the rapid divergence caused by RIP, thus preventing the recombination among repeated sequences and protecting the genome against gross chromosome rearrangements. Consistent with this interpretation, only remaining of transposons have been detected in *N. crassa* and *A. immersus*. The RIP and MIP processes may not be common to all fungi but signs of the RIP processes in some species may reflect the occurrence of this process in an ancestral or cryptic sexual stage, or the existence of a similar process to RIP in vegetative cells (Daboussi and Capy, 2003).

Transposable Element Dynamics in the Genome

The phylogenetic distribution and analysis of transposable elements in the main fungus groups, Ascomycota, Basidiomycota and Zygomycota suggest that they are old components of the fungal genome transmitted vertically, although the possibility of horizontal transmission should not be discarded as has been reported in several studies. The sporadic distribution of some elements and the variation in copy number reflect competition among elements, elimination, self regulation and regulation by the host. These aspects, along with the extensive DNA polymorphism which often occurs, have been used to investigate population structure and epidemiology of fungal pathogenic strains.

The dynamics of fungal transposons have been extensively analyzed in *F. oxysporum*, the *fot1* element being present in most of *F. oxysporum* strains with a copy number varying from zero to more than 100. The phylogeny of this element indicates that it is an old component of the genome and transferred vertically. The high number of homogeneous copies for structure and sequences of nucleotides probably reflects a recent amplification from a master copy. Regarding the *F. oxysporum impala* element, highly divergent families with a constant number of copies coexist in the genome (Hua-van *et al.*, 1998). These facts indicate that transposons can be kept in the host genome by different strategies. The absence of copies in various strains of *F. oxysporum* is probably due to elimination by natural selection and/or genetic drift. Other factors, such as rearrangements and silencing mechanisms may be involved in transposon dynamics leading to their reduction or inactivation, although this may be counterbalanced by the introduction of new elements by horizontal transmission (Dobinson *et al.*, 1993; Daboussi *et al.*, 2002).

Transposable Elements as Genetic Tools: Gene Isolation and Analysis of Population Structure

Transposons act as insertional mutagens and genes altered in this way can be cloned as sequences that flank the transposon insertion sites and are part of the gene of interest (Daboussi, 1996). The *fot1* and *impala* elements in *F. oxysporum*, *restless* in *Tolypocladium inflatum*, and *maggy* in *M. grisea* are autonomous elements that have been used as gene traps in their natural hosts and tested for their transposition skill in heterologous species. The use of transposons for gene cloning can be exemplified by the cloning of a nitrate metabolism regulator gene in *Tolypocladium inflatum* (Kempken and Kück, 2000). A high proportion of mutant in *F. oxysporum* was recovered by *impala* transposition, showing the efficiency of transposition in pathogenicity mutant generation of the fungi (Migheli *et al.*, 2000).

The elements of the *Tc1/mariner* family, *fot1* and *impala*, have also been tested in different species. The *fot1* transposition was demonstrated in *A. nidulans* (Li Destri *et al.*, 2001) while the *impala* element is capable of transposition in several ascomycetes species, for example *F. moniliforme* (Hua-van *et al.*, 2001b), *M. grisea* (Villalba *et al.*, 2001), *A. nidulans* (Li Destri *et al.*, 2001), *A. fumigatus* (Firon *et al.*, 2003), *C. gloeosporioides* (Li Destri *et al.*, 2002), and *P. griseoroseum* (De Queiroz and Daboussi, 2003). The isolation of genes of interest, such as a gene involved in *M. grisea* pathogenicity (Villalba *et al.*, 2001), genes involved in *A. nidulans* development and metabolism (Brocard-Masson, 2001) and different genes essential for the growth of *A. fumigatus* (Firon *et al.*, 2003) support the development of insertional mutagenesis tools in filamentous fungi. Other elements have also shown activity in heterologous species, *e.g. maggy* in *Colletotrichum lagenarium* and *Pyricularia zingheri* (Nakayashiki *et al.*, 1999) and *restless* in *N. crassa* and *P. chrysogenum* (Windhofer *et al.*, 2002).

Transposons, in addition to use as tools for cloning genes of interest, have also been used as markers for detection of specific races of phytopathogenic fungi in infected plant tissues and in the study of population dynamics and evolution (Daboussi and Langin, 1994; Daboussi and Capy, 2003). From the epidemiological point of view, it is important to understand how the specific populations of determined hosts are organized and how they are altered. For this, the conservation and dispersion of transposable elements in these fungi have given important markers in the study of biology of pathogen populations in plants and animals.

Transposons have been used to distinguish genetically divergent populations because they can mark specific genotypes that have a common ancestor (Dobinson *et al.*, 1993; Giraud *et al.*, 1997; He *et al.*, 1996; Kachroo *et al.*, 1994; Mouyna *et al.*, 1996; Shull and Hamer, 1996; Zhu and Oudemans, 2000). In *F. oxysporum* f. sp. *Elaeidis* (an oil palm pathogen) the *palm* transposon was used to identify subpopulations of the pathogen, showing that the recent appearance of the disease in South America probably occurred by the introduction of an African isolate. This study also showed the presence of the *palm* element in all the

pathogenic isolates, and its absence in all the non-pathogenic isolates, indicating that populations may be marked by transposons (Mouyna *et al.*, 1996).

Diagnostic tools based on PCR were developed to detect pathogenic *F. oxysporum* races causing carnation wilt. This strategy is based on the genetic characterization of a collection of strains using different transposons and in the cloning and sequencing of regions that flank insertion sites of these elements. Those seemingly related to a specific race or pathogenic form are used to construct specific primers for fast pathogen identification (Chiocchetti *et al.*, 1999). Analysis of *Pyricularia grisea* populations using different transposable elements has led to the understanding of the evolution of host-specific forms, showing the clonal organization of *P. grisea* populations that infect rice and the possibility of new strains of the pathogen emerging as independent strains (Dobinson *et al.*, 1993; Kachroo *et al.*, 1994; Shull and Hamer, 1996).

Conclusions

Many types of transposable elements have been described in several fungi species, indicating that they are old components of their genomes. With the genome sequencing of different Ascomycota and Basidiomycota species, new transposons will continue to be discovered. Genomic analysis will be very useful for understanding the impact of transposons on the evolution of the fungal genome and also for the development of better diagnostic tools. The study of transposons in fungi has contributed to the understanding of important questions concerning their biology, such as genetic silencing and movement mechanisms. Another important point is the isolation of genes by the transposon trap strategy. New tools are being developed using transposon engineering. Furthermore, because many fungi (along with some algae) are coenocytic such organisms represent a unique environment for transposable elements and can contribute to the study of horizontal genetic transference processes in diverse species.

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