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 Aspergillus nidulans and Aspergillus nidulans 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...
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 element structures (LINEs) and short dispersed nuclear element structures (SINEs). Class II elements (DNA transposons) are flanked by two inverted terminal repetitions (ITRs) 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 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 (Cambarei 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 bio-type 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...
et al., 1994; Langin et al., 1994; Langin and Kistler, 2000; Hua-van et al., 2001; Deschamps et al., 1999; Migheli et al., 1999; Hua-van et al., 2000; Rosevitch et al., 2001ab; Villalba et al., 2001; Davière 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 chloride (Anaya and Roncero, 1996). The fot1 and impala elements have also been used to assess 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-

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

<table>
<thead>
<tr>
<th>Element class</th>
<th>Element group</th>
<th>Element</th>
<th>Host fungus</th>
<th>References</th>
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<tr>
<td>Class I transposons</td>
<td>LTR retrotransposon</td>
<td>foret</td>
<td>Fusarium oxysporum</td>
<td>Julien et al., 1992</td>
</tr>
<tr>
<td></td>
<td>Metaviridae (gypsy)</td>
<td>skippy</td>
<td>F. oxysporum</td>
<td>Anaya and Roncero, 1995</td>
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<tr>
<td></td>
<td></td>
<td>grh</td>
<td>Magnaporthe grisea</td>
<td>Dobinson et al., 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>muggy</td>
<td>M. grisea</td>
<td>Farman et al., 1996b</td>
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<td></td>
<td>mgl3</td>
<td>M. grisea</td>
<td>Kang, 2001</td>
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<td></td>
<td>cft-1</td>
<td>Cladosporium fulvum</td>
<td>MeHale et al., 1992</td>
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<tr>
<td></td>
<td></td>
<td>egret</td>
<td>Colletotrichum gloeosporioides</td>
<td>Zhu and Oudemans, 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>boty</td>
<td>Botrytis cinerea</td>
<td>Dolez et al., 1995</td>
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<td>real</td>
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<td>Kaneko et al., 2000</td>
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<td>dane 1, 2</td>
<td>Aspergillus nidulans</td>
<td>Nielsen et al., 2001</td>
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<td></td>
<td></td>
<td>afut</td>
<td>A. fumigatus</td>
<td>Neuvgüle et al., 1996</td>
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<td>mars4</td>
<td>Ascochauls immersus</td>
<td>Goyon et al., 1996</td>
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<td></td>
<td></td>
<td>dahl1</td>
<td>Neurospora crassa</td>
<td>Bibbins et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>yet1</td>
<td>Podospora anserina</td>
<td>Hamann et al., 2000b</td>
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<td></td>
<td></td>
<td>mary1</td>
<td>Tricholoma matsutake</td>
<td>Murata and Yamada, 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>prtl</td>
<td>Phycymye blakesleanus</td>
<td>Ruiz-Pérez et al., 1996</td>
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<tr>
<td>LTR retrotransposon</td>
<td>mars 2, 3</td>
<td>tcen</td>
<td>N. crassa</td>
<td>Cambareri et al., 1998</td>
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<td></td>
<td></td>
<td>nht2</td>
<td>Nectria haematococa</td>
<td>Shiflett et al., 2002</td>
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<tr>
<td>Pseudoviridae (copia)</td>
<td></td>
<td>tad</td>
<td>N. crassa</td>
<td>Kinsey and Helber, 1989</td>
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<td>non LTR retrotransposon (LINE)</td>
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<td>M. grisea</td>
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<td>M. grisea</td>
<td>Hame et al., 1989</td>
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<td>cg1</td>
<td>C. gloeosporioides</td>
<td>He et al., 1996</td>
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<td></td>
<td>mars1</td>
<td>A. immersus</td>
<td>Goyon et al., 1996</td>
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<tr>
<td></td>
<td></td>
<td>mary2</td>
<td>T. matsutake</td>
<td>Murata et al., 2001</td>
</tr>
<tr>
<td>non LTR retrotransposon (SINE)</td>
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<td>nrs1</td>
<td>N. haematococa</td>
<td>Kim et al., 1995</td>
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<td></td>
<td>foxy</td>
<td>F. oxysporum</td>
<td>Mes et al., 2000</td>
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<td></td>
<td>mgr1</td>
<td>M. grisea</td>
<td>Kachroo et al., 1995</td>
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<td></td>
<td>egr1</td>
<td>Erysiphe graminis</td>
<td>Wei et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>egh1</td>
<td>E. graminis</td>
<td>Rasmussen et al., 1993</td>
</tr>
</tbody>
</table>

1Classification based on reviews by Daboussi, 1996; Kempken and Kück, 1998 and Daboussi and Capy, 2003. T. matsutake is a basidiomycete and P. blakesleanus a zygomycete, the remaining species are ascomycetes.
pathogenic mutant were isolated and it was shown that by insertion of the \textit{impala} element a pathogenicity gene could be cloned and sequenced (ORP1) which is essential for the penetration of \textit{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 \textit{Tc1/mariner} superfamily are described in Table 2.

The \textit{hAT} superfamily was defined based on the similarity between the maize \textit{Ac} elements and the \textit{hobo} element of \textit{Drosophila}. This superfamily is well represented in fungi, and has been identified in both the Ascomycota and Basidiomycota (square 2). Elements belonging to the \textit{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 \textit{hop}, was identified in the \textit{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 \textit{F. oxysporum} \textit{mimp} elements (Hua-Van et al., 2000) and the \textit{guest} element of on \textit{N. crassa} (Yeadon and Catcheside, 1995). These elements are REMARKERS of non-autonomous DNA transposons and their mobilization depends on the transposase produced by other class II elements (Feschottes et al., 2002).

\section*{Identification Strategies for Transposable Elements}

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

1) 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 \textit{foret1} and \textit{palm} transposons of \textit{F. oxysporum} (Julien et al., 1992; Mouyna et al., 1996) and the \textit{cgf1} and \textit{cgret} elements of \textit{C. gloeosporioides} (He et al., 1996; Zhu and Oudemans, 2000).

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Element class & Element superfamily & Elements & Host fungus & References \\
\hline
Class II transposons & \textit{Tc1/Mariner} & \textit{fot1} & \textit{Fusarium oxysporum} & Daboussi et al., 1992 \\
(DNA mediated elements) & & \textit{impala} & \textit{F. oxysporum} & Langin et al., 1995 \\
& & \textit{fot2} & \textit{F. oxysporum} & Daboussi and Langin, 1994 \\
& & \textit{fot3, fot4} & \textit{Nectria haematococa} & Hua-van et al., 2000 \\
& & \textit{flipper} & \textit{Botrytis cinerea} & Levis et al., 1997 \\
& & \textit{tan1} & \textit{Aspergillus niger} & Nyyssonen et al., 1996 \\
& & \textit{vader} & \textit{A. niger} & Anutan et al., 1996 \\
& & \textit{an1} & \textit{A. niger} & Glayzer et al., 1995 \\
& & \textit{pot2} & \textit{Magnaporthe grisea} & Kachroo et al., 1994 \\
& & \textit{megr586} & \textit{M. grisea} & Farman et al., 1996a \\
& & \textit{fcl1} & \textit{Cochliobolus carbonum} & Panaccione et al., 1996 \\
& & \textit{nhl1} & \textit{Nectria haematococa} & Enkerli et al., 1997 \\
& & \textit{pat} & \textit{Podospora anserina} & Hamann et al., 2000a \\
& & \textit{hupfer} & \textit{Beauveria bassiana} & Maurer et al., 1997 \\
& & \textit{pccl} & \textit{Phanerochaete chrysosporium} & Gaskell et al., 1995 \\
& & \textit{hAT} & & \\
& & \textit{restless} & \textit{Toxophaladium inflatum} & Kempken and Kück, 1996 \\
& & \textit{foil} & \textit{F. oxysporum} & Gómez-Gómez et al., 1999 \\
& & \textit{fot1} & \textit{F. oxysporum} & Okuda et al., 1998 \\
& & \textit{hornet1,2,3} & \textit{F. oxysporum} & Hua-Van et al., 2000 \\
& & \textit{palm} & \textit{F. oxysporum} & Mouyna et al., 1996 \\
& & \textit{crypt1} & \textit{Cryphonectria parasitica} & Linder-Basso et al., 2001 \\
& & \textit{ascot} & \textit{Ascobolus immersus} & Colot and Rossignol, 1995 \\
& & \textit{tasco} & \textit{A. immersus} & Goyon et al., 1996 \\
& & \textit{scooter} & \textit{Schizophyllum commune} & Fowler and Mitton, 2000 \\
& & \textit{abrl} & \textit{Agaricus bisporus} & Sonnenberg et al., 1999 \\
& & \textit{Mutator} & & \\
& & \textit{hop} & \textit{F. oxysporum} & Chalvet et al., 2003 \\
& & \textit{MITE} & & \\
& & \textit{mimp} & \textit{F. oxysporum} & Hua-van et al., 2000 \\
& & \textit{guest} & \textit{N. crassa} & Yeadon and Catcheside, 1995 \\
\hline
\end{tabular}
\caption{General classification of fungal class II transposable elements.}
\end{table}

\footnote{Classification based in previous reviews of Daboussi, 1996; Kempken and Kück, 1998 and Daboussi and Capy, 2003. \textit{S. commune}, \textit{A. bisporus} and \textit{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 chloride (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* (Glazzer et al., 1995); vader in *A. niger* var. *awamori* (Amutan et al., 1996); flipper in *Botrytis cinerea* (Levis et al., 1997); hupper in * Beauveria bassiana* (Maurer et al., 1997); and fof1 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 yety 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, Ascomobatus immersus* and *M. grisea* (Cogoni, 2001; Faugerong, 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-
transposons. 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 (Miglioli 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 strategy. New tools are being developed using transposon engineering. Furthermore, because many fungi (along with some algae) are coenocytic such organisms represent a strategy.

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