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Transposition of autonomous and engineered *impala* transposons in *Fusarium oxysporum* and a related species

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Abstract The *impala* transposon of *Fusarium oxysporum* is an active element. We demonstrated that the *imp160* copy, transposed into the gene encoding nitrate reductase, is an autonomous element, since it excises from this gene and reinserts at a new genomic position in backgrounds free of active elements. An element in which the transposase gene was replaced by a hygromycin B resistance gene was used (1) to demonstrate the absence of endogenous transposase in several *F. oxysporum* strains and (2) to check the ability of different genomic copies of *impala* to transactivate this defective element. This two-component system allowed the identification of autonomous elements in two *impala* subfamilies and revealed that transactivation can occur between highly divergent elements. We also demonstrate that the autonomous copy transposes in a closely related species complex, *F. moniliforme*, in a fashion similar to that observed in *F. oxysporum*. The ability of *impala* to function as a two-component system and to transpose in a heterologous host promises further advances in our understanding of the factors that modulate transposition efficiency and demonstrates the potential of *impala* as a means of establishing a transposon tagging system for a wide range of fungal species.

Key words *Fusarium oxysporum* · *Tc1-mariner* transposon · Autonomous copies · Transactivation · Heterologous transposition

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Introduction

Transposons of the *Tc1-mariner* superfamily are ubiquitous in eukaryotic genomes. The wide distribution of this family, as well as some phylogenetic analyses, suggest that *Tc1-mariner* elements are ancient components of eukaryotic genomes and could have used horizontal transfer as a means of expansion (Robertson and Lampe 1995). This hypothesis is supported by the fact that the transposition process is largely independent of host-specific factors, as demonstrated by in vitro transposition assays that only required the presence of the transposase (Lampe et al. 1996; Vos et al. 1996, Tosi and Beverley 2000). Moreover, several recent reports indicate that these elements transpose in widely diverse species. *Mos1*, *Himar1*, *Tc1* or *Tc3* elements have been shown to function in a large number of hosts, including insects (Coates et al. 1997, 1998), fishes (Fadool et al. 1998; Raz et al. 1998), birds (Sherman et al. 1998), mammalian cells (Luo et al. 1998; Schouten et al. 1998; Zhang et al. 1998), protozoans (Gueiros-Filho and Beverley 1997) and even prokaryotes (Rubin et al. 1999). This family has therefore received considerable attention as a source of tools for transgenesis.

Impala, a transposable element identified in the phytopathogenic fungus *Fusarium oxysporum* (Langin et al. 1995), is a member of the *Tc1-mariner* superfamily. It is typically 1.3 kb long, with near-perfect 37-bp inverted terminal repeats (ITRs), and contains a single ORF potentially capable of encoding a 340-amino acid transposase (Hua-Van et al. 1998). Like all *Tc1-mariner* elements, it transposes by an excision-reinsertion mechanism and specifically inserts into a TA dinucleotide,

which is duplicated upon insertion (Daboussi and Langin 1994). The first *impala* element, *imp160*, was identified as an insertion (mutation *niaD160*) in the *niaD* gene of *Aspergillus nidulans* that had been introduced into strain FOM24 of *F. oxysporum* (Daboussi et al. 1989). The transposed copy *imp160* is able to excise from *niaD*, leading to the restoration of nitrate reductase activity. It frequently reinserts into new genomic positions (Daboussi 1996; Migheli et al. 2000). Strain FOM24 contains nine different copies of *impala* which form a polymorphic family comprising three subfamilies named E, D and F. Only subfamilies E and D contain full-length elements with an uninterrupted long ORF encoding a protein with intact functional domains which is potentially active (Hua-Van et al. 1998). The additional *imp160* copy within *niaD* was previously thought to be inactive because two frameshift mutations disrupted the ORF (Langin et al. 1995). However automatic sequencing revealed that these frameshifts were sequencing artifacts due to GC compression. *Imp160* is actually identical to the genomic *impE* copy and is likely to derive from a duplicative transposition of *impE* (Hua-Van et al. 1998).

Autonomy of *impala* elements was investigated using a phenotypic assay based on the restoration of nitrate reductase activity upon excision of the inserted *imp160* copy. Excision frequency is reflected by the number of colonies able to utilize nitrate that arise from plates spread with a fixed number of mutant spores. This assay was used to identify autonomous copies of another Class II element, *Fot1*, also found inserted within the *niaD* gene but at a different position (Migheli et al. 1999). In the present report, we confirm that *imp160* is able to transpose in a strain of *F. oxysporum* that is free of endogenous elements and can also move in the related *F. moniliforme* complex, and show that a defective element can be transactivated by three different *impala* elements.

Materials and methods

Fungal strains and growth conditions

F. oxysporum strains used in these assays were derived from the following strains, collected in the field and obtained from C. Alabouvette (INRA-Dijon, France). FOM24 and FOM150 are pathogenic to melon, FOL15 is pathogenic to tomato and the FO5 strain is not known to be pathogenic. FOM150 is closely related to FOM24 and contains all *impala* elements found in FOM24, except *impF*, as determined by Southern analysis. *NiaD160* is a nitrate reductase-deficient mutant derived from the transformant TR7 in strain FOM24, and resulted from the insertion of an *impala* copy into the *niaD* gene of *A. nidulans* (Langin et al. 1995). FOM150 *nia9*, FOL15 *nia50* and FO5 *nia13* are nitrate reductase-deficient mutants selected on the basis of resistance to chlorate, as described in Daboussi et al. (1989), and were used as recipient strains in transformation experiments. A *nia* mutant, S68, derived from a strain isolated from superficially sterilized seeds of an asymptomatic maize inbred line in Brazil, which has been assigned to the *Fusarium moniliforme* complex by Dr. D. M. Massa Lima (Department of Mycology, Universidade Federal de Pernambuco, Brazil), was used for heterologous transposition experiments.

Complete medium [potato dextrose agar (PDA)], selective minimal medium (MMN, containing nitrate as the sole nitrogen source) and growth conditions are described in Daboussi-Bareyre (1980).

Plasmids

pNI160 contains the *A. nidulans niaD* gene, interrupted by *imp160*. The *Eco*RI fragment containing *niaD::imp160*, cloned from the genome of the mutant *niaD160* (plasmid pIN160, Langin et al. 1995), was inserted in the *Eco*RI site of the plasmid p11ΔNdeI, which carries the wild-type *niaD* gene (see Fig. 1). pNIH was constructed from pNI160 by first subcloning in pUC18 a *Bam*HI-*Apa*I fragment extending from the *niaD* promoter to the beginning of the *niaD* gene, and including *imp160*. The resulting plasmid was then digested with *Xho*I and *Sty*I, removing a 1-kb fragment containing most of the transposase gene, and leaving only the first 136 and last 252 bp of *impala*. After end-filling with Klenow polymerase, the plasmid was ligated to a *Hpa*I fragment of pCB1004 (Carroll et al. 1994) containing the *E. coli hph* gene for hygromycin B phosphotransferase under the control of the *A. nidulans trpC* promoter. The resulting plasmid was then digested with *Bam*HI and *Apa*I, and the fragment containing the modified element was used to replace the *Bam*HI-*Apa*I fragment in pNI160, giving rise to the plasmid pNIH. Plasmids pImpA, pImpC, pImpD and pImpE correspond to FOM24 genomic fragments containing *impala* elements, cloned in pMLC28 (for pImpA and pImpE) or pUC19 (for pImpC and pImpD), using the enzyme *Eco*RI or *Sph*I (pImpE).

Transformation experiments

Transformation experiments were done according to Langin et al. (1990). pNI160 was co-transformed with plasmid pAN7.1 (Punt et al. 1987), which confers hygromycin B resistance. pNIH was

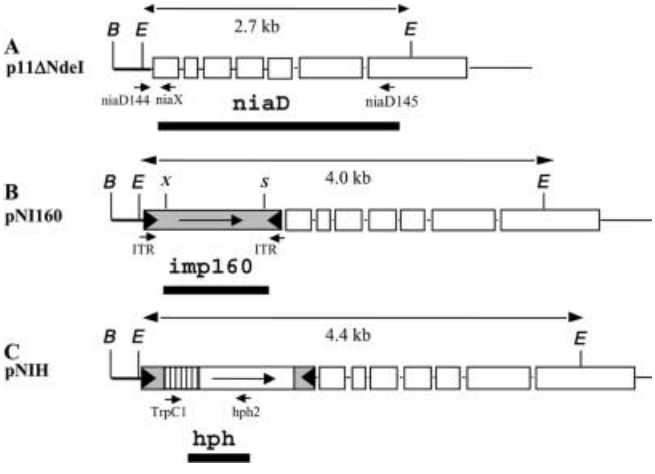

Fig. 1A–C Structures of the different constructs used. Plasmid p11Δ*Nde*I **A**, containing the wild-type *niaD* gene of *Aspergillus nidulans* (exons are represented as *open boxes* and the promoter as a *thick black line*) served as the basis for plasmids pNI160 **B** and pNIH **C**. The *shaded rectangle with black triangles* indicates the natural transposon *imp160*, inserted in the 5′ untranslated region of *niaD*. In pNIH, the modified transposon contains the coding sequence of the bacterial gene *hph* (hygromycin B resistance, *open rectangle with arrow*) driven by the *A. nidulans trpC* promoter (*striped rectangle*). The *arrows below* the maps indicate the primers used in this study. The fragments used as probes are marked by a *thick line below* each structure. In each case the size of the *Eco*RI fragment is given. E, *Eco*RI; B, *Bam*HI; Sp, *Sph*I. Only the *Xho*I (*X*) and *Sty*I (*S*) sites used to insert the *hph* gene are indicated

used either alone or with one of the plasmids containing *impala* elements from strain FOM24. Hygromycin B-resistant transformants were selected on nitrate minimal medium (MMN) containing glutamine and hygromycin B (Sigma, St Louis, Mo.). Co-transformants were identified by hybridization of their DNA with the central region of *impala* as a probe, according to Migheli et al. (1999).

Selection of revertants and determination of excision frequency

Spores of co-transformants were recovered, by washing and filtration, from a single-spore culture grown on solid medium, and were either spread at different dilutions on nitrate minimal agar, or inoculated into nitrate minimal broth (Migheli et al. 1999). Revertants appeared after several days of incubation at 26 °C. The excision level was estimated after 25 days by counting the number of isolated aerial mycelia on plates inoculated with 10^3 spores. For revertants obtained in liquid medium, the frequency of reversion could not be estimated, since each tube could contain one or several independent revertants. For molecular analysis, one revertant was isolated from each tube in order to compare independent events. The reinsertion frequency was calculated as the number of revertants containing an *impala* copy in a new genomic position/total number of revertants analyzed ×100.

Rapid PCR screening for excision events

One-half of a mycelium culture of the revertant, grown in a 55-mm petri dish, was placed in 500 µl of lysis buffer (0.2% SDS, 50 mM EDTA), incubated at 65 °C for 10–30 min and then centrifuged for 15 min. DNA was precipitated from the supernatant by adding an equal volume of isopropanol. The DNA pellet was washed with 70% ethanol and resuspended in 50 µl of TE (10 mM TRIS-HCl pH 8, 1 mM EDTA). Aliquots (2 µl) were taken for PCR amplification of excision sites using the primers niaD144 (5′-GTTCATGCCGTGGTCGCTGCG-3′) and niaX (5′-ATCTAG-ACT- TAGGCCTCGG-3′), which anneal to sequences on either side of the *impala* copy. PCR was done in a 100-µl volume, using each primer at 0.8 µM and each dNTP at 100 µM, with 1 U of Taq polymerase. The amplification program was 1 min at 94 °C, 30 s at 60 °C and 30 s at 72 °C for 30 cycles.

DNA preparation and Southern analysis

Genomic DNA was prepared using a standard miniprep extraction method (Langin et al. 1990). An aliquot (10 µg) of genomic DNA was digested with *Eco*RI, fractionated in a 0.8% agarose gel and transferred to a nylon membrane (Amersham). Membranes were probed first with a *niaD* probe, then stripped and hybridized with *impala* or *hph* probes. Probes were obtained by ^{32}P labelling (Pharmacia T7 random priming kit) of 50 ng of DNA template produced by PCR with the primers ITR (5′-CAGTGGGGGGCAAAAAG-TAT-3′) for *impala*, trpC1 (5′-CTTCTTAAGTTCGCCCTTCC-3′) and hph2 (5′-GCCATCGGTCCAGACGGCCGC- GC-3′) for *hph*, and niaD144 and niaD145 (see Migheli et al. 1999) for *niaD*.

Amplification of reinsertion sites by inverse PCR

Reinsertion sites were cloned using inverse PCR. A 500-ng aliquot of total genomic DNA of a given revertant was digested with a restriction enzyme, then purified by phenol/chloroform extraction and precipitated with ethanol. The DNA was self-circularized using the Amersham DNA Ligation System. Ligated DNA was then purified as above and resuspended in 20 µl of distilled water. A 10-µl portion was used in PCR experiments with the primers Div3 (5′-GTTTCGGCTAGCCTCGATCG-3′) and Div5 (5′-GAT-CGGGTTAGGCCGGACCG-3′), which are nested within the element and directed towards flanking regions. The amplification program utilized was as described by Warren et al. (1994).

DNA sequencing

Sequencing was performed with a 373 Applied Biosystems automatic sequencer using either the Dye Terminator ABI Prism sequencing kit (Applied Biosystems, Foster City, Calif.), or manually using the Amersham Sequenase PCR Product system. Primers were the same as those used for PCR.

Results

Identification of an autonomous copy of *impala*

Transposition of the *imp160* copy inserted within the leader sequence of the *niaD* gene 10 bp upstream of the initiator ATG (Langin et al. 1995, see Fig. 1) was demonstrated using a phenotypic assay for excision. Excision of *imp160* restores the activity of the *niaD* gene, thus allowing the fungal strain to utilize nitrate. Excision events were easily visualized on nitrate minimal medium as patches of aerial mycelium (revertants) in a background of sparse mycelium (mutants). To determine whether *imp160* was an autonomous element, or was excised through the action of a transposase coded by other genomic copies, we used the strain FO5, which was shown to lack endogenous *impala* elements by Southern and PCR analyses. A *nia* mutant of this strain, FO5 *nia13*, was selected according to Daboussi et al. (1989), and used as the recipient strain for transformation. Plasmid pNI160 containing the *niaD* gene interrupted by *imp160* (Fig. 1B) was introduced by genetic transformation, together with the plasmid pAN7.1 (Punt et al. 1987), which confers hygromycin B resistance. Hygromycin B-resistant transformants were then screened for the presence of pNI160 to select co-transformants containing intact *niaD::imp160* constructs. In the same way, we also transformed another strain, FOL15 *nia50*, which is pathogenic to tomato and contains several *impala* elements, as revealed by a Southern analysis (not shown). Four of these elements were cloned by PCR and appeared to be inactivated by deletions and frameshifts (A. Hua-Van, T. Langin and M-J Daboussi, unpublished results).

In both genetic backgrounds, we isolated several co-transformants that were able to revert to nitrate utilization. Molecular analysis was done by Southern hybridization (Fig. 2A) or using a rapid PCR-based screen (Fig. 2B). Using the *niaD* gene as a probe, the presence of a 2.7-kb *Eco*RI fragment in most revertants was confirmed, indicating that they resulted from element excision. A larger number of revertants was analyzed by PCR using primers flanking the element, and showed a PCR product of the size expected for an empty insertion site.

To exclude the possibility that excision was promoted by a transposase supplied by an endogenous element that might have been too divergent to be detected in our control experiments (as has been reported for the *hobo* element; Atkinson et al. 1993; Handler and Gomez 1995), we constructed a defective copy of *impala*. The transposase gene of *imp160* inserted within the *niaD*

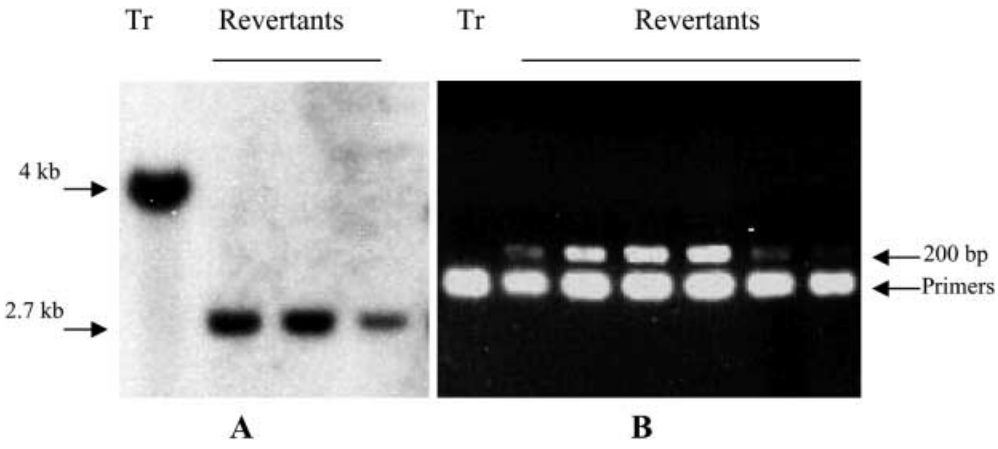

Fig. 2A, B Molecular analysis of excision events in revertants. **A** Southern analysis of genomic DNA from one pNI160 transformant (Tr) and three revertants, digested with *Eco*RI and hybridized with a *niaD* probe. **B** PCR analysis using primers (niaD144 and niaDX) in *niaD*, allowing amplification of a 200-bp fragment corresponding to the excision site (empty site)

gene was replaced by the *hph* gene, which confers resistance to hygromycin. This copy, *imp160::hph*, carried by the plasmid pNIH (Fig. 1C), was introduced into the *F. oxysporum* strains FO5 *nia13* and FOL15 *nia50*. In both genetic backgrounds we were able to isolate several co-transformants, but no revertants resulting from excision could be isolated, even after several weeks of culture on nitrate minimal medium (see below). In contrast, this defective, marked element was able to excise and reinsert in strain FOM150 *nia9*, which contains active *impala* elements (data not shown).

Transactivation of a defective *impala* element by genomic copies of intact elements

Strain FOM24 harbors four full-length *impala* elements in its genome, *impA*, *impC*, *impD* and *impE*, each potentially encoding a active transposase (Hua-Van et al. 1998). Sequence analysis revealed that the putative product of each of these contained all domains known to be required for transposition in other *Tc1-mariner* elements – the nuclear localization signal, the putative dimerization domain, the catalytic domain known as the DDE domain, and the potential α helices corresponding to the DNA binding domain (Hua-Van et al. 1998). Three elements, *impA*, *impC* and *impE* (subfamily E, to which *imp160* also belongs), encode very similar transposases differing by only two or three amino acids. These transposases differ from the predicted *impD* transposase (subfamily D) by 37 or 38 amino acid substitutions, 17 of which are conservative.

The defective *imp160::hph* element was introduced by co-transformation into the FO5 *nia13* strain, together with either *impA*, *impE*, *impC* or *impD*. Molecular analysis indicated that the co-transformants most often contained only one copy of pNIH and one copy of the plasmid carrying the transposase. The two co-transformants obtained with *impA* were unable to revert. It was later determined that integration in the genome had disrupted the *niaD* sequences in pNIH, precluding selection for excision by phenotype (data not shown). More transformants carrying *impA* must be obtained in order to measure the transposase activity of this copy. For each combination with pImpE, pImpC, or pImpD, 3–4 co-transformants giving rise to revertants were recovered.

A reversion assay based on the observation of reversion events on MMN plates seeded with 10^3 transformant spores 25 days after inoculation revealed different levels of reversion. While the *niaD160* mutant (used as a positive control) reproducibly gave a high level of reversion (>100 revertants/plate), the transformants obtained using either *impE* (4) or *impC* (4) as the source of transposase gave rise to 15–30 revertants/plate. Two transformants containing the divergent copy, *impD*, produced few revertants (1–3/plate), at a level similar to the transformant used as a negative control (FO5 transformant containing plasmid pNIH but no transposase source). A third one yielded a larger number of Nia^+ colonies (>50/plate), but molecular analysis revealed that these reversions did not result from excision of *imp::hph* (see below).

For each of these transformants several revertants were analyzed either by Southern hybridization or by PCR as described above. Most of the revertants (Fig. 3A, probe *niaD)* exhibited the expected 2.7-kb wild-type *niaD* fragment. PCR analysis of a larger number of revertants (about 90) confirmed *impala* excision in most revertants derived from *impC*- and *impE*- containing co-transformants, and in more than 50% of revertants recovered from the two *impD* co-transformants showing a low reversion level. In contrast, we did not obtain any evidence of *imp::hph* excision in revertants originating from the *impD* co-transformant which was characterized by a higher level of reversion. The ability of these latter revertants to utilize nitrate probably resulted from complex rearrangements triggered by the integration of two *impala* elements less than 5 kb from each other in inverse orientation. These phenotypic revertants are not considered further here, and a detailed analysis of them will be described in a later report (A. Hua-Van, T. Langin and M.J. Daboussi, in preparation). Finally, a PCR experiment on revertants from the strain used as a negative control confirmed that reversions were not due to excision but probably to reversion of the

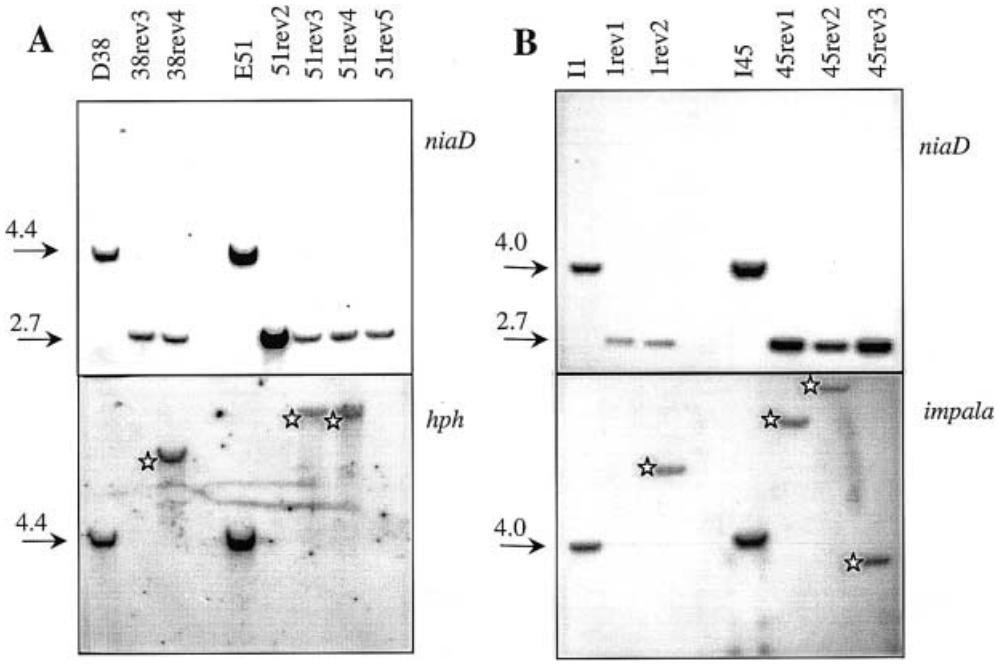

Fig. 3A, B Southern analysis of transformants and corresponding revertants. In all Southern blots, DNA was digested with *Eco*RI. The *stars* indicate reinsertion of excised copies. **A** Transformants of the *F. oxysporum* strain FO5 *nia13* containing pNIH and pImpD (D38) or pNIH and pImpE (E51), and revertants, hybridized with a *niaD* (*upper panel*) and a *hph* (*lower panel*) probe. The locations of the different probes are indicated in Fig. 1. **B** *F. moniliforme* transformants containing plasmid pNI160 (I1 and I45) and revertants, hybridized with a *niaD* (*upper panel*) and an *impala* (*lower panel*) probe

endogenous *F. oxysporum nia* gene. Hence, excision events were only observed when a transposase source (*impE*, *impC* or *impD*) was present.

Excision and reinsertion site analysis

For each condition, several excision sites were amplified by PCR and sequenced (Fig. 4A). We never observed the restoration of the wild-type sequence of *niaD* gene, but a footprint of 5 additional nucleotides (nt) was generally present, two corresponding to the duplicated target site TA and three to one of the ends of the element. These footprints are similar to those observed for *imp160* excision in mutant *niaD160* (Langin et al. 1995), and resemble footprints of other *Tc1-mariner*-like elements (Eide and Anderson 1988; Coates et al. 1995; Arcà et al. 1997). Other types of alterations, such as a 4-nt footprint, 1-bp deletion, or 1-bp substitution, were also observed, although less frequently.

The reinsertion of *impala* was checked using a probe corresponding to the whole element (for pNI160 transformants) or to the *hph* gene (for pNIH co-transformants, see Fig. 3A, bottom panel). A new hybridizing band corresponding to a reinserted element was observed in thirteen of the twenty-four pNI160 revertants and three out of twelve pNIH revertants. Due to the small number of revertants analyzed, the reinsertion frequencies (54% and 25%, respectively) are not significantly different from that previously estimated for revertants from mutant *niaD160* (about 75%–25/34; Migheli et al. 2000). One reinsertion site was cloned by inverse PCR from both a FOL15 and a FO5 revertant. The sequences of these sites confirmed that they represented new insertion sites. In both cases, *impala* was reinserted into a TA dinucleotide, as expected for a *mariner*-like element (Fig. 4A).

Heterologous transposition

The ability of *impala* to excise and reinsert in other *Fusarium* species was assayed in the closely related species complex *F. moniliforme*. The strain used as a transformation recipient appears to be devoid of *impala* elements, as revealed by Southern blot and PCR analysis under relaxed stringency conditions. The plasmid pNI160 was co-introduced with pAN7.1 into the *nia* mutant S68. Several spontaneously reverting co-transformants were isolated as described before. PCR experiments and Southern analysis showed that *impala* had excised and reinserted. Two examples of transformants and revertants are shown in Fig. 3B. Reinsertion of the excised copy was observed in 80% of the revertants (9 among 11 revertants analyzed).

Excision sites of some revertants were cloned and sequenced as before. They contained a footprint very similar to that observed in *F. oxysporum* (Fig. 4B), composed of 5 nt, of which two nucleotides represent the target site duplication and the other three are derived from one end of the element.

Discussion

The goal of this study was to identify autonomous *impala* elements in order to develop a two-element transposon tagging system. For this purpose, we used different versions of the *impala* element in different genetic backgrounds. We designed a two-component system

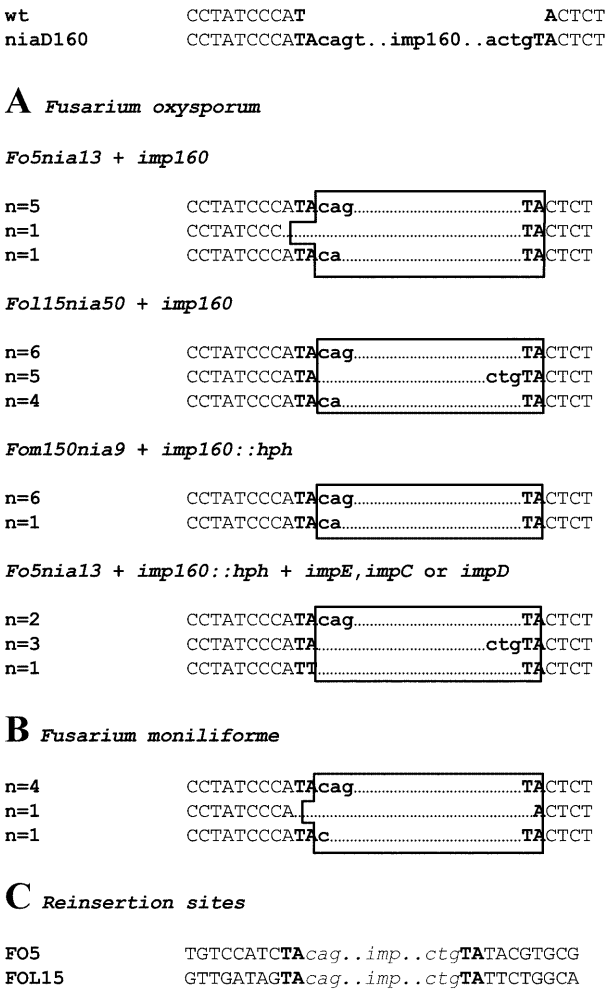

Fig. 4A, B Structure of impala excision sites in *F. oxysporum* revertants **A** and in *F. moniliforme* revertants **B**. Flanking sequences of two reinserted impala elements. Sequences of the wild-type gene (wt) and the original insertion mutant (*niaD160*) are given at the *top*. n = number of revertants with the indicated sequence. *Bold capitals:* duplicated target site TA. *Bold lower cases:* impala ends. Nucleotide insertion, relative to the wild type, are *framed*. **C**. *Sequences* of two reinsertion sites. *impala* sequences are in italics and the duplicated target sites are in *bold*

involving an active *impala* element and a defective element carrying a dominant selectable marker to facilitate monitoring of excision and to check for reinsertion of the excised copy. Moreover, we have shown that *impala* is able to transpose in a heterologous related species.

Autonomous elements that belong to different subfamilies coexist and transactivate

Imp160, the first *impala* copy to be identified, by virtue of its transposition into the *niaD* gene, can excise and reinsert in two strains that are devoid of endogenous elements. The defective element, *imp::hph*, in which most of the transposase gene has been substituted by the dominant marker *hph*, is unable to transpose in these genetic backgrounds, whereas it does move in a genetic background containing a source of transposase. This indicates that all *cis*-acting elements required for excision and insertion (recognition and binding sites) are present in the modified copy, and therefore are located in subterminal regions of *impala* (first 136 bp and last 252 bp). These assays led to the following conclusions: (1) neither of the two strains contains an endogenous transposase source; (2) the endogeneous *impala* elements in FOL15 are all inactive; (3) *imp160* is an autonomous element; and (4) the defective element can be complemented in trans by genomic elements.

The *imp::hph* element was used to demonstrate the activity of genomic *impala* elements belonging to subfamilies E and D. We have shown that among the members of subfamily E (including *imp160*), *impE* and *impC* encode an active transposase. The *impE* transposase is identical to that of *imp160* and differs from *impC* transposase at two positions (T290A and M305I; Hua-Van et al. 1998). These changes do not appear to affect the level of excision. The activity of *impA* element, also from the E subfamily, could not be determined because the *niaD* gene was disrupted during insertion into the genome of the two transformants analyzed, thus preventing selection of any revertants. However we suspect that this copy is active as well, since its transposase contains only two amino acid changes relative to the *impE* transposase (I35F and A247G), both located outside the critical domains.

Surprisingly, the *impD* transposase, which differs by 37–38 amino acids from transposases of the E subfamily, also transactivated *imp160::hph*. Transactivation between distantly related elements has been described for *P* elements of *Scaptomyza pallida* and *Drosophila melanogaster* (Simonelig and Anxolabéhère 1991), and the *D. melanogaster hobo* element can be transactivated by endogenous elements in *Musca domestica* (Atkinson et al. 1993). In our study, transactivation between E and D elements was found to be rare. This could result from the particular integration sites used in the few transformants analyzed. It is also quite plausible that the level of nucleotide divergence (more than 20% between *impD* and *impE*), particularly in the ITRs and subterminal regions (Hua-Van et al. 1998), could affect the efficiency of the recognition and binding processes required for a complete excision event.

The lower level of excision in FO5 transformants bearing a single active copy, compared to a genetic background such as FOM24, which contains at least three active elements, could be due to a lower transposase concentration, related to the number of copies, or to enhanced transcription in the region of the genomic *imp160* insertion in FOM24.

The *imp160* element is able to transpose in a heterologous host

Excision and reinsertion of *imp160* have been demonstrated in a strain of the *F. moniliforme* species complex,

which is closely related to *F. oxysporum* (Guadet et al. 1989). In the two co-transformants analyzed, the reinsertion frequency (up to 80%) appears to be in the same range as that observed in strain *niaD160*. The similarity of excision footprints reflects the use of the same excision process, dependent on the function of the transposase and also the same excision site repair mechanism, which reflects the model of direct ligation and repair proposed for *Tc1-mariner* elements (Arcà et al. 1997).

Tc1-mariner elements are active in a wide variety of organisms (Gueiros-Filho and Beverley 1997; Ivics et al. 1997; Fadool et al. 1998; Luo et al. 1998; Raz et al. 1998; Schouten et al. 1998; Sherman et al. 1998; Zhang et al. 1998; Rubin et al. 1999). The presence of *Tc1-mariner*-like elements in several filamentous fungi (Glayzer et al. 1995; Daboussi 1996; Maurer et al. 1997) suggests that their ability to transpose is not very dependent on host factors. Therefore, we assume that *impala* might be active in a broad range of species. The availability of *nia* mutants in many fungi (Daboussi et al. 1989) provides a means of directly assaying for *impala* transposition by screening for a simple change in phenotype. Confirmation of *impala* activity in a broad range of hosts could have tremendous practical significance, since it would permit the development of gene tagging in many fungi. To date transposition of fungal elements in heterologous species has been demonstrated only for the MAGGY retroelement of *Pyricularia grisea* (Nakayashiki et al. 1999).

Development of a two-component transposon tagging system

Gene tagging by a transposon has recently been achieved in fungi with the autonomous *Restless* element of *Tolypocladium inflatum* (Kempken and Kück 2000). The efficiency of *impala* transposition in generating mutants affected in pathogenicity suggests that *impala* also has great potential as a tagging system (Migheli et al. 2000). However, a potential disadvantage of using an autonomous element for gene tagging is the instability of the resulting insertions. Mutations can be stabilized easily in a two-element system composed of a non-autonomous element, which lacks its own transposase but is capable of transposition, and a transposase source (Bryan et al. 1990; Sundaresan 1996). Transposition of the defective element *imp::hph* catalyzed by different sources of transposase provides the basis of a two-element tagging system. Excision of *imp::hph* can be monitored by assaying the expression of the *niaD* gene, which confers the ability to grow on nitrate as the sole nitrogen source. Reinsertion of excised elements can be selected through the hygromycin B resistance of $NiaD^+$ revertants. This allows the recovery of many revertants, although transposition is less frequent than in the original mutant *niaD160*. To make the *impala* element a practical alternative for gene tagging, it will be necessary to improve the transposition frequency. The identification of *cis*-acting regulatory motifs would facilitate the development of more active elements. Manipulation of transposase expression may also permit us to increase the frequency of transposition.

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