Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1929–1933, March 1989
Genetics

Isolation of a transposable element from *Neurospora crassa*

(*am* gene/glutamate dehydrogenase/spontaneous mutation)

JOHN A. KINSEY AND JENNIFER HELBER

Department of Microbiology, Molecular Genetics, and Immunology, University of Kansas Medical School, Kansas City, KS 66103

Communicated by David D. Perkins, December 8, 1988 (received for review September 27, 1988)

ABSTRACT **A *Neurospora crassa* strain from Adiopodoumé, Ivory Coast, contains multiple copies of a transposable element, Tad. The element was detected as a 7-kilobase insertion in two independently isolated spontaneous forward mutants of the *am* (glutamate dehydrogenase) gene. Laboratory strains do not contain Tad. All progeny from crosses of the Adiopodoumé strain to laboratory strains contain multiple copies. When the element was inserted in *am*, target sequences of 14 and 17 base pairs were duplicated in the two cases analyzed. One mutation, caused by the insertion of Tad at the beginning of the *am* coding sequence, is genetically stable. The other mutation, caused by insertion upstream of the transcriptional start site, has a reversion frequency of 2.5×10^{-3}. Precise excisions of Tad have not been found.**

Insertion of transposable elements constitutes a major source of spontaneous mutation in a variety of organisms ranging from prokaryotes to yeasts and higher eukaryotes (1–7). In filamentous fungi, there have been reports of several genetically defined elements in *Ascobolus* that appear to represent classical transposons (8–12); however, none of these has been characterized at the molecular level. In *Neurospora*, two elements that insert in the mitochondrial genome have been described and characterized at the molecular level (13–15). These elements do not appear to act as transposons in the nucleus. Recently, an element, *pogo*, has been described that is associated with a telomere of linkage group V of *Neurospora crassa* (16). It was suggested that it is a transposable element based upon the facts that multiple copies exist in the genome and that these copies are found in different locations in different strains.

We have looked for transposable elements associated with spontaneous mutation at the *am* locus of *Neurospora*. The *am* gene was chosen for this kind of analysis because the gene has been cloned and well characterized at the molecular level (17, 18) and there is a simple direct selection procedure for *am* mutants (19). Our initial experiments were done with standard laboratory strains; however, out of 80 spontaneous mutants analyzed, none appeared to be due to a transposable element (D. Sherman, B. Cheung, and J.A.K., unpublished data). As an alternative approach we decided to look among strains collected from nature in the hope that active transposable elements might be present in these undomesticated strains. David Perkins (personal communication) suggested that there might be an interesting element in Adiopodoumé, a *N. crassa* strain collected from the Ivory Coast. Newmeyer and Galeazzi (20) had observed that there was some element in the genetic background of Adiopodoumé that has a profound effect on the nature of breakdown products from the unstable nontandem duplication *Dp(IL→IR)H4250*. Perkins (personal communication) had also observed that translocations were probably more frequent in crosses involving Adiopodoumé than in crosses involving only standard strains. Below we describe the isolation of a 7-kilobase (kb) transposable element from the Adiopodoumé strain.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Strains, Media, and Mutant Selection. Strains were either from our laboratory stock collection or from the Fungal Genetics Stock Center (FGSC), Department of Microbiology, University of Kansas Medical Center, Kansas City. The Adiopodoumé strain (FGSC no. 430) was isolated from leaves collected near the biological research station at Adiopodoumé, Ivory Coast, in 1955. It was deposited in the FGSC in 1960 and originally given the designation North Africa I.

Standard *Neurospora* media were used (21). Supplementation of minimal medium with glycine (1.5 mg/ml) was used to inhibit the leaky growth of *am* mutants. The direct selection procedure was used for the isolation of spontaneous *am* mutants (19).

DNA Blots and Hybridization. One microgram of each digested genomic DNA was electrophoresed in a 0.7% agarose gel in Tris acetate buffer at 15–20 V for 16–18 hr. The DNA was transferred from the gel to GeneScreen*Plus* (NEN) by capillary transfer, hybridized (1% $NaDodSO_4$/1 M NaCl/10% dextran sulfate containing 100 μg of salmon sperm DNA per ml; 65°C), and rinsed (1% $NaDodSO_4$/15 mM NaCl/1.5 mM sodium citrate, pH 7.0; 25°C) as recommended by the manufacturer. Hybridization probes were labeled with [α-^{32}P]dCTP by the method of Feinberg and Vogelstein (22).

Library Construction. Genomic DNA, digested to completion with *Bam*HI, was ligated to *Bam*HI-digested λ L47 DNA (23). The ligation mix was packaged *in vitro* by commercial packaging extracts (Gigapack Gold, Stratagene). Recombinant phage were selected on *Escherichia coli* strain Q359 (23). The resulting lysates were plated on strain Q358 (5000 plaque-forming units per plate). Plaques that contained *am* sequences were identified by using the jr-2 fragment as a probe (see Fig. 1).

RESULTS

Isolation of Spontaneous Mutants in Adiopodoumé Hybrid Strains. The procedure for selection of *am* forward mutants requires a strain with the *lys-1* marker. The Adiopodoumé strain (FGSC 430) was therefore crossed to J852, a laboratory strain that has the genetic constitution *lys-1 ure-2; cot-1; rec-3 a*. From among the progeny of this cross, nine independent *lys-1* strains were subjected to selection of spontaneous *am* mutants as described (19). Table 1 summarizes the results of these individual *am* selection experiments. A total of 33 *am* mutants were isolated, with an average mutant frequency of 4.3×10^{-7}. This value is similar to the average mutant frequency of 2.5×10^{-7} obtained with more extensive studies carried out with various laboratory strains that do not contain Tad (D. Sherman, B. Cheung, and J.A.K., unpublished data). This indicates that there is no obvious mutator activity for the *am* gene in Adiopodoumé-derived strains.

Table 1. Frequency of *am* mutations in small cultures derived from individual *lys-1* ascospores from the cross of Adiopodoumé to J852 (*lys-1 ure-2; cot-1; rec-3 a*)

Spore	Conidia plated, no. × 10^{-6}	*am* colonies, no.	*am* frequency × 10^{7}
1	4.5	3	7
2	1.5	0	—
3	5.1	5	10
5	3.6	1	3
6	14	8	6
8	26	3	1
10	3.3	1	3
12	7.5	4	5
16	11	8	7

To detect mutations that might have been caused by insertion of transposable elements, DNA samples from these 33 mutant strains were screened by means of genomic Southern analysis for restriction fragment size alterations at the *am* locus. Fig. 1 *Upper* shows the *am* locus with a restriction map of the surrounding region. Genomic DNA from the mutants was digested with a variety of enzymes, and Southern blots were probed with fragments from around the *am* locus. Only four mutants showed detectable alterations at the *am* locus. Fig. 1 *Lower* shows a *Pst* I digest of genomic DNA from these mutants and the strains from which they were derived. The Southern blot was probed with the labeled jr-1 9.1-kb *Hin*dIII fragment (Fig. 1 *Upper*). This probe hybridizes to the 5.8-kb *am*-containing fragment as well as to the 4.5-kb downstream *Pst* I fragment. The *am*(s16-1) mutant (lane a) shows no size alteration under these conditions; however, *Bam*HI digestion shows the 2.7-kb *am*-containing *Bam*HI fragment to be fused with the upstream *Bam*HI fragment (data not shown). The *Pst* I digest shown here, as well as digests employing other enzymes (data not shown), indicates no major alteration in other restriction fragment sizes. This suggests that the upstream *Bam*HI site has been either lost or altered in *am*(s16-1). The *am*(s5-1) mutation (lane d) involves a deletion of 0.6 kb including part of the *am* coding sequences. It was derived from spore 5 (lane e). The other two mutant strains, *am*(s3-2) (lane f) and *am*(s8-2) (lane c), were derived respectively from spore 3 (lane g) and spore 8 (lane g). Each has a mutation caused by the insertion of a 7-kb element (Tad) into the *am* gene. We will refer to *am*(s3-2) and *am*(s8-2) as 3-2 and 8-2, respectively.

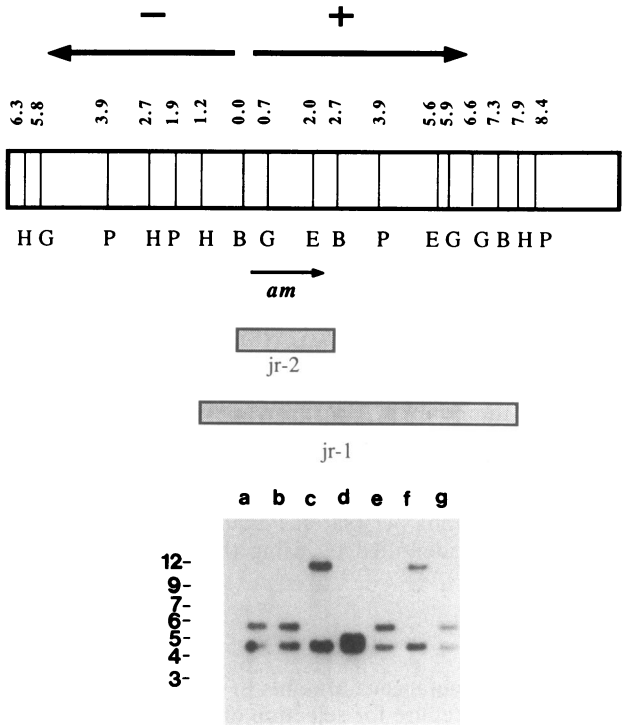

FIG. 1. (*Upper*) Restriction map of the region of linkage group V surrounding the *am* locus. The direction of transcription and the length of the transcript are indicated by the arrow below the map. The *Bam*HI site upstream of the *am* gene is arbitrarily given a coordinate designation of 0.0 kb and other sites are measured upstream (−) or downstream (+) from this site. Stippled bars below the map show fragments (jr-1 and jr-2) used as probes in this study. H, *Hin*dIII; G, *Bgl* II; P, *Pst* I; B, *Bam*HI; E, *Eco*RI. (*Lower*) Southern blot of genomic DNA from spontaneous mutants and the strains from which they were derived. The DNAs were digested with *Pst* I and probed with ^{32}P-labeled jr-1 fragment. Fragment size standards, in kilobases, are shown at left. Lanes: a, *am*(s16); b, spore 8 [from which *am*(s8-2) was derived]; c, *am*(s8-2); d, *am*(s5-1); e, spore 5 [from which *am*(s5-1) was derived]; f, *am*(s3-2); g, spore 3 [from which *am*(s3-2) was derived].

Cloning of the Tad Elements from Mutants 3-2 and 8-2. Genomic libraries were constructed with DNA from both the 3-2 and the 8-2 mutant strains. Preliminary Southern analysis indicated that *Bam*HI does not cut within the Tad element; thus the *Bam*HI fragment that contains the *am* gene is increased in size to 9.7 kb in the two mutants. A complete *Bam*HI digest of each genomic DNA was ligated into the λ replacement vector L-47 (23). From each library 50,000 plaque-forming units were screened using labeled jr-2 fragment as probe. The 3-2 library gave six positive clones and the 8-2 library yielded eight. Several positive clones were purified from each library, and the 9.7-kb insert of each was subcloned into the pBS KS^{+} plasmid cloning vector (catalog no. 212207; Stratagene). Restriction mapping of the subclones indicated that all of the primary clones from a given library were identical.

Fig. 2 shows a restriction map of the 9.7-kb fragment cloned from the 3-2 mutant. As expected from the Southern analysis, it contains a 7-kb element inserted into the *am* gene. The element from the 8-2 mutant has been mapped less extensively. However, the broad internal structure appears to be very similar. In particular, the positions of the internal *Eco*RI sites are conserved. We carried out sequence analysis at the ends of each of the elements to determine their locations precisely in the *am* gene and to determine whether there had been any target sequence duplication associated with the insertions. Fig. 3 shows the result of this analysis in schematic fashion. The 3-2 mutation involves the insertion of a Tad element 68 bp upstream of the major transcriptional start site at coordinate position 248. This places the element just upstream of the possible promoter element TATAAA. The 3-2 mutation resulted in a 17-bp duplication of target sequence. The 8-2 mutation involves the insertion of a Tad element at the beginning of the coding sequences in such a

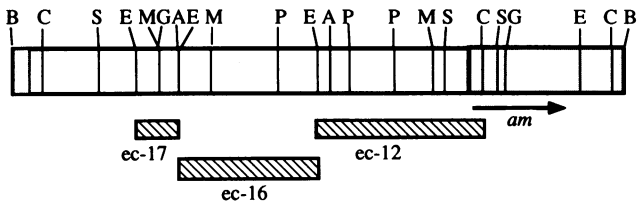

FIG. 2. Restriction map of the 9.7-kb *Bam*HI fragment cloned from strain J1511 [*am*(s3-2)]. The stippled region represents the normal sequence from this region of linkage group V. Hatched bars below the map show various fragments used as probes in this study. B, *Bam*HI; C, *Cla* I; S, *Sst* II; E, *Eco*RI; M, *Sma* I; G, *Bgl* II; A, *Sac* I; K, *Kpn* I; P, *Pvu* II.

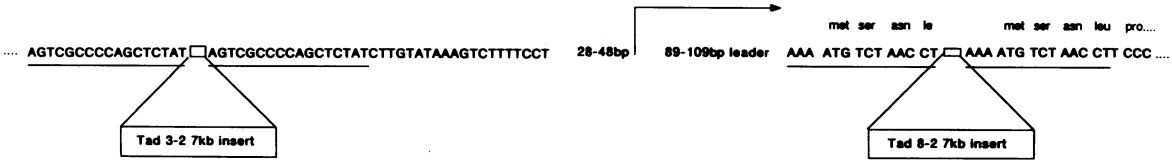

FIG. 3. Location of the Tad 3-2 and Tad 8-2 inserts in the *am* gene. The sequence shown here begins at +0.163 kb (see Fig. 1 *Upper*). Underlined sequences are present once in normal sequence but are duplicated in the mutant strains. For simplicity, both insertions are shown on one map although each mutation involves only one of the two insertions. The bent arrow indicates the transcriptional start site and direction of transcription (taken from ref. 18). Transcription of the *am* gene has a number of start sites (commonly referred to as stuttering), which span about 20 base pairs (bp) (18). The indicated 28- to 48-bp spacing between the first block of sequence and the bent arrow reflects this stuttering. For the same reason the length of the untranslated segment upstream of the second block of sequence is also variable as indicated.

position that the start codon has been duplicated as part of an overall 14-bp duplication of target sequences.

Tad Is Not Found in Standard Laboratory Strains. Since Tad appears to be a transposable element, it was of interest to determine whether it is present in multiple copies in Adiopodoumé and laboratory strains. Fig. 4 shows the results of genomic Southern analysis of DNAs from three strains digested with *Eco*RI and probed with the cloned Tad 3-2 fragment. It is clear that Adiopodoumé (lane a) and a hybrid derived from it (lane b) have multiple copies of Tad. All hybrid strains tested have patterns similar to the hybrid shown, although the absolute pattern of bands is different from hybrid to hybrid. There is a pronounced band at 2.1 kb, which corresponds in size to the larger internal *Eco*RI fragment found in both Tad 3-2 and Tad 8-2. This suggests that many of the copies of Tad have similar internal structure to that of Tad 3-2 and 8-2. In contrast to the Adiopodoumé-derived strains, the Oak Ridge strain (lane c) appears to have no copies of Tad. A variety of other laboratory strains of either Oak Ridge, Emerson, or mixed genetic backgrounds (15 strains in all) have been examined and all were found to lack Tad elements, although under conditions of reduced hybridization stringency it is possible to detect some distantly related sequences in most strains tested. In addition, 328 other strains collected from nature have been examined, and most of them appear not to contain Tad elements. A few strains have been found to have Tad-related sequences in low copy number.

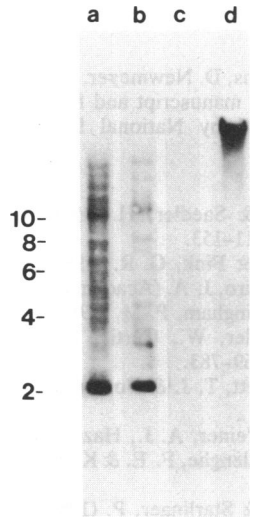

FIG. 4. Southern blot of genomic DNA from three strains. DNA was either digested with *Eco*RI (lanes a–c) or undigested (lane d). The blot was probed with the 9.7-kb *Bam*HI fragment cloned from strain J1518 [*am*(s3-2)]. Lanes: a and d, Adiopodoumé (FGSC 430); b, strain J1568 [an *am*(s3-2) hybrid strain derived by twice backcrossing the original *am*(s3-2) mutant strain to OR8-1a]; c, laboratory wild type 74-OR23-1VA (FGSC 2489).

To determine whether the Adiopodoumé strain contains any extrachromosomal Tad elements, undigested Adiopodoumé DNA was probed with the cloned 9.7-kb *Bam*HI fragment from *am*(s3-2) (Fig. 4, lane d). It is clear that all Tad sequences are present in high molecular weight DNA. There was no evidence of bands corresponding to either linear or covalently closed circular 7-kb DNA, even when the autoradiogram was greatly overexposed. This suggests that Tad elements are normally resident in chromosomal DNA, although it does not rule out the possibility of a transient extrachromosomal existence.

Tad Elements Are Not Found in Mitochondrial DNA. Since a variety of unique elements are known to be associated with mitochondria in *Neurospora*, it was of interest to determine whether the Tad element was mitochondrial in origin. Taylor *et al.* (24) have examined mitochondrial DNA from a variety of strains collected from nature. The Adiopodoumé strain was included in this survey. Examination of their restriction mapping data suggested that no complete Tad elements could be resident in the mitochondrial genome of Adiopodoumé. To determine whether there were any Tad-related sequences present in the mitochondrial DNA of Adiopodoumé, we digested a sample, kindly supplied by John Taylor, with *Eco*RI and subjected the digest to Southern analysis next to an *Eco*RI digest of genomic Adiopodoumé DNA. A cloned Tad element was used as probe. No Tad sequences were found in the mitochondrial DNA (data not shown).

Segregation of Tad Elements in Crosses. To follow the segregation of Tad sequences, we prepared DNA from members of several tetrads isolated from a cross of a standard laboratory wild-type strain (OR8-1a) to a strain (J1646) containing the 3-2 mutation. The DNAs were digested with *Eco*RI and subjected to Southern analysis. These Southern blots were sequentially probed with the 9.1-kb *am* fragment (jr-1), the internal 2.1-kb *Eco*RI fragment from Tad 3-2 (ec-16), and a 2.5-kb *Eco*RI–*Cla* I fragment from the right end of Tad 3-2 (ec-12). The blots were stripped between hybridizations with different probes. The results for a typical tetrad along with the 3-2 parent are shown in Fig. 5 (DNA from only one of each pair of sister spores is shown).

When the jr-1 (*am*) fragment was used as a probe each tetrad showed the bands expected if *am*, without (20.5 and 3.6 kb) and with (3.6 and 4.1 kb, not well resolved in this gel) the Tad insert, were segregating in a typical 2:2 fashion (Fig. 5*A*), indicating normal segregation for the copy of Tad present at the *am* locus. The 9.5-kb band, visible in all lanes, is a downstream *Eco*RI fragment. When the ec-16 probe was used, a single 2.1-kb band of similar intensity in all lanes was predominant (Fig. 5*B*). This corresponds to the expected band size for Tad 3-2 and 8-2 and again suggests that most of the multiple copies of Tad have similar internal structures at least with respect to the *Eco*RI sites. Similar results were obtained when the internal 0.5-kb *Eco*RI fragment, ec-17, was used as probe (data not shown).

The ec-12 fragment should hybridize to *Eco*RI fragments from one end of each copy of Tad. The size of each

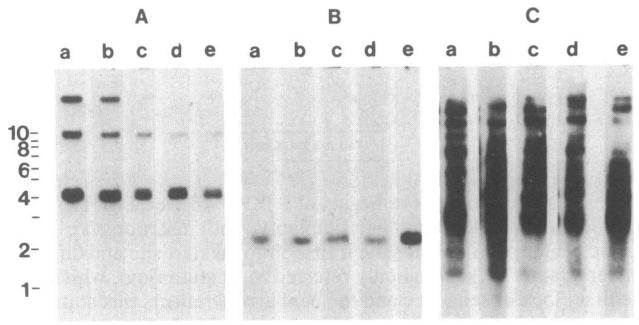

FIG. 5. Southern blot of genomic DNA from members of a tetrad isolated from a cross of laboratory wild-type strain OR8-1a (FGSC 2490) to strain J1646 [*am*(s3-2) *ure-2 A*]. DNA was digested with *Eco*RI and probed sequentially with ^{32}P-labeled jr-1 (*A*), ec-16 (*B*), and ec-12 (*C*). See Figs. 1 and 2 for descriptions of probe fragments. The lanes in all panels are the same. Lanes a–d contain DNA from tetrad members; lane e contains DNA from the *am* parent. Lanes: a, strain J1566 (*am*$^+$ *A*); b, strain 1565 (*am*$^+$ *a*); c, strain J1564 (*am ure-2 a*); d, strain J1562 (*am ure-2 A*); e, parental strain J1646 (*am ure-2 a*).

end-fragment will depend upon the location of the nearest *Eco*RI site at the particular chromosomal location of each element. When the ec-12 fragment was used as probe each strain showed multiple bands, as expected if all progeny had received multiple copies of Tad (Fig. 5*C*).

The 3-2 Mutation Is Very Unstable. It is well known that many mutations caused by transposable elements are unstable. Therefore the 3-2 and 8-2 mutant strains were screened for spontaneous revertability. We have screened $>10^8$ conidia from the 8-2 strain without detecting any revertants. Thus the 8-2 mutation appears to be quite stable. In contrast, the 3-2 mutation is very unstable, reverting to prototrophy with a frequency of $\approx 2.5 \times 10^{-3}$. The 3-2 mutant strain was crossed to a standard laboratory wild-type strain, and *am* progeny from this cross were examined for reversion. Virtually all of the *am* progeny were themselves unstable with a frequency similar to that of the *am* parent. However, an occasional *am* progeny was stable with respect to *am* phenotype, even though the Tad element was still present in the same position (data not shown).

DNA isolated from 12 revertants of the unstable 3-2 mutation was examined by means of genomic Southern blots. None involved a precise excision of the Tad element (data not shown). As an apparent consequence of this, none of the revertants had wild-type levels of glutamate dehydrogenase. We have examined the "revertants" for stability and found that some of the revertants are stable, whereas others are highly unstable, giving *am* conidia with a frequency similar to the reversion frequency of the 3-2 mutation. The explanation for this is not known.

DISCUSSION

We have shown that a transposable element, Tad, is present in the *N. crassa* strain Adiopodoumé and in hybrids derived from it but is not present in commonly used laboratory strains. The presence of multiple copies of a transposable element might be expected to indicate a mutator function. However, despite the fact that Adiopodoumé and its derived hybrids have multiple copies of Tad, the spontaneous mutant frequency for *am* is not significantly greater in these strains than in strains without Tad. The *am* mutations reported here occurred in cells dividing by mitosis. Due to the infertility of *lys-1* × *lys-1* crosses, it is not possible to select for *am* mutations that occur during meiosis. Thus there remains the possibility that Tad could have a mutator function during the meiotic process.

Tad elements from two independently isolated *am* mutants were cloned. We have shown that each cloned element is 7 kb long. As judged by their restriction maps, the two have very similar internal structure. Each of the insertion events that created the two mutations involved the duplication of target sequence as is characteristic for mutations caused by transposable elements. The target-site duplication was rather long in both cases, but the absolute length of the duplication was different for the two events (14 bp versus 17 bp). Preliminary sequence analysis of the Tad elements (unpublished data) suggests that there are neither short inverted repeats nor long direct repeats at the ends of Tad elements. Certain features of Tad, such as the relatively large size, the apparent lack of terminal repeats, and the variable size of target-sequence duplication accompanying insertion, are reminiscent of the I elements of *Drosophila* (25) and the LINES elements of mammals (26). Both of these elements are thought to be retrotransposons. However, we have no direct evidence to indicate that the Tad elements are retrotransposons.

The presence of multiple Tad elements in Adiopodoumé and hybrids derived from it suggests a possible explanation for some of the observations of Newmeyer and Galeazzi (20). They observed that some factor(s) in the Adiopodoumé genetic background caused breakdown of *Dp(IL→IR)H4250* by apparently random breaks, rather than the precise removal of duplicated sequence commonly seen with standard strains. A possible explanation for this observation is that recombination occurred between dispersed Tad elements, as has been observed for Ty elements in yeast (27). The boundaries of lost segments would then presumably depend upon the alignment of Tad elements at various locations within the duplicated sequences rather than the precise alignment of the entire duplication. Similarly the higher frequency of spontaneous chromosomal aberrations seen by Perkins (personal communication) in crosses involving Adiopodoumé might involve ectopic pairing and recombination between Tad elements at different chromosomal sites.

To our knowledge, the Tad elements represent the first active nuclear transposons isolated from filamentous fungi. It will be of considerable interest to determine whether related elements exist in other fungi or whether the Tad elements can function in related genera. Tad elements may prove useful either as cloning vectors or for transposon tagging of genes to be cloned.

We thank D. Perkins, D. Newmeyer, D. Stadler, and E. Selker for critically reading the manuscript and for helpful suggestions. This work was supported by National Institutes of Health Grant GM23967.

1. Starlinger, P. & Saedler, H. (1976) *Curr. Top. Microbiol. Immunol.* **75,** 111–153.
2. Roeder, G. S. & Fink, G. R. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 299–328.
3. Zachar, Z. & Bingham, P. M. (1982) *Cell* **30,** 529–541.
4. Cote, B., Bender, W., Curtis, D. & Chovnick, A. (1986) *Genetics* **112,** 769–783.
5. Kidd, S., Lockett, T. J. & Young, M. W. (1983) *Cell* **31,** 421–433.
6. Scott, M. P., Weiner, A. J., Hazelrigg, T. I., Polisky, B. A., Pirrotta, V., Scalenghe, F. E. & Kaufman, T. C. (1983) *Cell* **34,** 763–776.
7. Döring, H. P. & Starlinger, P. (1986) *Annu. Rev. Genet.* **20,** 175–200.
8. Decaris, B., Francou, F., Lefort, C. & Rizet, G. (1978) *Mol. Gen. Genet.* **162,** 69–81.
9. Decaris, B., Lefort, C., Francou, F. & Rizet, G. (1979) *Mol. Gen. Genet.* **170,** 191–202.
10. Rizet, G., Lefort, C., Decaris, B., Francou, F. & Kouassi, A. (1979) *Mol. Gen. Genet.* **175,** 293–303.
11. Decaris, B. (1981) *Mol. Gen. Genet.* **184,** 434–439.

12. Nicolas, A., Hamza, H., Mekki-Berrada, A., Kalogeropoulos, A. & Rossignol, J. L. (1987) *Genetics* **116,** 33–43.
13. Akins, R. A., Kelly, R. L. & Lambowitz, A. M. (1986) *Cell* **47,** 505–516.
14. Bertrand, H., Griffiths, A. J. F., Court, D. A. & Cheng, C. K. (1986) *Cell* **47,** 829–837.
15. Bertrand, H. (1986) in *Extrachromosomal Elements in Lower Eukaryotes*, eds. Wickner, R. B., Hinnebusch, A., Lambowitz, A. M., Gunsalus, I. C. & Hollaender, A. (Plenum, New York), pp. 93–102.
16. Schechtman, M. G. (1987) *Mol. Cell. Biol.* **7,** 3168–3177.
17. Kinnaird, J. H., Keighren, M. A., Kinsey, J. A., Eaton, M. & Fincham, J. R. S. (1982) *Gene* **20,** 287–396.
18. Kinnaird, J. H. & Fincham, J. R. S. (1983) *Gene* **26,** 253–260.
19. Kinsey, J. A. (1977) *J. Bacteriol.* **132,** 751–756.
20. Newmeyer, D. & Galeazzi, D. R. (1977) *Genetics* **85,** 461–487.
21. Davis, R. H. & de Serres, F. J. (1970) *Methods Enzymol.* **17,** 79–143.
22. Feinberg, A. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
23. Loenen, W. A. M. & Brammar, W. J. (1980) *Gene* **10,** 249–259.
24. Taylor, J. W., Smolich, B. D. & May, G. (1986) *Evolution* **40,** 716–739.
25. Fawcett, D. H., Lister, C. K., Kellett, E. & Finnegan, D. J. (1986) *Cell* **47,** 1007–1015.
26. Singer, M. F. & Skowronski, J. (1985) *Trends Biochem. Sci.* **10,** 119–122.
27. Roeder, G. S. & Fink, G. R. (1980) *Cell* **21,** 239–249.