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Behavior of a *Drosophila melanogaster* Transposable Element in *Saccharomyces cerevisiae*

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The *Drosophila melanogaster* transposable element 412 is transiently unstable in *Saccharomyces cerevisiae* when present on a freely replicating plasmid. The 412 element undergoes recombination to form two circular molecules, a 412 deletion plasmid and, presumably, a 412 circle. The 412 deletion plasmid contains a single long terminal repeat which most likely is the result of homologous recombination within the long terminal repeats. This recombination occurs at or shortly after transformation and is independent of both the *RAD52* gene product and the *F1p* gene of 2 μ m DNA.

Transposable genetic elements have been discovered in both procaryotic and eucaryotic organisms (reviewed in references 5 and 11) and may be a general feature of the genome. The 412 transposable elements of *Drosophila melanogaster* are copia-like elements (5) and consist of a central region of DNA of about 6.5 kilobases (kb) flanked by identical direct repeat sequences or long terminal repeats (LTRs) of 481 or 571 base pairs (16). These elements are structurally similar to the endogenous proviruses in the genomes of chickens and mice and contain many if not all the signal sequences necessary for an abbreviated viral life cycle (16). Transposition of 412 elements could occur by reverse transcription of full-length 412 RNAs to form circular DNA molecules which might integrate into the genome. A 412 element could excise from the genome by reciprocal recom-

his3 Δ 1 leu2-3, 112 trp1-289 ura3-52 [cir⁺]) was transformed (1) with plasmid pDY740 containing a 10.5-kb *Bam*HI fragment of *D. melanogaster* DNA, including a complete 412 element (Fig. 1). Transformants were examined for 412 integration into the genome. As the 412 element lacks *Sal*I and *Bam*HI endonuclease restriction sites, integration of the 412 element into the yeast genome will generate novel (nonplasmid) restriction fragments larger than the 412 element. DNA was isolated from transformed cells (14), digested in the appropriate buffer (4) with either *Sal*I or *Bam*HI, and electrophoresed on a 0.8% agarose Tris-borate (0.089 M Tris base-0.089 boric acid-2.5 mM EDTA) gel. Southern transfer was onto nitrocellulose (13), and DNA probes were ³²P-labeled by nick translation (9). In Fig. 2a and b, blots were probed with ³²P-labeled pOR708, a pBR322

TABLE 1. Plasmid rearrangements in different yeast strains

Strain	Genotype	No. of plasmids			Total ^b
		Transforming plasmid	Deletion form	Other ^a	
pDY740 transformed					
DBY747	[cir ⁻] <i>RAD52</i>	56	55	1	112 (4)
LL20A	[cir ⁰] <i>RAD52</i>	45	47	3	95 (4)
XS95.6c	[cir ⁺] <i>rad52</i>	39	28	1	68 (6)
pAB732 transformed					
DBY747	[cir ⁺] <i>RAD52</i>	97	1	7	105 (4)

^a Other rearranged plasmids included intermolecular recombinants with 2 μ m DNA.

^b Number of transformed lines examined is shown in parentheses; total is the summation of lines examined.

bination between its LTRs. This would leave one LTR in the chromosome and generate a circular molecule with the other LTR.

We have examined the behavior of 412 in *Saccharomyces cerevisiae* as a possible means of investigating the mechanism of 412 transposition. *S. cerevisiae* DBY747 (a *ste*⁺

derivative containing the same 10.5-kb *Bam*HI fragment as in pDY740. The *Sal*I digest was also probed with *LEU2* sequences as a genomic reference. The single-copy *LEU2* sequence was detected as a 2.6-kb band, and the plasmid-borne 412 element was present in an 8.7-kb *Sal*I fragment and a 10.5-kb *Bam*HI fragment. The faint 10.5-kb band in the *Sal*I digestion is due to partial digestion of the 10.5-kb deletion plasmid (see below). Two novel fragments were also detected, a 1.7-kb *Sal*I fragment and a 3.5-kb *Bam*HI fragment. With the exception of the plasmid-derived 8.7-kb *Sal*I

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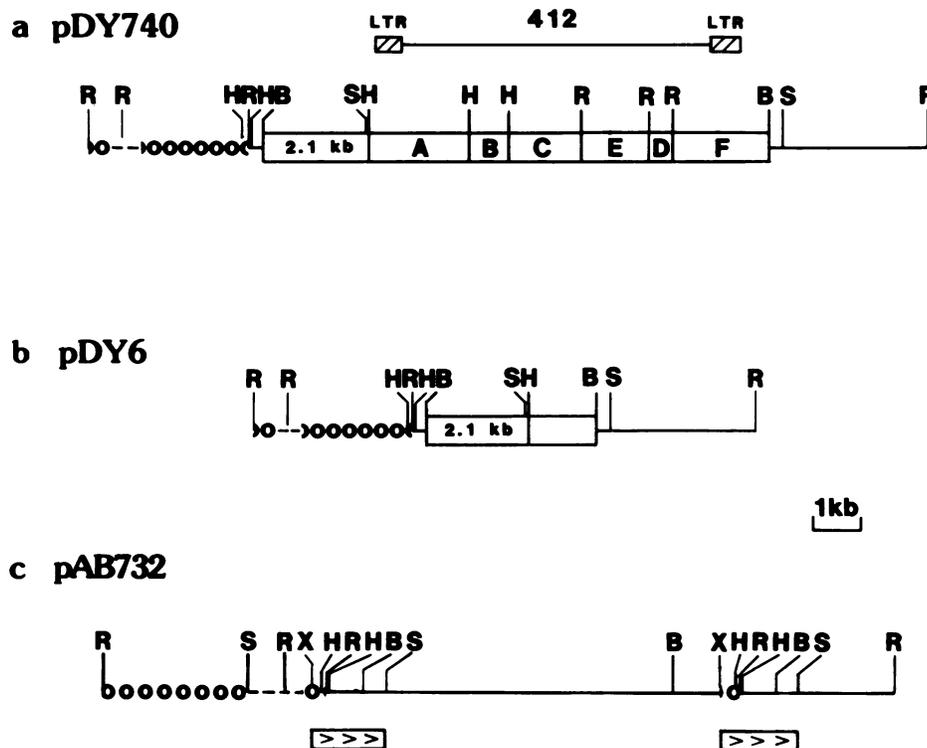


FIG. 1. Restriction map of *E. coli*-yeast shuttle plasmids. (a) Plasmid pDY740 was derived from the yeast shuttle vector pJDB207 (1). It contains an intact *412* element embedded in *D. melanogaster* chromosomal DNA. Fragments A through F contain portions of the *412* element and are defined by *Hind*III, *Eco*RI, and *Bam*HI restriction endonuclease sites. The 2.1-kb *Bam*HI-*Hind*III to the left of the *412* element consists solely of *D. melanogaster* chromosomal DNA. The cloned *D. melanogaster* DNA is contained on a single 10.5-kb *Bam*HI fragment. (b) Plasmid pDY6 was derived from pDY740 by *in vivo* deletion of the *412* element. A single *412* LTR remains embedded in *D. melanogaster* chromosomal DNA. (c) Plasmid pAB732 was a kind gift from F. Stahl. It contains a 7.6-kb insert containing a portion of *lacZ* of *E. coli* flanked by direct repeats of a 1.3-kb sequence made up of the small *Eco*RI-*Bam*HI fragment of pBR322 and a portion of 2- μ m DNA. Open box, *D. melanogaster* DNA; solid line, pAT153 sequence; open circle, 2- μ m DNA; broken line, *LEU2* structural gene; open box with arrows, duplicated sequence. B, *Bam*HI; H, *Hind*III; R, *Eco*RI; S, *Sall*.

and 10.5-kb *Bam*HI fragments, there were no bands greater than 7.5 kb which bore homology to *412*. We conclude that there is no detectable transposition of the intact *412* element into the yeast genome.

The novel *Sall* and *Bam*HI fragments were smaller than a *412* element, and the intensity of hybridization was greater than that of the single-copy *LEU2* gene. These data suggest that the novel fragments were present in multiple copies and that they did not originate from a simple transposition of an intact *412* element. Southern blots containing *Bam*HI-digested genomic DNA were probed with the pBR322 derivative pAT153 and an internal *412* fragment (fragment C in Fig. 1a). There was no hybridization of either probe to the 3.5-kb *Bam*HI fragment (Fig. 2c). The pAT153 probe hybridized to the 6.9-kb vector fragment from pDY740, while an internal *412* sequence hybridized to the original 10.5-kb insert. The size of the novel *Bam*HI fragment and its lack of hybridization with these two probes suggested that it was derived from the 10.5-kb *D. melanogaster* *Bam*HI fragment by the loss of the *412* element. The *Bam*HI fragment was 0.5 kb longer than expected if the complete *412* element had been excised ($10.5 - 7.5 = 3.0$ kb). This discrepancy may be due to the presence of a single LTR (0.5 kb) within the 3.5-kb fragment. The size of the novel *Sall* fragment (1.7 kb) was also consistent with the loss of the entire *412* element except for a single LTR.

These results predicted the presence of a smaller plasmid

(10.5 kb) in the transformed cells. DNA from transformed yeast was used to transform *Escherichia coli* HB101 (2) to ampicillin resistance on plates with 50 μ g of L broth per ml. The major plasmids isolated were the transforming plasmid (pDY740) and smaller 10.5-kb plasmids. The smaller plasmids were identical and the same as pDY740 except for a deletion in the 10.5-kb *D. melanogaster* *Bam*HI fragment (Fig. 1). We have studied one deletion plasmid, pDY6, to determine more precisely the *D. melanogaster* sequences it contained. In Southern transfer experiments with pDY6 as a probe, no hybridization to the internal *412* fragments was detected (Fig. 3), thus confirming the loss of most of the internal *412* sequences.

The *Hind*III-*Bam*HI fragment containing the *412* deletion was subcloned into pAT153 to determine whether an LTR was present and detect any further rearrangements. We compared the resulting plasmid, pDK353, with the A and F fragments of pOR708 (Fig. 4). The results of restriction and heteroduplex analysis (data not shown) indicate that this *Hind*III-*Bam*HI fragment is a fusion of A and F fragments and contains a single LTR. This suggests that the *412* deletion was the result of homologous recombination between LTRs.

In the Southern blots of DNA from newly transformed yeast, the intensity of hybridization to the 3.5-kb *Bam*HI fragment was similar to that of the 10.5-kb *Bam*HI fragment (Fig. 2b), suggesting that deletion plasmids were present in

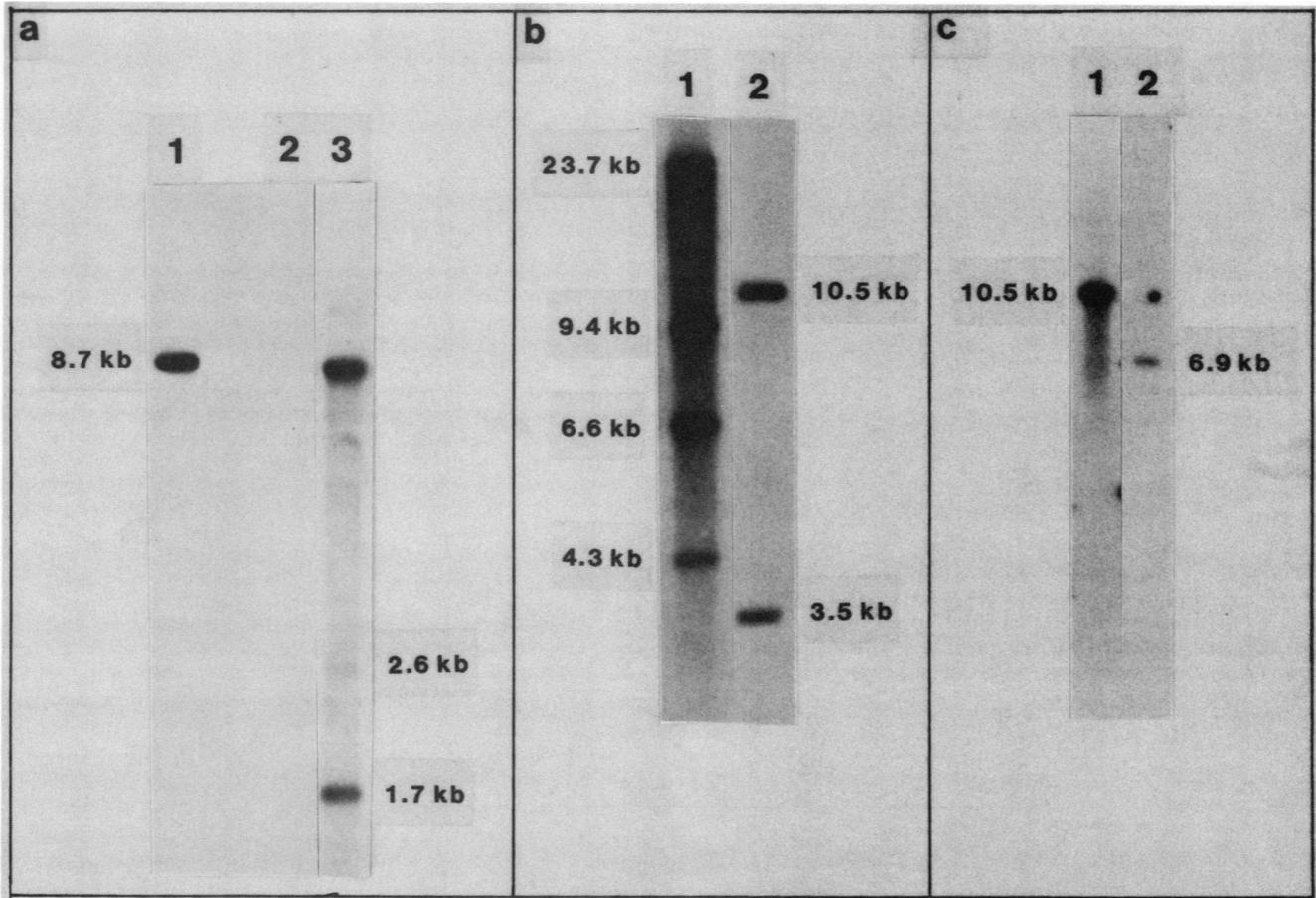


FIG. 2. Southern blot analysis of the yeast strain DBY747 transformed with the *412*-containing plasmid pDY740. (a) Southern blot of DNA isolated from DBY747 transformed lines probed with the *412* 10.5-kb *Bam*HI fragment and *LEU2*-containing fragment. DNA was digested with restriction enzyme *Sall*. Lanes: 1, plasmid pDY740; 2, yeast genomic DNA (5 µg) from DBY747; 3, genomic DNA (5 µg) from DBY747 transformed with pDY740. (b) Southern blot of DNA from DBY747 transformed with pDY740. Yeast DNA (5 µg) was digested with *Bam*HI and probed with the 10.5-kb *Bam*HI fragment. Lanes: 1, lambda phage DNA digested with *Hind*III; 2, DBY747 transformed with pDY740. (c) Southern blot of DNA isolated from DBY747 transformed with pDY740. DNA was digested with *Bam*HI and probed with the *412* fragment (lane 1) and pAT153 (lane 2).

the transformants in approximately equal amounts of pDY740. We have determined the proportion of deletion plasmids by isolating total yeast DNA from four newly transformed individuals and using it to transform *E. coli* HB101 to ampicillin resistance. Plasmids were extracted from 20 to 30 *E. coli* transformants for each yeast DNA isolation and individually sized by electrophoresis on 0.8% agarose gels. Representatives from each size class were further examined by restriction mapping. The proportion of deleted plasmids in the four individual transformants ranged from 42 to 64%; of 112 plasmids, 55 were deleted (Table 1). In contrast, control plasmid pAB732, which contains a 7.6-kb insert flanked by direct repeats of 1.3 kb (Fig. 1c), had only 1% (1 of 105) of the plasmids deleted by recombination within the direct repeats after transformation into DBY747 (Table 1). The *412* results were surprising since intermolecular (15) and intramolecular (7) recombination of plasmids in yeast rarely occurs unless stimulated by plasmid breakage. A notable exception is the *Flp* recombination system of 2µm (3). Plasmid pDY740 was tested in the 2µm plasmid minus strain LL20A (α *leu2-3*, *112 his3-11*, *15 ade*⁻ [*cir*⁰]). From 5 transformants, a total of 91 plasmids were examined; of these plasmids, 43 (60%) carried the *412*

deletion (Table 1). The pleiotropic recombination mutation *rad52* (6, 8) was also tested; of the plasmids from five independent XS95-6c (α *rad52-1 ura3-52 leu2-3*, *112 trp1 his3Δ1*) transformants, 41% contained the *412* specific deletion (Table 1).

The most striking feature of the *412* element in yeast is the rapid loss of the element from the plasmid in homologous recombination between the LTRs. Despite this, strains retaining the original transforming plasmid could still be isolated after long-term culturing of transformed DBY747. If recombination were to continue at a high frequency, only deletion plasmids should be recovered. Thus, the instability of the plasmid-borne *412* element appears to be a transient phenomenon which occurs shortly after or during transformation of the host cells. The proportion of deletion plasmids after long-term culture is probably dependent on random segregation.

The frequency of *412* excision appears to be much greater in yeast than in *D. melanogaster* (12). This may be due to the evolution of a mechanism(s) which suppresses mitotic recombination between the LTRs of copialike elements in *D. melanogaster* and which may not exist in yeast. The genome of *S. cerevisiae* contains transposable elements, Ty ele-

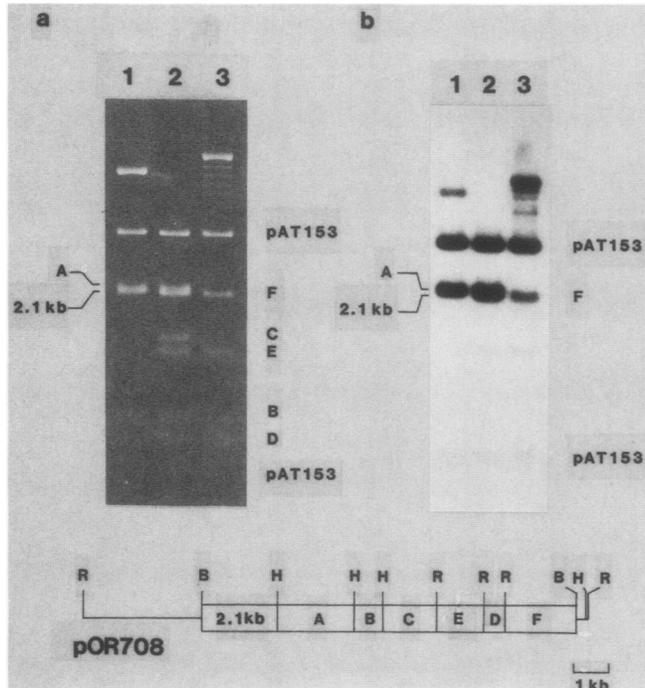


FIG. 3. Identification of the *D. melanogaster* sequence present in pDY6 by Southern blot analysis. (a) Ethidium bromide-stained gel pattern of the restriction endonuclease-digested pOR708. Lanes: 1, *Bam*HI and *Hind*III; 2, *Bam*HI, *Hind*III, and *Eco*RI; 3, *Bam*HI and *Eco*RI. (b) Autoradiograph of a Southern blot of restriction endonuclease-digested pOR708 probed with pDY6. Lanes 1 through 3 are the same as in panel a. (c) Restriction map of pOR708 plasmid. The *Bam*HI-*Hind*III 2.1 kb fragment flanking 412 consists solely of *D. melanogaster* chromosomal DNA. The positions of the various fragments of pOR708 are indicated. Solid line, pAT153 sequence; open box, *D. melanogaster* DNA; B, *Bam*HI; H, *Hind*III, R, *Eco*RI.

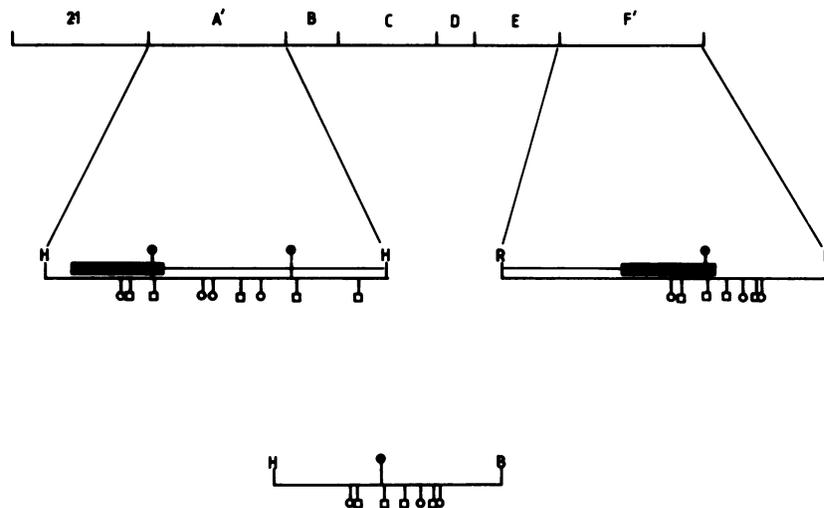


FIG. 4. Comparison of restriction maps of the 412 deletion fragment from pDY6 with the 412 A and F fragments. The pAT153-derived plasmids pOR702 and pOR724 contain the A and F fragments, respectively. The position of the *Hpa*I (●), *Hpa*II (○), and *Hin*I (□) restriction sites have been previously determined (16). The position of the *Hpa*I, *Hpa*II, and *Hin*I sites in pDK353 were determined by a series of single and double digestions. In addition, the restriction fragment pattern from single enzyme digestions of pDK353, pOR702 (fragment A), and pOR724 (fragment F) were directly compared by gel electrophoresis. The restriction map of the *Hind*III-*Bam*HI fragment containing the 412 deletion is consistent with a fusion of A and F in which all of 412 is deleted except for a single LTR. ■, LTR.

ments, which resemble copia-like elements (reviewed in reference 10). These have LTRs known as δ sequences. There are more solo δ s than δ s associated with complete Ty elements, suggesting that δ - δ recombination can occur relatively frequently. This may be related to the recombination between 412 LTRs which we have observed. Alternatively and more likely, the yeast mitotic recombination system may be stimulated by transformation to recognize sequences on the transforming plasmid and cause recombination between the LTRs.

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ADDENDUM IN PROOF

In Fig. 4, the order of the D and E fragments should be reversed (as in Fig. 3).

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