Behavior of a Drosophila melanogaster transposable element in Saccharomyces cerevisiae

Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Molecular and Cellular Biology

Publisher Rights Statement:
Freely available via Pub Med.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and/or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Behavior of a *Drosophila melanogaster* Transposable Element in *Saccharomyces cerevisiae*

DEBORAH K. HOSHIZAKI*† AND DAVID J. FINNEGAN

Department of Molecular Biology, University of Edinburgh, Edinburgh, Scotland EH9 3JR

Received 23 January 1985/Accepted 3 July 1985

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 Flp gene of 2 μm 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 recombination 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 BamHI fragment as in pDY740. The SalI 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 *SalI* fragment and a 10.5-kb *BamHI* fragment. The faint 10.5-kb band in the *SalI* 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 *SalI* fragment and a 3.5-kb *BamHI* fragment. With the exception of the plasmid-derived 8.7-kb *SalI*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Transforming plasmid</th>
<th>Deletion form</th>
<th>Other*</th>
<th>Total*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDY740 transformed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBY747</td>
<td>[cir+] RAD52</td>
<td>56</td>
<td>55</td>
<td>1</td>
<td>112 (4)</td>
</tr>
<tr>
<td>LL20A</td>
<td>[cir+] RAD52</td>
<td>45</td>
<td>47</td>
<td>3</td>
<td>95 (4)</td>
</tr>
<tr>
<td>XS95.6c</td>
<td>[cir+] rad52</td>
<td>39</td>
<td>28</td>
<td>1</td>
<td>68 (6)</td>
</tr>
<tr>
<td>pAB732 transformed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBY747</td>
<td>[cir+] RAD52</td>
<td>97</td>
<td>1</td>
<td>7</td>
<td>105 (4)</td>
</tr>
</tbody>
</table>

* Other rearranged plasmids included intermolecular recombinants with 2 μm DNA.

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

† Present address: Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461.

* Corresponding author.
and 10.5-kb BamHI 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 BamHI 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 BamHI-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 BamHI 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 BamHI fragment and its lack of hybridization with these two probes suggested that it was derived from the 10.5-kb D. melanogaster BamHI fragment by the loss of the 412 element. The BamHI 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 μg 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 BamHI 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 HindIII-BamHI 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 HindIII-BamHI 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 betweenLTRs.

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

---

**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 HindIII, EcoRI, and BamHI restriction endonuclease sites. The 2.1-kb BamHI-HindIII 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 BamHI 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. Shahi. 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 EcoRI-BamHI fragment of pBR322 and a portion of 2μm DNA. Open box, D. melanogaster DNA; solid line, pAT153 sequence; open circle, 2μm DNA; broken line, LEU2 structural gene; open box with arrows, duplicated sequence. B, BamHI; H, HindIII; R, EcoRI; S, Sall.
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 (a 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 (a rad52-1 ura3-52 leu2-3, 112 trpl 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. 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 BamHI 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 BamHI and probed with the 10.5-kb BamHI fragment. Lanes: 1, lambda phage DNA digested with HindIII; 2, DBY747 transformed with pDY740. (c) Southern blot of DNA isolated from DBY747 transformed with pDY740. DNA was digested with BamHI and probed with the 412 fragment (lane 1) and pAT153 (lane 2).
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, *BamHI* and *HindIII*; 2, *BamHI*, *HindIII*, and *EcoRI*; 3, *BamHI* and *EcoRI*. (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 *BamHI*-*HindIII* 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, *BamHI*; H, *HindIII*; R, *EcoRI*.

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 *HpaI* (●), *HpaII* (○), and *HindIII* (□) restriction sites have been previously determined (16). The position of the *HpaI*, *HpaII*, and *HindIII* 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 *HindIII*-*BamHI* 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 δs than δs 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.

This work was supported by a project grant from the Medical Research Council to D.J.F. and an American Cancer Society postdoctoral fellowship grant (PF-1905) to D.K.H.

We thank A. Cioffi for typing the manuscript.

**ADDENDUM IN PROOF**

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