of part of the tRNA occurs, providing the genetic material that is inserted by non-homologous recombination events.

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Haber and Moore reply — We do not yet know how a specific segment of Ty1 can be captured at the sites of double-strand breaks, and the suggestion by Lauermann provides one plausible explanation of the source of the putative cDNA segment. But we feel that the details of the events that we characterized favour our original interpretation.

The intermediate suggested by Lauermann would contain cDNA initiated at the primers PPT1 or PPT2 and should contain all of the long-terminal repeat (U3-R-U5), but three of the four repairs we studied contained R and U5 but lacked any U3. We prefer an explanation in which the initial minus-strand strong-stop cDNA, beginning at the primer-binding site and including only U5 and R, was the source of the captured DNA. The abundance of this intermediate might be greater among (often mutant) natural Ty1 elements than for a construct carrying an inducible Ty1 gene. This is consistent with our observation that expression of Ty1 messenger RNA from a galactose-inducible promoter had little effect on the frequency of these largely endogenous events[2].

Lauermann's explanation is more likely for the insertion of *HIS3* derived from cDNA of a galactose-inducible Ty1*mhis3AI* construct observed by Teng *et al.*[1], but more recent results from Teng and Gabriel suggest that this may not be the case (see below).

The covalent ligation of RNA with DNA during insertion by DNA replication is not entirely new. It has recently been documented for group II introns in a reverse splicing reaction[8]. But such an RNA–DNA junction is not a necessary intermediate in the process. It would suffice for the tRNA–cDNA segment to be used as a template by one 3′ end of the broken DNA, to copy these sequences. The fact that all of the Ty1 sequences containing R and U5 are inserted in one orientation supports this idea (ref. 2 and see below).

Clearly additional experiments are needed to determine which cDNA intermediate is used as the source of the inserted DNA, and to establish how the non-homologous junctions are formed.

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Teng and Gabriel reply — Lauermann has suggested that the observed[1,2] Ty1 insertions associated with the primer-binding site may be derived from plus-strand cDNAs, whose 3′ ends are templated by primer tRNA present at the 5′ end of minus-strand cDNA. Further, he suggests specific experiments to verify his hypothesis. Recent data from our laboratory bear directly on these proposals.

We examined histidine prototroph formation among survivors of HO cutting in yeast strains carrying wild-type, ppt1$^-$ mutant, or ppt1$^-$/ppt2$^-$ double mutant versions of a *GAL1*-Ty1 element marked with *mhis3AI*. We screened 52 independently targeted histidine prototrophs (Fig. 2a) by polymerase chain reaction (PCR) and identified nine (17%) in which U3 sequences were closely linked to either the right (Z1) or left (Yα) side of the HO cut site at *MATα*. A strong bias for linkage with the left side of the HO cut site was seen (eight of nine prototrophs, Table 1). By sequencing these eight junctional segments, we determined that each contained between four and ten bases of the primer-binding site separating U5 from the *MAT* segment. These sequences were indistinguishable from those observed by Moore and Haber[2] in wild-type yeast. A potential base complementarity of 7/10 between the left end of the HO cut site and the primer-binding site (Fig. 2b) may underlie this strong insertion bias.

Our findings do not support Lauermann's prediction that insertions derived from the ppt1$^-$/ppt2$^-$ mutant version of Ty1 will lack associated primer-binding-site sequences. Instead our results suggest that minus-strand cDNA with its attached tRNA — the expected Ty1 replication intermediate in the ppt1$^-$/ppt2$^-$ double mutant strain — is the most likely substrate for insertional repair.

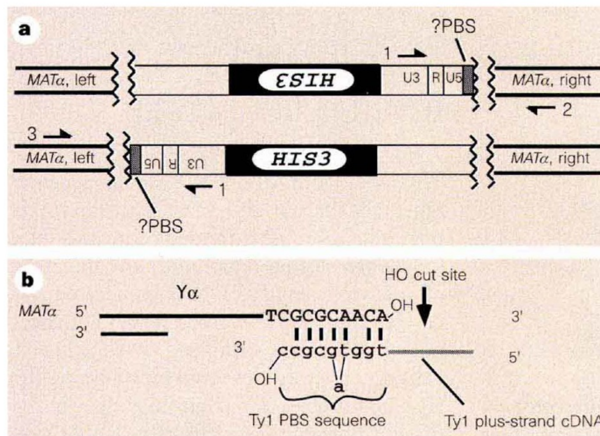

Figure 2 **Preferential association of Ty1 primer-binding-site (PBS) sequences with one side of the HO cut site in *MATα*. a, The two possible orientations of *HIS3* insertions at the HO cut site in *MATα*, and a PCR strategy for identifying the orientation of insertions in which the Ty1 U3 sequence is closely linked to the *MAT* locus. b, Potential alignment of the 3′ terminal bases to the left of the HO cut site in *MATα* and the Ty1 plus-strand cDNA terminating in the PBS.**

Table 1 Results from PCR analysis

GAL1-Ty1 associated element	No. analysed	No. associated with *MATα*, L	No with *MATα*, R
Wild type	26	3	1
ppt1$^-$ mutant	11	1	0
ppt1$^-$/ppt2$^-$ mutant	15	4	0

Tabulation of results from the PCR analysis shown in Fig. 2a, using independent *MATα* targeted insertion events derived from a variety of Ty1 constructs, carrying wild-type, ppt1$^-$ mutant or ppt1$^-$/ppt2$^-$ mutant versions of a *GAL1*-Ty1 element marked with *mhis3AI*. L, left (primers 1 and 3); R, right (primers 1 and 2). Targeted *HIS3* insertions were obtained as described in ref. 1.

A plausible model for repair in these cases would involve plus-strand synthesis initiated from the right end of the cut site, using minus-strand cDNA as the template. Plus-strand synthesis would end with partial copying of the attached tRNA. After RNaseH cleavage of the tRNA–DNA duplex, the terminal primer-binding-site region would be exposed and could be anchored to the left end of the HO cut site by complementary base pairing. Our data indicate that, even with wild-type Ty1 constructs, insertions may be formed from intermediates or 'dead-end' products of the replication process, as ends containing primer-binding sites are not expected to be found in mature double-stranded Ty1 DNA.

Our new results cannot distinguish between repairs initiated from plus-strand or minus-strand cDNA, or even from RNA templates. However, the primer-binding site–HO cut site complementarity suggests that the observed junctional 'hotspot' results from terminal base pairing and consequent stabilization of the two single-strand ends. Yeast may use various substrates during insertional repair of double-strand breaks, and our data show that minus-strand Ty1 cDNA linked to its primer tRNA might be one such intermediate. Further work is needed to determine the relative contributions of different repair substrates, including plus-strand cDNA as suggested by Lauermann.

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