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Genetics

Efficient translational frameshifting occurs within a conserved sequence of the overlap between the two genes of a yeast Ty*1* transposon

(retrovirus-like transposable element/*Saccharomyces cerevisiae*/β-galactosidase gene fusions/nuclease S1 mapping)

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ABSTRACT **The Ty (*t*ransposon *y*east) family of retroviral-like transposons include two genes, *TYA* and *TYB*, analogous to the *gag* and *pol* genes of metazoan retroviruses. *TYB* lies downstream of *TYA*, the two genes overlapping by 38 base pairs. The primary translation product of *TYB* is a TYA/TYB fusion protein whose expression has been inferred to occur by translational frameshifting within the overlap region. We show that the event leading to expression of *TYB* is very efficient, resulting in 20% read-through into *TYB* from *TYA*. We demonstrate that the Ty mRNA is colinear with the DNA sequence of the element, eliminating any pretranslational model for *TYB* expression. Frameshifting requires no particular sequence of the upstream *TYA* gene, nor any global RNA structure. Surprisingly, it can be promoted by a 14-base-pair oligonucleotide of the overlap region. The ability of this oligonucleotide to function is inhibited when it is positioned immediately downstream of an initiator AUG. We conclude that the *TYB* gene is expressed by an efficient ribosomal frameshifting event requiring a small oligonucleotide sequence derived from the *TYA/TYB* overlap region.**

The Ty (*t*ransposon *y*east) elements of the yeast *Saccharomyces cerevisiae* are a family of about 30 dispersed retroviral-like transposons (1, 2). Ty elements include two open reading frames, termed *TYA* and *TYB* (3), which are analogous respectively to the *gag* and *pol* genes of metazoan retroviruses. The similarity in structure and organization reflects the fact that Ty elements, like retroviruses, replicate via an RNA intermediate during transposition (2). The primary translation product of retroviral *pol* genes and *TYB* are fusions to the product of the upstream gene, *gag* and *TYA*. In the case of most of the avian and mammalian retroviruses the *gag*, *pol*, and sometimes *pro* (encoding retroviral protease) genes overlap; similarly *TYB* overlaps the last 38–44 base pairs (bp) of *TYA*. For Rous sarcoma virus (RSV) (4) and mouse mammary tumor virus (MMTV) (5, 6), translational frameshifting has been shown explicitly to produce the fusion peptides. It is likely, given their similarity to retroviruses, that Ty elements also express their *pol* analog by translational frameshifts; however, the evidence for this is circumstantial. Nuclease S1 protection experiments eliminate the possibility that *TYB* expression involves RNA splicing (3, 7, 8). The experiments are sufficiently sensitive to detect a very small discontinuity between the RNA and the DNA [5–11 nucleotides (nt)]; these experiments do not exclude the possibility that expression of *TYB* occurs by RNA editing.

Wilson *et al.* (8) have demonstrated that a 125-bp region of the element Ty*1-15*, extending from a position 7 bp into the overlap through a *Bgl* II site 94 bp downstream of the overlap, is sufficient to promote normal *TYA/TYB* expression. They also showed that a deletion removing the first 24 nt of the overlap eliminates *TYA/TYB* expression. They speculated that a short region of the overlap that is conserved between the Ty*1* and Ty*2* class elements, partially extending into the first 24 nt, is required for frameshifting but provided no evidence for this conclusion. Here we show that *TYB* expression is very efficient, 20% that of *TYA*. A construction that expresses this level of the TYA/TYB fusion peptide encodes a single RNA colinear with the DNA, eliminating any pretranslational model for production of the fusion. Finally a 14-nt sequence of the *TYA/TYB* overlap is both necessary and sufficient to promote normal levels of *TYB* expression.

MATERIALS AND METHODS

Strains and Media. The yeast strain used in this work is 387-1D (α *his4Δ38 ura3-52 trp1-289 HOL1-1*). DNA transformations of yeast were performed by the lithium acetate method (9). This strain, when transformed with *URA3*$^+$-containing plasmids, grows on SD minimal medium supplemented with histidine and tryptophan, 20 μg/ml each (10).

Oligonucleotide Mutagenesis and Plasmid Constructions. Mutations introducing unique *Bam*HI sites within the *TYA* gene of the Ty*1-912* element (7) were constructed by oligonucleotide mutagenesis. The oligonucleotides correspond to positions 1591–1612 (oligonucleotide 1: 5′-TTCACCTTAGGATCCAGGAACTTA-3′), 1549–1572 (oligonucleotide 2: 5′-AATCAACTACGGATCCGATTCAAT-3′), and 286–306 (oligonucleotide 3: 5′-TTAAAAATGGATCCCAACAA-3′). Introduced *Bam*HI sites are italic. These oligonucleotides were used to prime the synthesis of a complementary strand by DNA polymerase large fragment on single-stranded DNA from appropriate phage M13 subclones. Ty::*lacZ* fusions or internal deletions made with the introduced *Bam*HI sites are referred to by the last wild-type base pair within the site: for oligonucleotide 1, position 1601 from the 5′ direction; for oligonucleotide 2, 1563 from the 3′ direction; and for oligonucleotide 3, 294 from the 3′ direction.

All plasmids used for yeast transformation carry the *URA3*$^+$ gene and the 2-μm origin of replication from pLGSD5 (11) and a 5′ truncated *lacZ* gene from either pLGSD5 or pMC1790 (12); the construction of these was done essentially as described (13). Sets of *lacZ* fusions were made by using internal *TYA* deletions that retained the entire *TYA/TYB* overlap (column 5 in Table 1) or the last 12 bp of the overlap (column 6 in Table 1). These were made by fusing a *Bgl* II site 88 bp past the overlap to the *Bam*HI site of

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Abbreviations: nt, nucleotide; RSV, Rous sarcoma virus; MMTV, mouse mammary tumor virus; Mo-MLV, Moloney murine leukemia virus; Ty, transposon yeast.

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Table 1. The 14-bp conserved region of the Ty*1-912* overlap is sufficient to promote frameshifting

Gene	Insertion position	Codons downstream of AUG, no.	β-Galactosidase activity, units* (% frameshifting†)			
				Oligonucleotide inserts		
			lacZ fusion‡	Region 1563–1702§	Region 1601–1702§	Consensus region 1596–1609§
Ty*2-917*	296	1	800	150 (19%)	0.2 (0.03%)	5 (0.6%)
Ty*2-917*	559	88	4091	ND	ND	1924 (47%)
Ty*2-917*	1072	259	51	ND	ND	4 (7.7%)
Ty*1-912*	388	31	3631	1090 (30%)	6.9 (0.2%)	209 (5.7%)
Ty*1-912*	1225	310	4593	1446 (32%)	4.7 (0.1%)	500 (11%)
HIS4	100	33	2133	ND	ND	472 (22%)

*Units are given in μmol of orthonitrophenyl galactoside cleaved per min per mg of protein.
†Ratio of units expressed by each construct to units expressed by the analogous *lacZ* fusion.
‡Plasmids either have no insert or, for *HIS4*, an insert of the consensus oligonucleotide such that *HIS4* and *lacZ* are in the same reading frame.
§Fusions are made such that *lacZ* is in the +1 reading frame with respect to the upstream gene. Oligonucleotide 1 = region 1601–1702; oligonucleotide 2 = region 1563–1702. ND, not determined.

pLGSD5 [the sequence of which is available (14)]. Complementary oligonucleotides, the "consensus oligonucleotides," were synthesized (5′-GATCCCCTTAGGCCAGGAACCC-3′ and 5′-GATCGGGTTCCTGGCCTAAGGG-3′), including a 15-nt region of the *TYA/TYB* overlap (positions 1595–1609). A set of fusions of the consensus oligonucleotide to *lacZ* were made by first inserting the double-stranded oligonucleotide into the unique *Bam*HI site of pMC1790 (12) and then transferring the oligonucleotide fused to *lacZ* into various Ty-*lacZ* fusion plasmids (described in *Results*). In each set of constructions, the position of the *lacZ* fusion junction is conserved.

β-Galactosidase Assay, Endonuclease S1 Mapping, and cDNA Sequencing. Three transformants of each plasmid were each assayed in triplicate for β-galactosidase activity as described (3). Total RNA was isolated from yeast transformants, and the amount of *Ty*-specific RNA present was assayed by using a uniformly labeled fragment derived from the *lacZ* gene as described (13). The sequence of the RNA corresponding to the minimal sequence capable of promoting frameshifting was determined by cDNA synthesis with avian myoblastosis virus reverse transcriptase (Life Sciences, Saint Petersburg, FL) in the presence of chain-terminating triphosphates essentially as described (15). Two primers were used, primer 1 homologous to a region 39–78 nt downstream of the cloning region (5′-CGAAAGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAAC-3′) and primer 2 homologous to a region 113–152 nt downstream (5′-CCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGA-3′).

RESULTS

The *TYA/TYB* Overlap Region Itself Directs Efficient Frameshifting. As a preliminary step toward understanding the expression of the *TYB* gene, we have measured *TYB* expression relative to that of *TYA*. An accurate measurement was obtained by using the mutant, Ty1-912Δ3500-oli1. In Ty1-912Δ3500, the *lacZ* gene is fused 2 kilobase pairs (kb) into *TYB*. The oligonucleotide 1 mutation inserts 2 bp after position 1601 within the *TYA/TYB* overlap region, creating a *Bam*HI restriction site and fusing the *TYA* and *TYB* reading frames. The insertion causes a 5-fold increase in β-galactosidase expression (1174 units of β-galactosidase compared to 231 units) without affecting mRNA accumulation (Fig. 1). Assuming that the insertion does nothing to disrupt normal translation of the overlap, this indicates that the putative *TYA/TYB* frameshift event occurs at a rate of 20%. Comparison of the β-galactosidase activities of transformants of three *TYA* fusions (Ty1-912Δ388, Ty1-912Δ613, and Ty1-912Δ1225) and two *TYB* fusions (Ty1-912Δ1708 and Ty1-912Δ3500) gave a similar indication (data not shown).

To test if deleting TYA sequences affected the level of *TYB* expression, we made in-frame deletions of *TYA1* (formerly *TYA-912* allele) extending from two different 5′ endpoints to a common 3′ endpoint. Ty1-912Δ388-1563 and Ty1-912Δ1225-1563 retain a region (positions 1563 to 1706) including the full *TYA/TYB* overlap. The deletions do not alter mRNA expression (Fig. 1). The efficiency of the putative frameshift equals the ratio of β-galactosidase activity directed by fusions with and without the overlap. Efficient frameshifting still occurs when >90% of the *TYA1* gene is deleted (Table 1, line 4, column 5). Indeed wild-type levels of frameshifting (19%) are observed even when the entire coding region of *TYA1* upstream of position 1563 is deleted (Table 1, first line). This construction, Ty917Δ296::Ty912Δ1563, fuses the region including the overlap to the ATG initiator codon of the *TYA2* gene, removing the entire *TYA* gene. Thus, the region from position 1563 to 1706 alone is capable of promoting efficient frameshifting. No other cis-acting sites

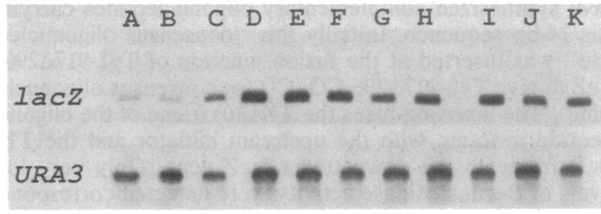

FIG. 1. Endonuclease S1 protection analysis. Total RNA (25 μg) isolated from each of 11 transformants was separately hybridized with two probes (an internal fragment of the *Escherichia coli lacZ* gene and of the *URA3* gene of yeast) and subjected to digestion with endonuclease S1 as described in *Materials and Methods*. The products are labeled "lacZ" and "URA3," respectively. In the case of the *URA3* probe, two fragments could be seen in the gel electrophoresis, one corresponding to protection of the probe by the ura3-52 transcript from the chromosome and the other to protection by the URA3$^+$ transcript from the plasmid. ura3-52 results from insertion of a Ty element into *URA3* and therefore can not protect the entire *URA3* gene from digestion. The ura3-52 protected fragment is shown in the figure. The amount of URA3$^+$ product was proportional to the amount of ura3-52 product in each case (data not shown). The lanes correspond to RNA from plasmids carrying the constructs Ty1-912Δ388 (lane A), Ty1-912Δ388-1601 (lane B), Ty1-912Δ388-1563 (lane C), Ty1-912Δ1225 (lane D), Ty1-912Δ1225-1601 (lane E), Ty1-912Δ1225-1563 (lane F), Ty1-912Δ1225-CO (lane G), Ty1-912Δ1595A (lane H), Ty1-912Δ1595B (lane I), Ty1-912Δ3500-oli1 (lane J; the *TYA-TYB* frame fusion), and Ty1-912Δ3500 (lane K).

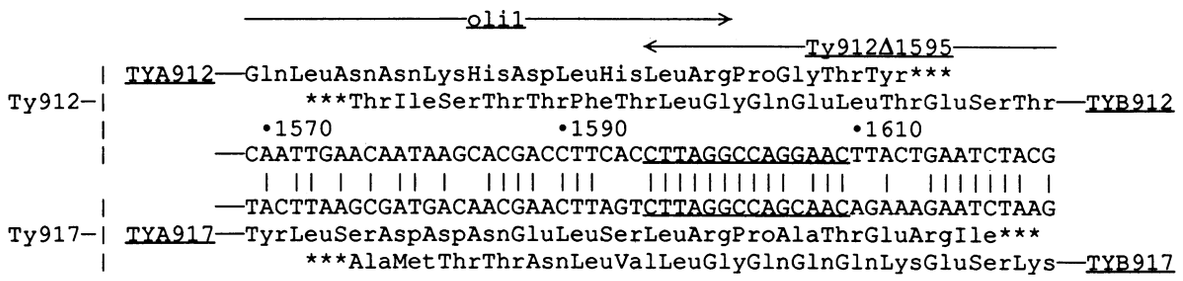

FIG. 2. Comparison of the Ty*1-912* and Ty*2-917 TYA/TYB* overlaps. The sequence of the region of the overlaps from Ty*1-912* and Ty*2-917* are shown aligned, vertical lines indicating identity, and with the Ty*1-912* sequence numbered (position 1 is the 5′ end of the element). The predicted *TYA* and *TYB* gene products are shown along with the sequences. Three deletions which end before or within the Ty*1-912* overlap are indicated above the sequences, the arrowhead corresponding to the last nucleotide removed by the deletion. A 14-bp region of homology (13/14) between the two overlaps is indicated by underlining. This region corresponds to the "consensus oligonucleotide" referred to in the text.

between positions 294 and 1563 are required for this process, nor is the overall secondary structure of the Ty message important.

A Conserved Sequence Within the Overlap Region of Ty Elements Is Necessary and Sufficient for *TYB* Expression. To further define the sequences required for frameshifting, we have made deletions of the Ty*1-912* overlap (positions 1576 to 1613) that retain either the last 12 bp or the first 20 bp of the overlap (see Fig. 2). Three internal *TYA* deletions (Ty1-917Δ296::Ty1-912Δ1601, Ty1-912Δ388-1601, and Ty1-912Δ1225-1601) that retain the 3′ 12 bp of the overlap express very low levels of β-galactosidase (Table 1, column 6). A deletion of the *TYA1* gene up to the first 20 bp of the overlap, position 1595, lacks the rest of the element. It directs little frameshifting, expressing 2053 units of β-galactosidase when *TYA1* is fused in frame with *lacZ* (Ty1-912Δ1595A) versus 13 units (0.6% frameshifting) when expression requires a +1 frameshift (Ty1-912Δ1595B). This shows that at least the region between the two deletions is necessary for efficient *TYB* expression.

As noted by Wilson *et al.* (8), though the *TYA/TYB* overlaps of Ty*1* and Ty*2* class elements include a region of 13-of-14-nucleotide homology, this region is partially removed by both of the above deletions (see Fig. 2). To examine the role of this conserved region in frameshifting, we have synthesized complementary oligonucleotides carrying this 14-bp sequence. Initially this "consensus oligonucleotide" was inserted at the fusion junction of Ty2-917Δ296::*lacZ* to give Ty2-917Δ296-CO (CO for consensus oligonucleotide). The insertion fuses the *TYA* (0) frame of the oligonucleotide in-frame with the upstream initiator and the *TYB* (+1) frame to the downstream *lacZ* gene. Only very low levels of β-galactosidase activity were detected corresponding to 0.6% frameshifting (Table 1, line 1, column 7); the amount of mRNA expressed is unchanged (data not shown). The consensus region corresponds to codons 4 to 8 of the fused gene; insertion this close to the translation initiation site could inhibit the frameshift event that normally occurs far downstream. To test this we inserted the oligonucleotide into Ty2-917::*lacZ* and Ty1-912::*lacZ* fusion junctions from 31 to 310 codons further downstream. All of these constructions allow much more efficient frameshifting. The amount of frameshifting varied from 6% to 47% among the constructions (Table 1, column 7). The reason for this variation is not clear but may involve some feature of local RNA structure near the deletion endpoints. We conclude that the conserved 14-bp sequence found within the overlap region of Ty elements is able to direct efficient frameshifting, although the ability to undergo frameshift may be dramatically reduced by proximity to the site of translational initiation.

To determine if this 14-bp sequence is sufficient to allow the frameshift, it was cloned into a vector lacking any Ty*1-912* sequences. The oligonucleotide was inserted into a *Bam*HI site at the junction of a *HIS4A::lacZ* fusion 33 codons downstream of the initiation codon. The insertion put *lacZ* either in the same reading frame as *HIS4*, or in the +1 reading frame. The 14-bp conserved sequence allowed 22% frameshifting in this context (Table 1, line 6), showing that the oligonucleotide is sufficient to promote high-level frameshifting even in the absence of any other Ty*1-912*-derived sequences.

Expression of *TYB1* Does Not Occur by RNA Editing. We and others (3, 7, 8) had shown that the TYA-TYB fusion protein is not expressed by RNA splicing. These experiments do not prove ribosomal frameshifting because two models are consistent with the results presented, ribosomal frameshifting and RNA editing. An RNA-editing model predicts two transcripts of the overlap region, one colinear with DNA and one in which *TYA* and *TYB* are fused by the insertion or deletion of several nucleotides. We tested the RNA-editing model by sequencing the mRNA produced by Ty1-912Δ1225-CO and HIS4-CO by primer-directed cDNA synthesis in the presence of dideoxynucleoside triphosphates. If the RNA-editing model is correct, cDNA sequencing of the mRNA should reveal two overlapping sequences differing by an oligonucleotide frameshift; the shifted sequence should be from 10% to 20% of the intensity of the colinear sequence. We used two 40-nt *lacZ* primers, one beginning 78 bp (primer 1) and the other 152 bp (primer 2) downstream of the Ty*1-912-lacZ* fusion junction. Deletions that remove more than the first 41 amino acids of β-galactosidase are enzymatically inactive (16); the same would be true of any RNA modification that were to occur *in vivo* in yeast. Primer 2 lies entirely downstream of this boundary, so no RNA modification could extend from the upstream overlap through any portion of its binding site and yield an active enzyme product.

The RNA sequence of the overlap shows no evidence of the predicted frameshift of the RNA sequence of the overlap region either of Ty1-912Δ1225-CO, sequenced by using primer 1 (Fig. 3) or primer 2 (data not shown), or of HIS4-CO, sequenced with either primer (data not shown). From overexposure of the sequence, we are confident that no frameshift of a RNA sequence is present at as much as 1% of the concentration of the colinear RNA. Since at least 99% of the mRNA expressed is colinear with the gene, the *TYA-TYB* fusion cannot be expressed by a pretranslational mechanism. We conclude that expression is by an efficient site-specific translational frameshift within the region defined by the consensus oligonucleotide.

DISCUSSION

Previous work had shown that the *TYB* gene product is expressed as a TYA/TYB fusion protein (3, 7). Circumstan-

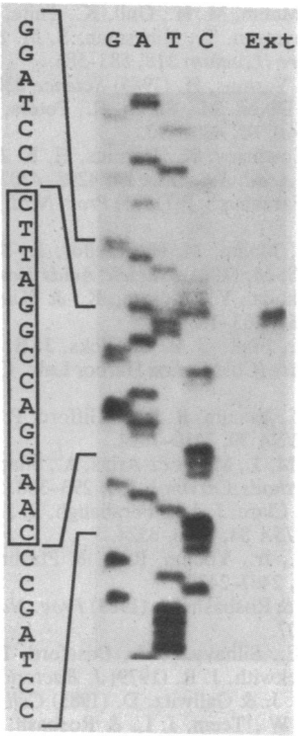

FIG. 3. cDNA sequences of the mRNA of consensus oligonucleotide constructions. Shown are the products of cDNA synthesis on the mRNA transcribed from Ty1-912Δ1225-CO. The reactions were done in the absence of dideoxynucleotides (labeled "EXT") or with each dideoxynucleotide-triphosphate ("G," "A," etc.). For simplicity the sequence is labeled with the sequence of the RNA template rather than the cDNA product and the sequence homologous to the 14-bp conserved region of the Ty*1-912* overlap is shown boxed.

tial evidence has accumulated to suggest that the fusion protein is expressed by a translational frameshifting mechanism. An obvious alternative mechanism, RNA splicing, is unlikely. The region of the *TYA/TYB* overlap is devoid of the characterized yeast consensus splicing signals (17–19), which appear to be required for any splicing of messengers. Also, endonuclease S1 analysis failed to detect any discontinuity in the overlap region (3, 7, 8); these experiments could detect a splice as small as 5–10 nt. This experiment does not rule out a transcriptional misincorporation model, such as transcriptional frameshifting or RNA editing, which could introduce a discontinuity as small as 1 nt. RNA editing controls the expression of the mitochondrial *coxII* gene of trypanosomes, adding 4 nt not encoded by the DNA to each message (20). *TYB* expression is less efficient, resulting in only about 20% TYA-TYB fusion peptide. Indirect sequencing of the mRNA shows that the TYB message is colinear with the DNA, eliminating any pretranslational model for *TYB* expression.

A translational event is responsible for expression of retroviral *gag-pol* gene fusion products. *In vitro* translation of RNA made *in vitro* with phage SP6 polymerase (4) showed that expression of RSV *pol* results from a −1 change in reading frame by ribosomes during translation of the overlap, estimated to occur *in vivo* at a frequency of about 5%. Similarly, MMTV uses two successive translational frameshifts to express a *gag/pro/pol* gene fusion peptide (5, 6), each of which occurs at about 25% efficiency. Moloney murine leukemia virus (Mo-MLV) *gag* and *pol* genes are translated in the same reading frame separated by a single UAG codon. Sequencing of Mo-MLV protease, which is synthesized across the *gag/pol* junction, shows that readthrough involves inefficient suppression by the insertion of glutamine at the UAG termination codon (21). Infection by Mo-MLV greatly stimulates the steady-state level of a natural glutamine-inserting suppressor tRNA presumably responsible for the suppression (22). The unifying feature of retroviral *gag/pol* expression is translational misincorporation. Whether the RSV and MMTV frameshifts are, like the Mo-MLV read-through, encoded by a suppressor tRNA is not known. For MMTV, the suppression model is less likely because even full overlaps are insufficient (6); the suppression model predicts that a short region of the overlap would be sufficient. Regardless of mechanism, it is likely that all of the retroviruses and retroviral-like transposons that include overlapping genes express them by a ribosomal frameshifting mechanism.

The frequency of translational errors resulting in frameshifting has been estimated *in vivo* in *E. coli* to average 3×10^{-5} events per codon and to vary from 5×10^{-4} to 3×10^{-6} (23, 24). The rate in yeast has not been accurately determined but should not be significantly different. Assuming that the *TYA/TYB* frameshift event occurs at a unique codon, its frequency is from 500-fold to 100,000-fold higher than expected of a random frameshift event. This unusually high level of frameshifting strongly implies a specific mechanism that enables the ribosome to efficiently shift frames within a very small region of the transcript. The nature of this mechanism is obscure but could take one of several forms: a specifically encoded four-base anticodon suppressor tRNA that recognizes a four-base codon within the overlap; a protein factor, perhaps Ty-encoded, that recognizes a region of the transcript of the overlap and promotes frameshifting; or a specific sequence "context" that drastically affects one or more components of the ribosome responsible for maintaining the normal three-base translocation frame. The fact that the unrelated mechanism of suppression of termination that operates for Mo-MLV involves a specific cell-encoded suppressor tRNA (22) lends credence to the notion that expression of the *TYB* gene should depend on a cell-encoded frameshift suppressor tRNA.

As an initial attempt to understand the nature of the frameshift event within the *TYA/TYB* overlap, we have determined the sequences necessary and sufficient for specific and efficient frameshifting. One formal possibility was that a sequence upstream of the overlap is required to enable the ribosome to subsequently undergo a frameshift, analogous to transcription antitermination in phage λ in which an interaction between RNA polymerase, N-protein, and various other factors at the *nut* site enables polymerase to transcribe through multiple terminators further downstream (25). Since removing the entire region from position 296 to position 1563 causes no reduction in the rate of frameshifting, no sequence within this region, nor any overall RNA secondary structure, is required for efficient frameshifting. Wilson *et al.* (8) similarly conclude that the region upstream of base pair 7 of the overlap of a Ty*1* element, Ty*1-15*, can be deleted without eliminating expression of *TYB*. Neither of these experiments eliminate the necessity of sequences immediately downstream of the overlap; in each case the construction made included the region up to a *Bgl* II site, position 1706 of Ty*1-912*, 93 bp downstream of the overlap.

Two deletions that separately delete the 5′ and 3′ halves of the overlap greatly reduce translational frameshifting, defining a minimal required region, position 1596 to 1601 of Ty*1-912*. This region is within a sequence almost completely conserved (13 of 14 nt) between the two classes of Ty elements. Wilson *et al.* (8) suggested that this conserved region is the site of frameshifting but gave no evidence to support that conclusion. To test this hypothesis, we synthesized a double-stranded oligonucleotide corresponding to the conserved region and inserted it into the fusion junctions of five Ty-*lacZ* fusions, at positions from 1 to 310 codons into

the translated region. Four of these promote high levels of frameshifting (from 6% to 47%). This supports the notion that the conserved sequence can promote frameshifting. In the fifth construct, Ty2-917Δ296-CO, the consensus regions encodes amino acids 4–8 of the predicted product. This construct promotes background levels of frameshifting. Since the consensus sequence allows frameshifting when inserted in a variety of other contexts, we hypothesized that the mere proximity to the initiation codon might inhibit frameshifting. Translation immediately after initiation might be fundamentally different from that during the elongation phase, and this difference could perturb the ribosome's frameshifting ability. This effect underscores the need to strictly control the distance between the frameshift site and the initiation codon. The conclusion that the last 14 nt of the Ty*1-15* overlap do not promote frameshifting (8) could be incorrect, since in the construction made to test this, the deleted overlap is 14 nt downstream of the initiator.

To test if the region carried on the consensus oligonucleotide was sufficient to promote frameshifting in the absence of any other Ty*1-912* sequences, we inserted it into a *HIS4A::lacZ* fusion. In this context the oligonucleotide was still able to promote wild-type levels of frameshifting. This experiment demonstrates that all of the signals necessary for Ty*1-912* frameshifting are present in the 14-nt conserved region of the overlap. It is interesting that Jacks *et al.* (6) have shown that oligonucleotides corresponding to either of the MMTV overlap regions cannot promote any detectable frameshifting. They concluded that the overlaps themselves are probably not sufficient to allow frameshifting and suggest that a conserved secondary structure, a stem–loop immediately following the frameshift site, is required. This has recently been shown to be true (26). The fact that a small segment of the Ty*1-912* overlap is competent to promote frameshifting suggests that, though expression of retroviruses and Ty elements is grossly similar, the frameshifts probably occur through distinct mechanisms. Significantly, Ty elements use a +1 frameshift, whereas retroviruses universally use a −1 frameshift.

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1. Cameron, J. R., Loh, E. Y. & Davis, R. W. (1979) *Cell* **16**, 739–751.
2. Boeke, J. D., Garfinkel, D. J., Styles, C. A. & Fink, G. R. (1985) *Cell* **40**, 491–500.
3. Mellor, J., Malim, M. H., Gull, K., Tuite, M. F., McCready, S. M., Dibayawan, T., Kingsman, S. M. & Kingsman, A. J. (1985) *Nature (London)* **318**, 583–586.
4. Jacks, T. & Varmus, H. (1985) *Science* **230**, 1237–1242.
5. Moore, R., Dixon, M., Smith, R., Peters, G. & Dickson, C. (1987) *J. Virol.* **61**, 480–490.
6. Jacks, T., Townsley, K., Varmus, H. E. & Majors, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4298–4302.
7. Clare, J. & Farabaugh, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2829–2833.
8. Wilson, W., Malim, M. H., Mellor, J., Kingsman, A. J. & Kingsman, S. M. (1986) *Nucleic Acids Res.* **14**, 7001–7016.
9. Ito, H., Jukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
10. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
11. Guarente, L., Yocum, R. R. & Gifford, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7410–7414.
12. Casadaban, M. J., Martinez-Arias, A., Shapira, S. K. & Chou, J. (1983) *Methods Enzymol.* **100**, 293–308.
13. Liao, X.-B., Clare, J. J. & Farabaugh, P. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8520–8524.
14. West, R. W., Jr., Yocum, R. R. & Ptashne, M. (1984) *Mol. Cell. Biol.* **4**, 2467–2478.
15. Teem, J. L. & Rosbash, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4403–4407.
16. Brickman, E., Silhavy, T. J., Bassford, P. J., Jr., Shuman, H. A. & Beckwith, J. R. (1979) *J. Bacteriol.* **139**, 13–18.
17. Langford, C. J. & Gallwitz, D. (1983) *Cell* **33**, 519–527.
18. Pikielny, C. W., Teem, J. L. & Rosbash, M. (1983) *Cell* **34**, 395–402.
19. Teem, J. L., Abovich, N., Kaufer, N. F., Schwindinger, W. F., Warner, J. R., Levy, A., Woolford, J., Leer, R. J., van Raamsdonk-Duin, M. M., Mager, W. H., Planta, R. J., Schultz, L., Friesen, J. D., Fried, H. & Rosbash, M. (1984) *Nucleic Acids Res.* **12**, 8295–8312.
20. Benne, R., Van Den Burg, J., Brakenhoff, J. P. J., Sloof, P., Van Boom, J. H. & Tromp, M. (1986) *Cell* **46**, 819–826.
21. Yoshinaka, Y., Katoh, I., Copeland, T. D. & Oroszlan, S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1618–1622.
22. Kuchino, Y., Beier, H., Adita, N. & Nishimura, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2668–2672.
23. Atkins, J. F., Elseviers, D. & Gorini, L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1192–1195.
24. Kurland, C. G. (1979) in *Nonsense Mutations and tRNA Suppressors*, eds. Celis, J. E. & Smith, J. D. (Academic, New York), pp. 97–108.
25. Friedman, D. I., Olson, E. R., Georgopoulos, C., Tilly, K., Herskowitz, I. & Banuett, F. (1984) *Microbiol. Rev.* **48**, 299–325.
26. Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J. & Varmus, H. E. (1988) *Nature (London)* **331**, 280–283.