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Genetics

Retrotransposon insertion induces an isozyme of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster*

(*blood* retrotransposon/polyadenylylation/evolution)

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ABSTRACT **The insertion of the *blood* retrotransposon into the untranslated region of exon 7 of the *sn*-glycerol-3-phosphate dehydrogenase-encoding gene (*Gpdh*) in *Drosophila melanogaster* induces a GPDH isozyme—GPDH-4—and alters the pattern of expression of the three normal isozymes—GPDH-1 to GPDH-3. The process of transcript terminus formation inside the retrotransposon insertion reduces the level of the *Gpdh* transcript that contains exon 8 and increases the level of the transcript that contains exons 1–7. The induced GPDH-4 isozyme is a translation product of the three transcripts that contain fragments of the *blood* retrotransposon. The mechanism of mutagenesis by the *blood* insertion is postulated to involve the pause or termination of transcription within the *blood* sequence, which in turn is caused by the interference of a DNA-binding protein with the RNA polymerase. Thus, we show the formation of a new functional GPDH protein by the insertion of a transposable element and discuss the evolutionary significance of this phenomenon.**

The phenotypic effect caused by the insertion of a transposable element (TE) into the genome depends on the site of the insertion and the structure of the element (1). Insertions in exons usually lead to gene inactivation by either disrupting the synthesis of the protein or giving rise to the formation of a truncated protein lacking normal activity (2). Insertions in regions controlling gene expression can change the amount of protein produced and the expression in different tissues or stages of development (1). The imprecise excision of TEs can change the structural integrity of a gene and give rise to altered gene products (3). It follows that, in general, transposon insertions and excisions give rise to loss of function mutations that are deleterious (2). Herein we describe a TE insertion that alters gene expression to produce a new functional protein.‡ To our knowledge, TE-induced effects of this kind have not been reported before, but they are particularly interesting because of their potential evolutionary significance.

We show that the expression of a *sn*-glycerol-3-phosphate dehydrogenase (*Gpdh*) allele extracted from a natural population of *Drosophila melanogaster* is altered to produce another GPDH isozyme by the insertion of a retrotransposon in a transcribed but untranslated region of the gene. The functional molecule of GPDH (EC 1.1.1.8) is a dimer (4). Three GPDH isozymes are expressed in a tissue- and developmentally specific manner (5): GPDH-3 is the only isozyme found in larvae and is also the predominant form in the adult abdomen. GPDH-1 is the major form in the adult thorax, and both GPDH-1 and GPDH-3 isozymes are present in the head in approximately equal amounts. GPDH-2 is found in small amounts in those adult tissues where both GPDH-1 and GPDH-3 are expressed (5).

All these isozymes are encoded by the same structural *Gpdh* gene (6). There are three classes of Gpdh mRNAs, which are generated by different ways of processing of the primary transcript (7). They differ in their 3′ termini. The transcript containing exons 1 through 6 codes for GPDH-3 and the transcript containing exons 1–6 and exon 8 codes for GPDH-1 (7–9). The product of the transcript that contains exons 1–7 has not been identified, but it has been suggested that this transcript might be coding for GPDH-2 (7). Exons 6, 7, and 8 include large untranslated areas, which code for 3′ untranslated regions of the mature transcripts. In the allele $Gpdh^{AKy33}$ (10), which was isolated from a natural population, the expression of the gene is changed so that it produces a new GPDH isozyme and alters the activity of the normal isozymes.

MATERIALS AND METHODS

***Drosophila* Strains.** The $Gpdh^{AKy33}$ allele was isolated from a natural population at Cardwell, Queensland, Australia, in 1988 (10). The allele has been detected on a single occasion and it is not known whether it has persisted in the population. Normal activity *Gpdh* alleles, *AC5* and *AC8*, used as controls were extracted from an Australian population at Cygnet, Tasmania, in 1984 (11).

Electrophoresis. The GPDH electrophoretic phenotypes were determined on cellulose acetate membranes as described (11), but the electrophoresis time was extended from 1 to 3 h to enhance the separation of the different isozymes.

Extraction of Nucleic Acids. The methods for the extraction of genomic DNA and total cellular RNA from adult flies were as described (12, 13). Poly(A)$^+$ RNA was extracted from 4-day-old adult flies by using the Quick Prep (Pharmacia) kit by the manufacturer's instructions.

Restriction Mapping. A restriction map of the $Gpdh^{AKy33}$ gene was obtained as described (14).

Northern Blot Analysis. The method has been described (13), but instead of nitrocellulose, Hybond-N nylon filters (Amersham) were used. Nick-translated labeled pGpdh(-5.3/7.7) plasmid, which contains the *D. melanogaster Gpdh* gene (S. Bartoszewski, personal communication), was used as a *Gpdh*-specific probe. An end-labeled oligonucleotide JG43 (5′-ACGTCTCAGCTTCTTTAGGG-3′), which is complementary to the noncoding part of exon 8, was used as an exon

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Abbreviations: TE, transposable element; RACE, rapid amplification of cDNA ends; LTR, long terminal repeat.

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‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X81126 and X81127).

8-specific probe. The hybridization intensities of the transcript bands were measured by using the PhosphorImager (Molecular Dynamics). To check the RNA loadings in the gel, the level of the *Adh*-specific transcript was assessed, as both the $Gpdh^{AKy33}$ stock and the control stock $Gpdh^{AC8}$ carry normal Adh^{S} alleles (15): the filters were stripped of radioactivity and reprobed with the nick-translated labeled plasmid pSAF-2 (16) that includes the *D. melanogaster Adh* gene sequences.

PCR Amplification and Sequencing of Genomic DNA. The conditions for the PCR and for the direct sequencing of the PCR products have been described (17).

Rapid Amplification of cDNA Ends (RACE) and Sequencing of RACE Products. RACE was carried out by the published protocol (18). The JG11 oligonucleotide primer (5′-AGAAGCTGCAGGGCCCACCCA-3′) that recognizes a sequence within exon 5 of the *Gpdh* gene was used as the gene-specific primer. PCR amplification was performed as described (17), with the following cycle parameters: denaturation of DNA at 94°C for 30 sec, annealing at 67°C for 1 min, and extension at 72°C for 1 min. Obtained RACE products were subcloned into the pBluescript phagemid (Stratagene) by using a *Pst* I restriction site in the JG11 recognition sequence and a *Bam*HI restriction site in the adaptor primer recognition sequence, and the double-stranded plasmid DNA was sequenced in both directions, as described (19).

RESULTS

Electrophoretic Phenotype and Tissue Distribution. The electrophoretic phenotype of $Gpdh^{AKy33}$ is shown in Fig. 1. In comparison with the $Gpdh^{AC5}$ ($Gpdh^{F}$) control, there is a new band of GPDH activity present in $Gpdh^{AKy33}$ (GPDH-4) that is less anodal than GPDH-3. The staining intensities of the other bands are also altered in $Gpdh^{AKy33}$.

In comparison with the control, GPDH-4 is expressed mainly in thoraces (Fig. 2). Levels of GPDH-1 are reduced in all the body parts investigated, but levels of GPDH-3 seem unchanged. Therefore, the mutation in $Gpdh^{AKy33}$ reduces the level of the GPDH-1 isozyme but does not affect the expression of the GPDH-3 isozyme and this is confirmed by the electrophoretic phenotype of the $Gpdh^{AKy33}$ third instar larvae, which does not differ from that of the control (Fig. 2).

Molecular Structure of the $Gpdh^{AKy33}$ Gene. Restriction mapping revealed a large insertion (≈8 kb) in the 3′ region of the $Gpdh^{AKy33}$ gene. The sequence of the $Gpdh^{AKy33}$ gene was obtained by direct sequencing of PCR fragments amplified from the $Gpdh^{AKy33}$ genomic DNA. Compared with sequences for normal activity alleles (8, 9), there were no changes, other than the insertion, that would affect either transcription or the sequence of the protein encoded by $Gpdh^{AKy33}$.

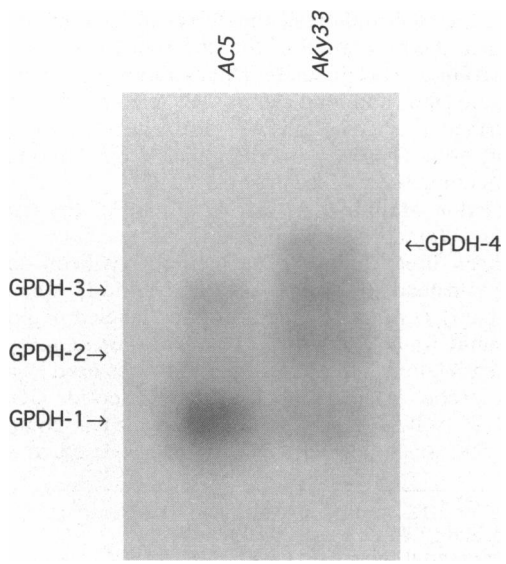

FIG. 1. Electrophoretic phenotype of an adult male fly homozygous for $Gpdh^{AKy33}$ compared to that of a fly homozygous for $Gpdh^{AC5}$. The electrophoresis was from cathode (top) to anode (bottom).

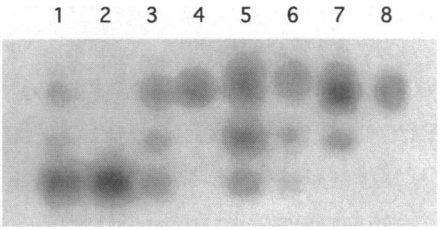

FIG. 2. Tissue and developmental distribution of the GPDH isozymes in $Gpdh^{AKy33}$ compared with that of the $Gpdh^{AC5}$ control. Lanes: 1, whole adult male fly; 2, thorax; 3, abdomen; 4, third instar larva (lanes 1–4, $Gpdh^{AC5}$ control); 5, whole adult male fly; 6, thorax; 7, abdomen; 8, third instar larva (lanes 5–8, $Gpdh^{AKy33}$).

The 5′ flanking region of the insertion was amplified by inverse PCR (20). A fragment bounded by a *Pst* I site in the 5′ part of the insertion and a *Pst* I site in exon 5 of the *Gpdh* gene was circularized for inverse PCR. The 3′ end of the insertion was also PCR-amplified. The PCR products obtained were directly sequenced and this established the position of the insertion in the $Gpdh^{AKy33}$ gene in a noncoding area 66 bp downstream from the stop codon at the 3′ end of exon 7. We sequenced 1079 bp of the 5′ part of the insertion (upstream from the *Pst* I site) and 174 bp of its 3′ part (downstream from a *Sal* I site in its 3′ region). A search (February 18, 1992) of the EMBL nucleotide sequence database (21) found that the insertion shared 99.5% identity with the *blood* retrotransposon, which was first discovered inserted into the white (*w*) locus in the white–*blood* (w^{bl}) allele of *D. melanogaster* (22). The insertion in $Gpdh^{AKy33}$ is flanked by a 4-bp ATAG target site duplication, which is consistent with the properties of *blood* (2). The transcriptional orientation of the inserted *blood* copy (22) in $Gpdh^{AKy33}$ is the same as that of the *Gpdh* gene. The exact position of the insertion is shown in Fig. 3.

Transcription of $Gpdh^{AKy33}$. To test whether the lower GPDH activity in $Gpdh^{AKy33}$ adults might result from a reduced level of the *Gpdh* transcript, the level of this transcript was compared with that in the $Gpdh^{AC8}$ normal activity allele.

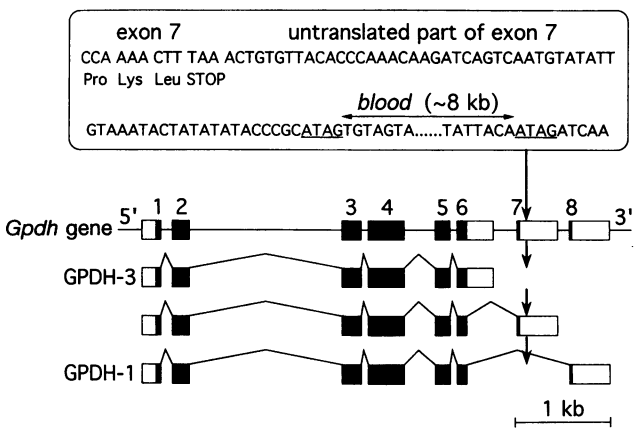

FIG. 3. Position of the *blood* insertion in the $Gpdh^{AKy33}$ gene. (*Lower*) In *D. melanogaster* the *Gpdh* gene has eight exons. Solid boxes indicate the coding regions; open boxes indicate the transcribed but untranslated regions. This gene normally expresses three classes of mRNAs that differ in their 3′ termini. The structure of the *Gpdh* locus is shown, based on published data (8, 9). (*Upper*) The sequence of the region in which *blood* is inserted is shown, with the target site duplication underlined.

The Northern blot analysis results showed that the relative level of the *Gpdh*-specific transcript in $Gpdh^{AKy33}$ was not significantly different from that in the $Gpdh^{AC8}$ control (Fig. 4*A*). This suggests that neither the transcription rate nor the mRNA stability is altered in $Gpdh^{AKy33}$.

As the intensity of the GPDH-1 isozymic band is reduced in $Gpdh^{AKy33}$ (Fig. 1), the level of the GPDH-1-encoding transcript was measured by using an exon 8-specific probe (JG43)—exon 8 is unique to those *Gpdh* transcripts that code for GPDH-1 (7). The level of the GPDH-1-encoding transcript in $Gpdh^{AKy33}$ adults was found to be 30.3 ± 4.1% of that of the $Gpdh^{AC8}$ control (Fig. 4*B*).

Northern blot analyses did not clearly separate the transcripts of the $Gpdh^{AKy33}$ gene, so we used a more sensitive method—RACE (18)—to amplify the 3′ ends of the $Gpdh^{AKy33}$ transcripts, since these are most likely to be affected by the *blood* insertion, which is located in the 3′ region of the $Gpdh^{AKy33}$ gene (Fig. 3). The obtained RACE products were separated by electrophoresis on a 2% agarose gel and the result is shown in Fig. 5. The three RACE products derived from the $Gpdh^{AC8}$ control represent the three normal *Gpdh* transcripts (7). From the sequence data for the 3′ termini of the normal *Gpdh* transcripts (9), it can be deduced that the smallest fragment (≈500 bp) should represent the GPDH-3-encoding transcript, the largest (≈640 bp) should represent the GPDH-1-encoding transcript, and the middle-sized fragment (≈540 bp) should represent the transcript containing exons 1–7. These predictions were confirmed by restriction digest and partial direct sequencing of these products.

In comparison with the control, the bands corresponding to those obtained from the transcripts coding for GPDH-1 and GPDH-3 are present in $Gpdh^{AKy33}$. The 540-bp fragment observed in $Gpdh^{AC8}$ is missing in $Gpdh^{AKy33}$. In addition, three additional RACE products were derived from $Gpdh^{AKy33}$, of ≈400 bp, 650 bp, and 850 bp (Fig. 5). All the RACE products obtained from $Gpdh^{AKy33}$ were sequenced and aligned to the genomic sequence of the $Gpdh^{AKy33}$ gene. The result is shown in diagrammatic form in Fig. 6. The structure of the transcript encoding GPDH-3 is unchanged in $Gpdh^{AKy33}$. Although the level of the GPDH-1-encoding transcript is reduced, its structure is unaltered, as all the *blood* sequences have been excised in the process of splicing.

But the structure of the transcript containing exons 1–7 is changed in $Gpdh^{AKy33}$. Instead of a single transcript, there are now three, differing from each other in the lengths of their 3′

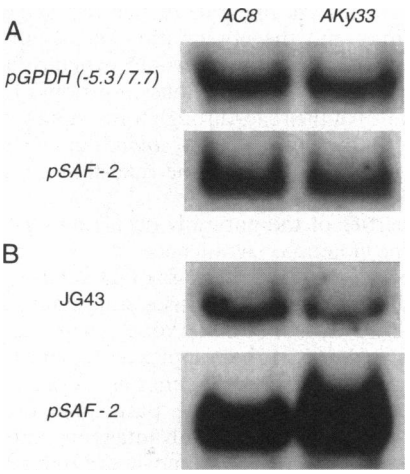

FIG. 4. Northern blot analysis of $Gpdh^{AKy33}$. (*A*) The level of the *Gpdh*-specific transcript. The filter was probed with the *Gpdh*-specific probe [pGpdh(-5.3/7.7)], then stripped of radioactivity, and reprobed with the *Adh*-specific probe (pSAF-2). (*B*) Level of the GPDH-1-encoding transcript. The filter was probed with the exon 8-specific probe (JG43), and the RNA loadings were checked as above.

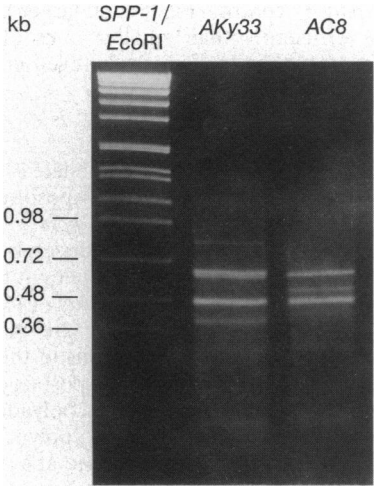

FIG. 5. Agarose gel showing the products of the RACE amplification (see text for details). *SPP-1* digested with *Eco*RI is a DNA size standard (Bresatec, Adelaid, Australia).

untranslated regions. Two of these transcripts utilize polyadenylylation sites in the 5′ long terminal repeat (LTR) of the *blood* retrotransposon, and the third, and largest, terminates inside the retrotransposon sequence, 156 bp downstream from the 3′ end of the *blood* 5′ LTR. This region of the *blood* insertion possesses an A-rich area ($A_{10}GA_3CA_5$). It has been reported (18) that sometimes the $(dT)_{17}$ stretch of the $(dT)_{17}$-adaptor primer binds very effectively to such sequences prior to reverse transcription. Thus it is possible that the 3′ terminus of the RACE product does not represent that of the corresponding transcript and that the correct polyadenylylation site is located further downstream. Two potential polyadenylylation sites are present in the *blood* sequence: one is located 72 bp and the other 109 bp downstream from the A-rich region. It is likely that the largest $Gpdh^{AKy33}$ transcript utilizes one of these sites.

Densitometry assays of the RACE products from Fig. 5 indicated at least a 5-fold increase in the level of the exon 7-specific transcripts in $Gpdh^{AKy33}$ in comparison with the $Gpdh^{AC8}$ control. Since two of the RACE products corresponding to these transcripts in $Gpdh^{AKy33}$ are larger than

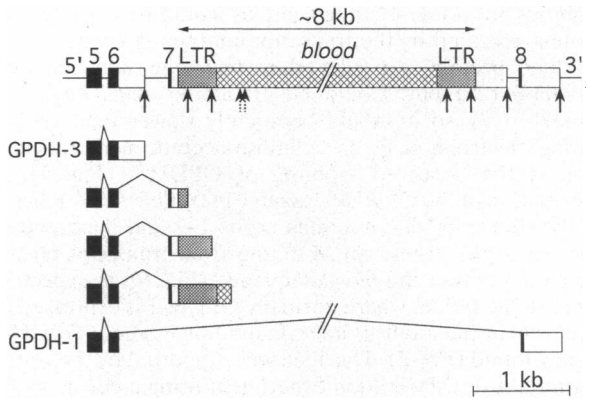

FIG. 6. Structure of the 3′ ends of the $Gpdh^{AKy33}$ transcripts. The upper diagram shows the structure of the $Gpdh^{AKy33}$ gene from exon 5 to exon 8. The plain arrows below the diagram indicate the positions of the polyadenylylation signals that are known to be utilized in the *Gpdh* gene (9) and in the *blood* retrotransposon (from the results of the RACE studies). The dashed arrows show the positions of polyadenylylation signals that we postulate to be utilized in the 5′ region of the *blood* retrotransposon. The diagrams below indicate the structures and the extent of the 3′ ends of the $Gpdh^{AKy33}$ transcripts as deduced from the sequences of the RACE products.

those in the $Gpdh^{AC8}$ control (Fig. 5) and larger fragments are amplified less efficiently than smaller ones (18), the true increase of the transcript level might be even greater.

DISCUSSION

The mutant phenotype of the $Gpdh^{AKy33}$ allele is caused solely by the insertion of the *blood* retrotransposon as no other changes were found in or near the $Gpdh^{AKy33}$ gene. The mechanism by which this phenotype has arisen is analogous to those responsible for the leaky mutant alleles at the *w* locus in *D. melanogaster*, which include white–*apricot* (w^a), white–*buff* (w^{bf}), and white–*blood* (w^{bl}) (2). They are all induced by retrotransposon insertions into the introns of the *w* gene (22, 23). The LTRs of the inserted retrotransposons contain polyadenylylation signals, and *w* transcripts polyadenylylated at those signals encode truncated (inactive) polypeptides. However, some transcripts are polyadenylylated at a normal site at the 3′ end of the *w* gene and the insertions are spliced out with the rest of the *w* intron. The resulting full-length transcript encodes a fully active polypeptide, but it is present at a lower level than in the wild-type w^+ allele (2).

The $Gpdh^{AKy33}$ phenotype is much more complex than those of the leaky mutant alleles at the *w* locus. This can be attributed to the position of the retrotransposon insertion in the $Gpdh^{AKy33}$ gene (Fig. 3). The *blood* retrotransposon is inserted 338 bp downstream from the polyadenylylation site utilized by the GPDH-3-encoding transcript, so neither the level nor the structure of this transcript is affected. But the transcript terminus formation within the *blood* sequence reduces the level of the GPDH-1-encoding transcript, which results in the decrease in the level of the GPDH-1 isozyme.

An important difference between the leaky *w* mutants and $Gpdh^{AKy33}$ is that the three *Gpdh* transcripts polyadenylylated inside the *blood* insertion might encode a functional polypeptide. They each contain exons 1–7 (Fig. 6) and in normal alleles the product of translation of this transcript has not been identified. It has been suggested that it might be coding for the GPDH-2 isozyme (7), but its developmental distribution does not match that of GPDH-2—for example, the transcript containing exons 1–7 is expressed in third instar larvae (7), while GPDH-2 is absent there (5). As no other $Gpdh^{AKy33}$-specific transcripts were detected, it is highly likely that the three transcripts containing exons 1–7 encode GPDH-4. This explanation is supported by the evidence that the electrophoretic mobility of GPDH-4 is the same as would be expected for a protein encoded by the transcript containing exons 1–7. The encoded protein is predicted to have the same charge as GPDH-3 (24) but should be 10 amino acids larger than GPDH-3 (7), so it should be slightly slower than GPDH-3 during electrophoresis on cellulose acetate membranes, and such is the observed mobility of GPDH-4 (Fig. 1). The distribution of the GPDH-4 isozyme in $Gpdh^{AKy33}$ matches that of the transcript that contains exons 1–7 and increased production of this transcript, resulting from transcript terminus formation within the *blood* sequence (Fig. 6), is expected to occur in the tissues where normally GPDH-1 is expressed (i.e., primarily in the adult thoraces), and this is where GPDH-4 is mainly found (Fig. 2). This idea was supported by the result of a transient transformation experiment using a construct lacking exon 8 but with exon 7 spliced to exons 1–6 that produced GPDH activity in a null activity line—after electrophoresis the single staining region was at a similar position to that of GPDH-4 from $Gpdh^{AKy33}$.

Our hypothesis concerning the origin of GPDH-4 is consistent with the suggestion that GPDH-2 is a heterodimer consisting of one GPDH-1 subunit and one GPDH-3 subunit (5). The increase in the staining intensity of the second most anodal band in $Gpdh^{AKy33}$ (Fig. 1) can be explained by heterodimer formation between GPDH-1 and GPDH-4. Heterodimer formation between GPDH-4 and GPDH-3 is not observed, most likely because these two isozymes are expressed in different tissues in $Gpdh^{AKy33}$ (Fig. 2).

As a transcript containing exons 1–7 has been identified in normal *Gpdh* alleles (7–9), it is possible that GPDH-4 is expressed in normal alleles but at levels below the limit of detection by standard staining.

The phenotype of another *blood*-induced allele, w^{bl}, is temperature-dependent, and it has been suggested that this property might be important in engineering temperature-sensitive mutants (22). We investigated the impact of temperature on the $Gpdh^{AKy33}$ phenotype, but no such effect was detected: flies raised at 18°C displayed exactly the same GPDH electrophoretic phenotype as those raised at 29°C (data not shown). This difference between w^{bl} and $Gpdh^{AKy33}$ could be a result of a different orientation of the inserted retrotransposon: in w^{bl}, *blood* is inserted in the transcriptional orientation opposite to that of the *w* gene (22), while in $Gpdh^{AKy33}$ the transcriptional orientation of the *blood* insertion is the same as that of *Gpdh*.

It is interesting that neither the Northern blot analysis nor the RACE analysis detected any transcripts that were polyadenylylated in the 3′ LTR of the *blood* retrotransposon, although this region is a direct repeat of the 5′ LTR and contains exactly the same polyadenylylation signals as the 5′ LTR (Fig. 6). Similarly in w^{bl}, polyadenylylation occurs in the 5′ LTR of the *blood* insertion and not in its 3′ LTR (22), even though the orientations of the *blood* insertions are different in w^{bl} and in $Gpdh^{AKy33}$. These findings suggest that the mechanism controlling the use of the polyadenylylation signals in the *blood* retrotransposon not only preferentially potentiates the polyadenylylation sites in the 5′ LTR but also is independent of the orientation of the inserted *blood* copy.

A model that would account for the above observations has been proposed to explain the use of polyadenylylation signals in mutant alleles induced by insertions of the *gypsy* retrotransposon (25). The protein encoded by the suppressor of Hairy-wing [*su(Hw)*] gene in *D. melanogaster* binds to multiple repeats of an octamer-like motif 5′-YRYTGCATAYYY-3′ in an internal non-LTR segment of *gypsy* (26). This pauses or terminates transcription, giving more opportunity for the utilization of upstream polyadenylylation signals by the RNA processing machinery. Two repeats of this motif with two mismatches each, separated by an 11-bp spacer, are present in the internal non-LTR part of *blood*, downstream from all of the polyadenylylation sites that are potentiated in *blood* (Fig. 7). These mismatches are likely to reduce the SU(HW) protein binding affinity and the potentiation of the upstream polyadenylylation sites (25), which would explain the apparently weaker effect of the *blood* insertions: insertions of *gypsy* usually reduce the level of the read-through transcript by 20- to 30-fold (27), whereas the insertions of *blood* in w^{bl} (22) and in $Gpdh^{AKy33}$ reduce the level of the read-through transcript by only ≈3-fold.

The properties of the naturally occurring $Gpdh^{AKy33}$ allele underline the potential significance of TEs for evolution in natural populations (28). The class of TEs that includes *blood* is structurally similar to retroviruses and related elements are found in a large variety of eukaryotic organisms (29). Retrotransposons are known to be complex mutagenic factors, which are able to modify the affected genes in various ways. There is evidence suggesting that in the past some of TE-induced mutations might have proven advantageous and survived in natural populations—there are known examples of eukaryotic genes containing fragments of TEs that are vital for the expression of those genes (30, 31). The expression of the GPDH-4 isozyme in $Gpdh^{AKy33}$, which is caused by the insertion of the *blood* retrotransposon, shows that TEs can induce the production of functional proteins, which could have distinctive properties and be of potential selective value. The case

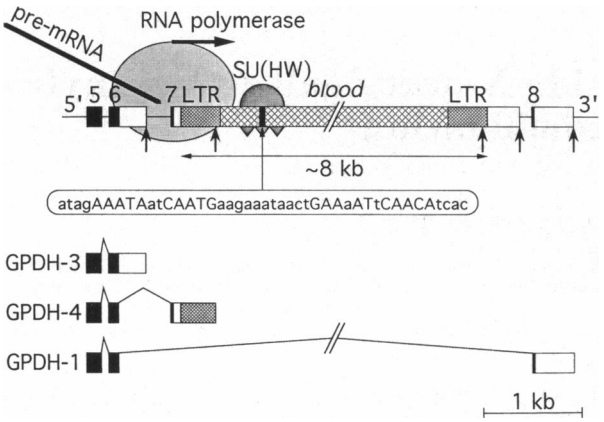

FIG. 7. Schematic representation of the proposed model of the *blood*-induced effects in $Gpdh^{AKy33}$. The symbols used are as in Fig. 6. To simplify the picture, only one polyadenylylation signal in each of the *blood* LTRs and only one of the transcripts polyadenylylated inside the *blood* insertion are shown. A solid bar indicates the position of the two repeated motifs that might serve as a binding site for the SU(HW) protein (25). Below is shown the sequence of this site with uppercase type indicating the consensus with the DNA sequence motif 5′-YRYTGCATAYYY-3′ recognized by the SU(HW) protein (26). The orientation of both repeats is opposite to the above consensus, but the SU(HW) recognition site functions in both orientations (25).

of $Gpdh^{AKy33}$ is unique in the sense that all the normal GPDH isozymes are still being expressed, and therefore, the production of GPDH-4 is an example of a true qualitative gain of function mutation induced by a TE insertion. The biochemical properties of GPDH-4 have not yet been investigated, but the isozyme does occur in specific tissues, particularly the thoracic flight muscles, and, as it differs in sequence from GPDH-1 and GPDH-3, it is likely to have different properties.

1. Smith, P. A. & Corces, V. G. (1991) *Adv. Genet.* **29,** 229–300.
2. Bingham, P. M. & Zachar, Z. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 485–502.
3. Finnegan, D. J. (1989) *Trends Genet.* **5,** 103–107.
4. Collier, G. E., Sullivan, D. T. & MacIntyre, R. J. (1976) *Biochim. Biophys. Acta* **429,** 316–323.
5. Wright, D. A. & Shaw, C. R. (1969) *Biochem. Genet.* **3,** 343–353.
6. O'Brien, S. J. & MacIntyre, R. J. (1972) *Genetics* **71,** 127–138.
7. Cook, J. L., Bewley, G. C. & Shaffer, J. B. (1988) *J. Biol. Chem.* **263,** 10858–10864.
8. Bewley, G. C., Cook, J. L., Kusakabe, S., Mukai, T., Rigby, D. L. & Chambers, G. K. (1989) *Nucleic Acids Res.* **17,** 8553–8567.
9. von Kalm, L., Weaver, J., DeMarco, J., MacIntyre, R. J. & Sullivan, D. T. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5020–5024.
10. Gibson, J. B., Cao, A., Symonds, J. & Reed, D. (1991) *Heredity* **66,** 75–82.
11. Gibson, J. B., Wilks, A. V., Cao, A. & Freeth, A. L. (1986) *Heredity* **56,** 227–235.
12. Chia, W., Savakis, C., Karp, R., Pelham, H. & Ashburner, M. (1985) *J. Mol. Biol.* **186,** 679–688.
13. Freeth, A. L., Gibson, J. B. & Wilks, A. V. (1988) *Genome* **30,** 25–30.
14. Symonds, J. E. & Gibson, J. B. (1992) *Biochem. Genet.* **30,** 169–188.
15. Gibson, J. B., Wilks, A. V. & Agrotis, A. (1992) *Mol. Biol. Evol.* **9,** 250–260.
16. Goldberg, D. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5794–5798.
17. Reed, D. S. & Gibson, J. B. (1993) *Proc. R. Soc. London B* **251,** 39–45.
18. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8998–9002.
19. Reed, D. S. & Gibson, J. B. (1994) *Biochem. Genet.* **32,** 161–179.
20. Triglia, T., Peterson, M. G. & Kemp, D. J. (1988) *Nucleic Acids Res.* **16,** 8186.
21. Wilbur, W. J. & Lipman, D. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 726–730.
22. Bingham, P. M. & Chapman, C. H. (1986) *EMBO J.* **5,** 3343–3351.
23. O'Hare, K., Murphy, C., Levis, R. & Rubin, G. M. (1984) *J. Mol. Biol.* **180,** 437–455.
24. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
25. Dorsett, D. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4373–4377.
26. Dorsett, D. (1991) *J. Cell. Biochem.*, Suppl. **15G,** 258.
27. Dorsett, D., Viglianti, G. A., Rutledge, B. J. & Meselson, M. (1989) *Genes Dev.* **3,** 454–468.
28. Biémont, C. (1992) *Genetica* **86,** 67–84.
29. Flavell, A. J. (1992) *Genetica* **86,** 203–214.
30. Goodchild, N. L., Wilkinson, D. A. & Mager, D. L. (1992) *Gene* **121,** 287–294.
31. Banki, K., Halladay, D. & Perl, A. (1994) *J. Biol. Chem.* **269,** 2847–2851.