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Genetics

Mutations in the *su(s)* gene affect RNA processing in *Drosophila melanogaster*

(transposable elements/RNA stability/suppression)

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ABSTRACT **We have studied the effect of mutations in the suppressor of sable [*su(s)*] gene on *P* element-induced yellow alleles. Two independent mutations tested, y^{76d28} and $y^{1\#7}$, contain a 1.1-kilobase (kb) *P* element inserted in the 5′ transcribed untranslated portion of the yellow gene. Sequences responsible for the $y^{1\#7}$ mutation are inserted in the same transcriptional orientation as yellow and cannot be processed by splicing, and this mutation is not suppressed by *su(s)* mutations. *P* element sequences are located in a transcriptional orientation opposite to that of the yellow gene in y^{76d28}; these sequences can be spliced from a composite *P* element–yellow mRNA, resulting in low accumulation of a functional 1.9-kb yellow transcript. The levels of both the putative precursor *P* element–yellow RNA and the 1.9-kb yellow transcript increase in y^{76d28} *su(s)* flies, suggesting that mutations in *su(s)* do not affect the efficiency of splicing of the *P* element sequences. Analysis of y^{76d28} cDNAs isolated from flies carrying a wild-type or mutant *su(s)* gene demonstrates that the choice of splice junctions to process *P* element sequences is unchanged in these different backgrounds, suggesting that mutations in *su(s)* do not affect the selection of donor and acceptor splice sites. We propose that the *su(s)* protein functions to control the stability of unprocessed RNA during the splicing reaction.**

Insertions of transposable elements into genes cause a wide variety of mutant phenotypes, dependent on both the location and the properties of a given element (1, 2). In some cases, transposable element-induced mutations can be affected by second-site modifier loci, causing either a more severe phenotype (enhancer loci) or complete or partial restoration of the wild-type phenotype (suppressor loci) (3).

Molecular characterization of the interactions of modifier loci and transposable element-induced mutations has indicated that modifier genes encode proteins that are involved in fundamental processes of gene expression. For example, the suppressor of Hairy-wing [*su(Hw)*] gene encodes a DNA-binding protein with hallmarks of a transcriptional activator (4, 5). Mutations in this gene reverse the phenotypic effects of a subset of *gypsy*-induced mutations (6). In the case of the yellow allele y^2, it appears that *gypsy* mutagenesis results from the binding of the *su(Hw)* protein to the transposable element, inactivating enhancer elements that reside distal to the site of *gypsy* insertion (7). Additionally, the suppressor of white-apricot [*su*(w^a)] locus, which reverses the phenotypic effect of a *copia* insertion into an intron in the white gene (8), encodes a protein with an RNA-binding domain. It has been proposed that this protein is involved in RNA processing events (9, 10).

Mutations in the suppressor of sable [*su(s)*] gene reverse the phenotype of mutations caused by insertion of the retrotransposon 412 (11). In the three suppressible vermilion alleles, v^1, v^2, and v^k, a *412* element is inserted into the transcribed untranslated region of the vermilion gene in the opposite transcriptional orientation. These flies accumulate a trace amount of a wild-type-sized vermilion transcript that increases in abundance in a *su(s)* mutant background. This wild-type-sized vermilion transcript is produced from the splicing of the *412* element from the precursor RNA (12). Recently, it has been reported that mutations in *su(s)* can affect non-retrotransposon-induced mutations. M. Simmons found that a *P*-induced allele of singed (sn^w) was suppressed by mutations in *su(s)* (13). This observation suggests that the mechanism of mutagenesis and not necessarily the properties of the transposable element is an important determinant of whether a given allele is suppressed by *su(s)* mutations.

To further understand the molecular basis of suppression by *su(s)*, we have used the molecularly well-characterized yellow gene as a model system. The yellow gene is required for pigmentation of larval and adult cuticle structures. This gene encodes a single 1.9-kilobase (kb) RNA that is differentially expressed during development as a result of several tissue-specific enhancer elements that reside in the 5′ flanking DNA and within the intron (14). A number of *P* element-induced alleles of the yellow gene have been isolated (15, 16). Here we report experiments testing the effects of mutations in the *su(s)* gene on two *P*-induced mutations at yellow, y^{76d28} and $y^{1\#7}$. Our results confirm that mutations in *su(s)* can affect non-retrotransposon-induced alleles and suggest that the *su(s)* protein is involved in RNA turnover during the splicing process.

MATERIALS AND METHODS

Isolation of *su(s)* Mutant Lines. *P*-induced *su(s)* mutations were induced on an X chromosome containing the ras^2 v^1 *m* mutations, as well as the yellow allele to be tested using an *MR* element to mobilize *P* elements as described in ref. 15. Flies were raised at 22°C and 70% relative humidity.

Characterization of Mutant Alleles. *Drosophila* DNA was isolated as described in ref. 17. Southern and Northern analysis were carried out as described in ref. 18. cDNA was isolated by using PCR amplification as described in ref. 19. PCR products were subcloned in pUC18. DNA sequence analysis of the cDNA was done by using the dideoxy chain termination method (20) with Sequenase (United States Biochemical).

RESULTS

Molecular Structure of *P* Element-Induced yellow Mutants. The effects of mutations in the *su(s)* locus were determined by using two different *P* element-induced mutations of the yellow gene designated y^{76d28} and $y^{1\#7}$. In each of these

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mutations, pigmentation in every tissue of the fly is affected. $y^{1\#7}$ is a null mutation, whereas in y^{76d28} flies all larval and adult cuticular structures are tan (intermediate between wild-type and null). In both y^{76d28} and $y^{1\#7}$, a 1.1-kb *KP* element is inserted 76 base pairs (bp) downstream of the transcription start site but in opposite orientations (Fig. 1). The direction of the *P* element insertion is of phenotypic consequence, since y^{76d28} flies are more darkly pigmented than $y^{1\#7}$ flies. Northern analysis of late pupal poly(A)$^+$ RNA isolated from y^{76d28} and $y^{1\#7}$ was done to determine the molecular basis for this phenotypic difference (Fig. 2). In y^{76d28} flies, two RNAs, 3.0 and 1.9 kb, accumulate at levels lower than the level of the wild-type 1.9-kb RNA. The sizes of these RNAs are consistent with the larger transcript being a *P* element–yellow composite RNA with the yellow intron removed, while the 1.9-kb RNA arises from further splicing of the 3.0-kb RNA removing *P* element sequences (see below). The 1.9-kb RNA is responsible for the production of yellow protein; the low level of pigmentation parallels the amount of 1.9-kb RNA. In $y^{1\#7}$ flies, where the *P* element is in same transcriptional orientation as the yellow gene, two RNAs accumulate whose size are 3.0 kb and 1.0 kb (Fig. 3). The 3.0-kb RNA is of identical size to the large RNA in y^{76d28}, suggesting it represents a composite *P* element–yellow mRNA. The smaller RNA hybridizes to sequences 5′ of the *P* element insertion (data not shown) and most likely results from initiation at the wild-type yellow promoter and termination within the *P* element. A summary of the structure of the yellow gene and the RNA species transcribed from y^{76d28} and $y^{1\#7}$ is shown in Fig. 1.

Effect of *su(s)* Mutations on the Phenotype of *P*-Induced yellow Alleles. Both *su(s)* and *y* map to X chromosome position 0.0, and they can be separated only rarely. One of us (M.M.G.) found that the *su(s)* locus is a hot spot for *P* insertion (see ref. 22). For this reason, we generated new *su(s)* alleles via *P* insertion, using the *P* mutagenesis system described by Hiraizumi (23). In this system, *P* element

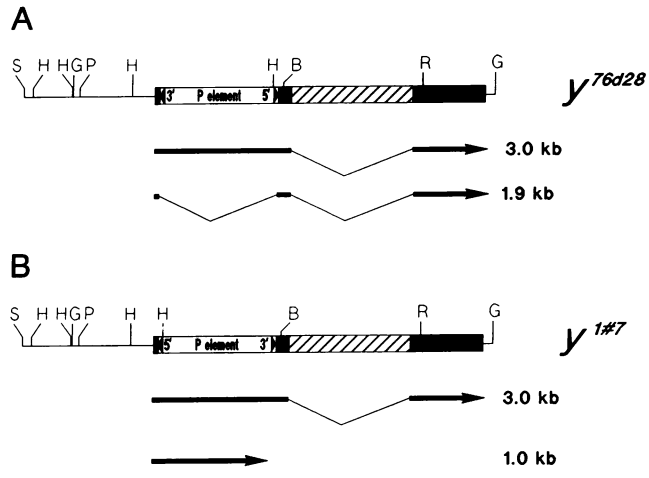

FIG. 1. DNA structure of *P* element-induced yellow mutations. (*A*) The top shows a restriction map of the y^{76d28} allele indicating the position of insertion of the 1.1-kb *P* element. The *P* element is inserted into the yellow gene 76 bp downstream of the transcription initiation site, in the opposite transcriptional orientation. *P* element 39-bp inverted repeats are represented by the arrowheads. Dark boxes represent exons and the hatched box indicates the position of the intron. The lower portion shows the structure of the 3.0- and 1.9-kb poly(A)$^+$ RNAs which accumulate in mid-to-late pupae. (*B*) The top shows the restriction map of $y^{1\#7}$. The same *P* element is inserted at the same position in the yellow gene as in y^{76d28} but in the opposite transcriptional orientation. The structure of the 3.0- and 1.0-kb RNAs expressed in this mutant are shown in the lower portion. Symbols for restriction enzymes are as follows: S, *Sal* I; H, *Hin*dIII; G, *Bgl* II; B, *Bam*HI; R, *Eco*RI.

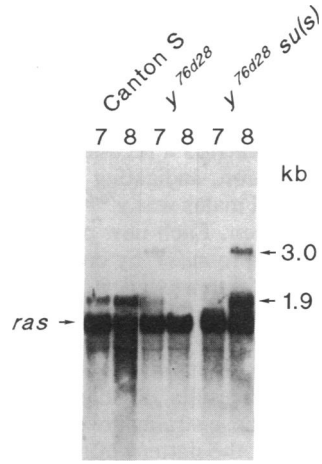

FIG. 2. Northern blot analysis of RNA accumulated in y^{76d28} and y^{76d28} $su(s)^g$. Ten micrograms of poly(A)$^+$ RNA was isolated from pupae at days 7 and 8 of development of Canton S, y^{76d28}, and y^{76d28} $su(s)^g$, electrophoresed on a 1.5% agarose/formaldehyde gel, blotted onto nitrocellulose, hybridized with a ^{32}P-labeled fragment of a yellow cDNA (18), and then probed with a DNA fragment containing the *Drosophila ras2* gene (21). The numbers at the top refer to the number of days the flies developed after embryo collection. The yellow cDNA hybridizes to two mRNAs in both y^{76d28} and y^{76d28} $su(s)^g$: 3.0 and 1.9 kb. The *ras2* gene hybridizes to a 1.6-kb RNA which serves as a control for the amount of RNA loaded per lane.

mobilization is under the control of a patroclinously inherited *MR* element linked to either the second or third chromosome. Males carrying an *MR* and an X chromosome with one of the two *y* mutations described above, as well as the linked eye color mutations raspberry-2 (ras^2) and vermilion-1 (v^1), were crossed to double-X females. The resultant male progeny, who inherited their X chromosome from their fathers, were screened for mutations in the *su(s)* gene. The eye color mutations ras^2 v^1 serve to monitor the occurrence of a *su(s)* mutation, allowing a clearer distinction between v^+ and *v* phenotypes. *su(s)*$^+$ ras^2 v^1 males exhibit an orange eye color; while *su(s)*$^-$ ras^2 v^1 males have a maroon eye color. This selection strategy has been previously used to identify *su(s)* mutations (24). Since y^{76d28} reverts to wild-type in the presence of an *MR* element at a frequency of about 1%, induced *su(s)* mutations were first identified through their suppression of v^1.

Among ≈5000 males scored in the y^{76d28} *P* mutagenesis screen, two independent males were recovered which carried

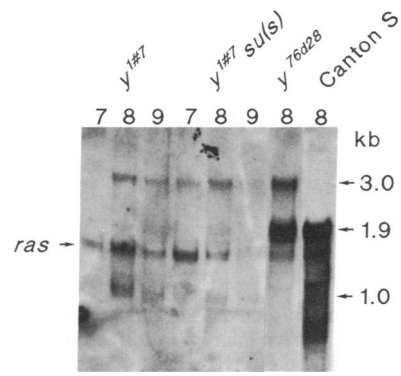

FIG. 3. Northern blot analysis of RNA accumulated in $y^{1\#7}$ and $y^{1\#7}$ $su(s)^{18\text{-}2}$. RNA was isolated from pupae on days 7, 8, and 9 of development. The Northern blot was prepared as described in the legend to Fig. 2. The yellow cDNA hybridizes to two RNAs, 3.0 and 1.0 kb, in $y^{1\#7}$ and $y^{1\#7}$ $su(s)^{18\text{-}2}$ flies. For a reference, y^{76d28} and Canton S RNAs isolated from pupae on day 8 of development were run in parallel. No wild-type RNA of 1.9 kb accumulates in $y^{1\#7}$ flies.

putative *su(s)* mutations. Both males had a maroon eye color and cuticle pigmentation darker than that of y^{76d28} but not quite wild-type in color. To verify the isolation of new *su(s)* mutations, each male was crossed to y^{76d28} ras^2 v^1 females. The resultant female progeny had orange eyes, as is expected if the parental males carried a recessive *su(s)* mutation and tan cuticle pigmentation, indicating that the yellow gene present in the parental males was y^{76d28} and had not reverted in the mutagenic screen. Each new putative *su(s)* mutation was tested for allelism to $su(s)^2$ by crossing to $su(s)^2$ ras^2 v^1 females. Since v^1 is suppressed in the female progeny, functional allelism was confirmed. The new *su(s)* mutations were designated $su(s)^g$ and $su(s)^{g2}$.

Southern analysis of genomic DNA was undertaken to confirm these genetic results. Results obtained from DNA isolated from one of these strains are shown in Fig. 4. These data demonstrate that the pattern of restriction fragments is unchanged when probed with yellow sequences. However, changes in the restriction fragment pattern were observed when hybridization was with *su(s)* sequences. The $su(s)^g$ allele induced in the y^{76d28} chromosome has an insertion into the wild-type 3.1-kb *su(s)* fragment, changing it to 4.0 kb. This fragment contains the 5′ end of the *su(s)* gene and is the region into which most *P* element insertions occur (22). These results indicate that the y^{76d28} chromosome contains a new mutation in the *su(s)* gene. The same conclusion was obtained from analysis of y^{76d28} $su(s)^{g2}$ genomic DNA (data not shown). Since all adult cuticular structures of *su(s)* y^{76d28} flies are more darkly pigmented than those of y^{76d28}, we conclude that mutations in *su(s)* are able to suppress the effects of the *P* element inserted in y^{76d28}.

Further evidence for this conclusion was obtained from the analysis of a spontaneous revertant of the $su(s)^g$ mutation. While the stock y^{76d28} $su(s)^g$ ras^2 v^1 was being maintained, a single male arose that had tan cuticle and orange eyes. When this male was crossed to double-X females, the resultant male offspring also carried tan cuticle and orange eyes. Southern analysis of genomic DNA isolated from these progeny showed that the restriction pattern of the *su(s)* gene was restored to wild type, while that of the *y* gene remained mutant (data not shown). These results confirm the correlation of the allelic state of the *su(s)* gene with phenotypic suppression of y^{76d28} and argue against background differences as a probable cause of this effect.

After *P* mutagenesis of $y^{1\#7}$ ras^2 v^1 males, two independent males, from the ≈5000 progeny screened, had maroon eye coloration but a yellow null cuticle pigmentation. Genetic analysis confirmed that these flies carried a mutation in the *su(s)* locus. The eye color in these flies was indistinguishable from that in the y^{76d28} $su(s)^g$ ras^2 v^1 flies, indicating that these new *su(s)* alleles are equivalent in their ability to suppress the v^1 mutation. The observed yellow null phenotype could result either from no change in the parental $y^{1\#7}$ gene or from imprecise *P* element excision associated with a deletion. To confirm that the state of the yellow locus was unchanged in these flies, genomic Southern analysis was carried out. Fig. 5 shows the results of these experiments. Southern blot analysis showed that the restriction fragment pattern of the yellow gene was identical to that of the parental $y^{1\#7}$ stock. However, in each case, the restriction pattern of the *su(s)* locus differed from that of wild type. The restriction pattern of the two alleles, $su(s)^{18\text{-}2}$ and $su(s)^{18\text{-}19}$, indicates the presence of an insertion into the same 3.1-kb fragment mentioned above. In $su(s)^{18\text{-}2}$ 3.2- and 4.0-kb bands are observed. In the case of $su(s)^{18\text{-}19}$, the 3.1-kb *su(s)* fragment is replaced by 3.0- and 3.4-kb fragments. These data indicate that these new *su(s)* mutations represent independent isolates. Since the phenotypes of $y^{1\#7}$ and $y^{1\#7}$ *su(s)* are indistinguishable, we conclude that the *P* element insertion in $y^{1\#7}$ is not suppressible by *su(s)* mutations.

Effect of *su(s)* Mutations on the Transcription of *P*-Induced yellow Alleles. Our results indicate that only a subset of *P*

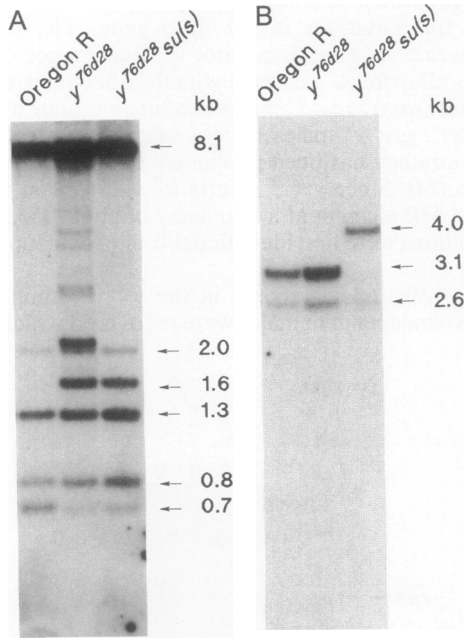

FIG. 4. (*A*) Southern analysis of genomic DNA isolated from y^{76d28} and y^{76d28} $su(s)^g$. Five micrograms of total genomic DNA was digested with *Hin*dIII and *Bam*HI, electrophoresed on a 1% agarose gel, blotted onto nitrocellulose, and probed with [^{32}P]DNA corresponding to the 6-kb *Sal* I–*Eco*RI fragment of the wild-type yellow locus (14). The numbers at the sides indicate the size of the hybridizing fragment. The additional higher molecular weight fragments seen in the y^{76d28} lane are due to partial digestion (for comparison, see ref. 15). (*B*) The Southern blot was prepared as described above except it was hybridized with the plasmid p4.1 containing sequences of the 5′ end of the *su(s)* gene (22).

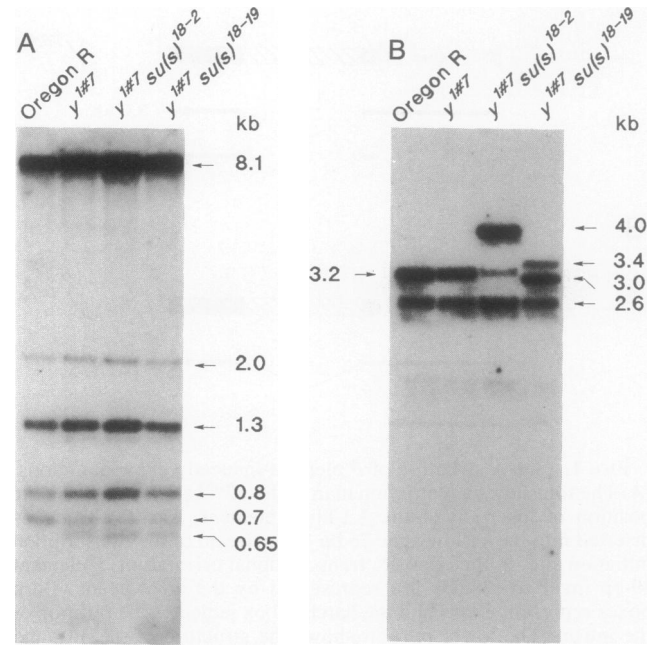

FIG. 5. Southern analysis of genomic DNA isolated from $y^{1\#7}$ and $y^{1\#7}$ *su(s)* double mutants. (*A*) Southern blot was prepared as described in the legend of Fig. 4 and hybridized with yellow sequences corresponding to the 6-kb *Sal* I–*Eco*RI fragment. (*B*) The Southern blot was hybridized with plasmid p4.1 containing the 5′ end of the *su(s)* gene.

element-induced mutations are suppressed by mutations in the *su(s)* locus. In both y^{76d28} and $y^{1\#7}$, the same *P* element is causing the mutation (15). The most striking difference between these two *P* element alleles is that the transcribed hybrid yellow–*P* element mRNA appears to be further processed to a functional mRNA in y^{76d28}, whereas this does not occur in $y^{1\#7}$. Northern analysis was done to determine the molecular basis for the increased pigmentation of y^{76d28} $su(s)^g$ flies. These results are shown in Fig. 2. Two *y* mRNAs accumulate in the y^{76d28} $su(s)^g$ flies. The 3.0-kb hybrid *P* element–yellow mRNA and the 1.9-kb wild-type size RNA both increase in abundance relative to that found in y^{76d28}, such that the ratio of the two transcripts is similar in y^{76d28} and y^{76d28} $su(s)^g$ flies. The increase in the 1.9-kb RNA is consistent with the resultant darker pigmentation in the adult cuticle.

The same analysis was done with poly(A)$^+$ RNA isolated from the $y^{1\#7}$ $su(s)^{18\text{-}2}$ flies in which the phenotype of the yellow gene is not affected by the presence of the *su(s)* mutation. Results from this experiment are shown in Fig. 3. The 3.0- and 1.0-kb RNAs present in $y^{1\#7}$ are also observed. However, the level of both mRNAs is the same as that found in the parental $y^{1\#7}$ strain. Similar results were obtained for $y^{1\#7}$ $su(s)^{18\text{-}19}$ (data not shown). Thus, two different *su(s)* alleles are unable to increase the level of RNA produced from the $y^{1\#7}$ gene. These results indicate mutations in the *su(s)* locus do not have a general affect on yellow gene expression.

Processing of *P* Element Sequences Is Not Affected by Mutations in *su(s)*. Northern analysis was extended by isolation of partial yellow cDNAs from y^{76d28} flies. Midpupal poly(A)$^+$ y^{76d28} RNA was reverse transcribed into single-stranded cDNA and yellow sequences were amplified from this mixture by using PCR. DNA sequence data obtained from 33 y^{76d28} cDNAs are summarized in Fig. 6. A number of different donor and acceptor sites are used to process the *P* element from the y^{76d28} transcript. The majority of the cDNAs (31/33) contain the splice donor site in the 39-bp inverted repeat of the *P* element. This donor site AAG|GTGGTC (class 1) has a 4/9 match to the 5′ splice site of the normal yellow intron ATG|GTAAGT and a 5/9 match to the consensus invertebrate 5′ splice junction AAG|GTAAGT described by Shapiro and Senapathy (25). In two cases, the 5′ donor sequence was found to be within the yellow sequence AAG|GCTAGA (classes 3 and 5). The most striking feature of this junction is the fact that it does not contain the most highly conserved GT. These donor sites can splice to a variety of acceptor sites which all reside in yellow sequences.

Splice junctions at the 3′ end are characterized by a pyrimidine-rich stretch preceding four highly conserved nucleotides at the splice junction CAG|G, the exact sequences being less highly conserved than the 5′ junction (26). In the PCR-amplified product of y^{76d28} mRNA, the most prevalent class of cDNAs (class 1, 31/33) was spliced at the sequence AACCCCCTATAG|C. This sequence is pyrimidine rich and contains the conserved AG at the junction point. The other two acceptor sites sequenced from the y_{76d28} cDNAs were CTTTGGCTTAAG|T (class 3, 1/33) and GCAATGTTC-CAG|G (class 5, 1/33). This later splice junction is downstream of the translation initiation site and so this mRNA would not produce a functional yellow protein.

PCR amplification of cDNA made from y^{76d28} $su(s)^g$ poly(A)$^+$ RNA was done to determine if the spliced junctions remained unchanged in the mutant background. In this case, 20 cDNA clones were obtained and sequenced. This analysis demonstrated that the splicing events in the double mutant paralleled those in y^{76d28}. Again, the primary 5′ donor site chosen was AAG|GTGGTC (classes 1, 2, and 4; 14/20). However, a greater proportion of these cDNAs contained the junction that does not contain the conserved GT: AAG|GCTAGA (6/20). The 3′ acceptor sites used were the same as found in a $su(s)^+$ background; class 1 had 6/20, class 2 had 3/20, class 4 had 5/20, and class 5 had 6/20. The fact that both classes 4 and 5 cause the loss of the translation initiation site suggests that about half of the 1.9-kb RNA that accumulates in these flies does not produce a functional protein.

DISCUSSION

Here we demonstrate that *P* element-induced mutations can be suppressed by mutations in the *su(s)* locus. We studied the effects of *su(s)* mutations on two different *P* element-induced alleles of the yellow gene and found that the mechanism of *P* mutagenesis influences whether a *P* element mutation can be affected. The yellow mutation y^{76d28}, which is suppressed by mutations in the *su(s)* gene, results from the insertion of a *P* element into the transcribed untranslated portion of the gene. Northern analysis and DNA sequencing of y^{76d28} cDNAs demonstrate that *P* element sequences are spliced to produce a wild-type 1.9-kb RNA. The predominant 5′ donor site is within the 39-bp inverted repeat of the *P* element, while all of

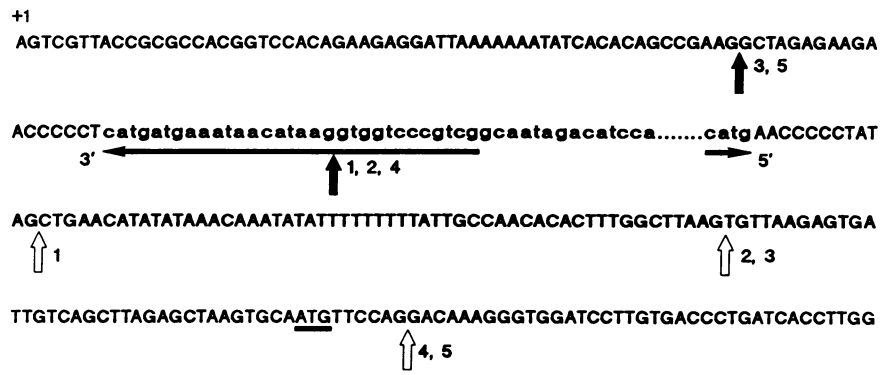

FIG. 6. Splice junctions surrounding the *P* element insertion in y^{76d28}. The DNA sequence of the y^{76d28} gene is shown. The uppercase letters represent yellow sequences beginning at the 5′ end of the mRNA (+1), and the lowercase letters are of the *P* element. A partial sequence of the *P* element is shown. Horizontal arrows indicate the extent and position of the *P* element 39-bp inverted repeats. The underlined ATG indicates the initiator methionine. Five different classes of cDNAs were obtained by PCR amplification of both y^{76d28} and y^{76d28} $su(s)^g$ poly(A)$^+$ RNA. The 5′ primer used in the cDNA amplification contained yellow sequences upstream of the *P* element insertion site 5′-CGGTACCACGGTCCACAGAAGAAGATTA (starting at position +12 relative to the transcription start site). The 3′ primer was 5′-GTCACAAGGATCCACCCTTTGTCCT, which is at position +118 relative to the start site of transcription and is downstream of the *P* element insertion site. Arrowheads under the DNA sequence indicate the position of the 5′ donor (filled-in arrow) and 3′ acceptor (empty arrow) sites. Numbers associated with each arrow indicate which donor and acceptor were used in a given class of cDNA.

the 3′ acceptor sites are within yellow sequences. The $y^{1\#7}$ mutation that results from insertion of the same *P* element at the exact location as the one in y^{76d28} but in the opposite transcriptional orientation is not suppressed by *su(s)* mutations. A 1.9-kb transcript is not detected in these flies even though the splice junctions are present in the precursor RNA. The lack of processing could result from differential efficiency of splicing caused by a different secondary structure in the $y^{1\#7}$ RNA. These results suggest that the mechanism by which mutations in the *su(s)* locus affects gene expression is by altering some process involved in splicing of heterogeneous nuclear RNA.

Both donor and acceptor sites used to remove *P* element sequences from a y^{76d28} precursor RNA do not conform well with the invertebrate donor consensus site (25). A comparison made between the choices of splice junctions in a *su(s)* mutant and wild-type background showed that the same splice acceptor and donor sites were used although the frequency differed. In a mutant *su(s)* background, we found more cDNAs containing the 5′ splice site lacking the consensus GT base pair. This may reflect that the selection of a given splice site is more relaxed in the absence of *su(s)* protein. However, since these cDNAs were isolated by using PCR amplification, this may not be an accurate representation of the abundance of a given mRNA in the pool.

Our data indicate that *su(s)* mutations reverse the mutant phenotype of y^{76d28} flies by increasing the level of precursor transcript from which to produce the wild-type mRNA. In the y^{76d28} flies, in addition to the 1.9-kb RNA, a 3.0-kb transcript accumulates that we suggest represents the precursor to the 1.9-kb species. Alternatively, the 3.0-kb RNA may represent a stable, aberrantly spliced transcript that is not further processed. We favor the precursor hypothesis for the following reasons. First, the size of this RNA is consistent with it being a *P* element–yellow hybrid RNA in which the normal yellow intron has been removed. Second, $y^{1\#7}$ flies also accumulate a 3.0-kb RNA. The simplest explanation for the identically sized transcript is that they represent an RNA in which just the normal yellow intron has been removed. If instead the 3.0-kb transcripts in y^{76d28} and $y^{1\#7}$ were both end products of an aberrant splicing event, these processes should be similar and thus the cryptic splice junction would have to reside in either yellow sequences or the 39-bp *P* inverted repeats since the precursor RNAs contain different sequences corresponding to the *P* element. It is difficult to imagine that $y^{1\#7}$ precursor would use this hypothetical splice junction but be incapable of using the junctions characterized in y^{76d28} to give rise to the 1.9-kb transcript. Finally, $y^{13\text{-}11}$ flies, which carry a smaller *P* element of 405 bp inserted at the same position in the transcribed untranslated portion of the gene as is y^{76d28}, accumulate two RNAs, 1.9 and 2.3 kb (15). The 2.3-kb RNA is again the predicted size of a *P* element–yellow hybrid precursor RNA. In $y^{13\text{-}11}$ flies, the level of 1.9-kb RNA is increased relative to that of y^{76d28}, restoring wild-type cuticle pigmentation. Concomitant with the increase in the 1.9-kb transcript is an increase in the 2.3-kb transcript. This is the expected result if this transcript is a precursor to the wild-type-sized transcript and supports the contention that in y^{76d28} the 3.0-kb transcript is the precursor for the 1.9-kb transcript.

The marked increase in both the putative 3.0-kb *P*–yellow mRNA precursor and the 1.9-kb wild-type-sized RNA in y^{76d28} $su(s)^{g}$ flies could be explained by two alternatives. The normal function of the *su(s)* protein may be to degrade unprocessed RNA or it may repress yellow transcription. The fact that mutations in the *su(s)* locus do not increase the yellow mRNA in $y^{1\#7}$ *su(s)* flies strongly argues that the function of the *su(s)* protein is to affect some event involved in RNA splicing. A comparison of the amount of yellow mRNA accumulated in a *su(s)* wild-type and mutant background supports this conclusion (data not shown).

What is the function of the *su(s)* protein? Our results argue against a role for the *su(s)* protein in decreasing the efficiency of splicing reactions at cryptic splice sites, since the level of the 1.9-kb RNA does not increase to a much greater extent than does the level of the putative precursor 3.0-kb species. In addition, the selection of splice junctions is not altered by *su(s)* mutations. We suggest that mutations in the *su(s)* gene cause a stabilization of unprocessed RNA that allows more time for splicing at abnormal splice junctions. It can be imagined that the *su(s)* protein affects RNA stability during mRNA processing by either preventing recognition of poor splice junctions or destabilizing spliceosome formation on cryptic sites, which leads to rapid degradation of the heterogeneous nuclear RNA. Consistent with this suggestion is the identification of a region in the *su(s)* protein that is homologous to domains of other *Drosophila* and vertebrate proteins involved in RNA processing (27).

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