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Interactions among the Gypsy Transposable Element and the Yellow and the Suppressor of Hairy-Wing Loci in *Drosophila melanogaster*

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We cloned and characterized the yellow locus of *Drosophila melanogaster*. We also studied its transcription pattern in the suppressible allele y^2, which is caused by the insertion of the transposable element gypsy, and the effect of mutations at the unlinked suppressor of Hairy-wing locus on the transcription of yellow RNAs. The gypsy element is transcribed in a temporal fashion that correlates with the pattern of expression of the yellow locus. We propose that the mutational effect of the gypsy element is due to developmentally specific transcriptional interference on yellow transcription. Mutations at the *su(Hw)* locus reverse this effect by altering the quantitative expression of gypsy.

The phenomenon of suppression, in which mutations at a suppressor locus are able to reverse the phenotype of other unlinked mutations, has been described in various eucaryotic organisms such as plants, yeasts, insects, and mammals (7, 17, 19, 25). This phenomenon is different from the translational nonsense suppression in bacteria, since the suppressor loci are recessive and act as null mutations. The suppressible alleles are always caused by spontaneous mutations and are associated with the insertion of transposable elements. The transposon acts in *cis* on the gene located nearby, whereas the suppressor loci act in *trans* to alter the stability of the transposon or the expression of the gene adjacent to it or both, thus reversing the mutant phenotype.

The suppressor of Hairy-wing locus [*su(Hw)*, 3-54.8] affects a wide variety of mutations in *Drosophila melanogaster* (see reference 15). All the suppressible alleles are spontaneous and caused by the insertion of the transposable element gypsy (19). This transposon has a retrovirus-like structure with two long terminal repeats (LTRs) which contain transcription initiation (TATATAA) and termination (AATAAA) signals (12). While other transposable elements, such as copia, seem to interfere with proper transcription termination (14), little is known about the molecular basis of gypsy-induced mutations or the mechanisms by which mutations at the *su(Hw)* locus reverse the phenotype of these mutations. To approach these problems, we cloned and characterized the forked (24) and yellow loci of *D. melanogaster* to use as a model system in which to ask questions related to the phenomenon of suppression. This paper describes the studies carried out on the yellow (*y*, 1-0.0) locus, which is involved in controlling the pattern of pigmentation of the body of the fly; the visible phenotypic effect of *y* mutations is an altered pigmentation of the adult body cuticle and its derivative structures. The color of the cuticle in *y* mutants ranges from yellow to brown, whereas wild-type cuticle appears gray to black. This altered yellowish pigment seems to be a qualitatively different form of the wild-type black melanin (26). The different yellow alleles have been divided into two phenotypic classes. Type 1 mutants are amorphic and display the *y* phenotype throughout the adult cuticle and also in the pigmented structures of the larva; type 2 mutants, on the other hand, exhibit a mutant phenotype only in some cuticular structures while showing a partial or complete wild-type expression in others (22). The alleles we examined in this study belong to this second group. Although many alleles of *y* are type 2 mutants, no two have exactly the same phenotype; in these various alleles at least 40 different structures can independently express the normal or mutant *y* phenotype. These pattern mosaics are probably the result of differential yellow gene expression in the various cell types which form the adult cuticle, a conclusion which requires that the *y* gene can be autonomously regulated in each cell type (21).

Alleles at the yellow locus have also been associated with behavioral defects, including reduced locomotor activity and a low level of male competitive mating success (5). The yellow gene(s) has been implicated in the control of tyrosine and 3,4-dihydroxyphenylalanine utilization in the biosynthetic pathways leading to the production of melanin, sclerotin, and catecholamines. An impairment of catecholamine production implies that the behavioral effects associated with the yellow mutation have a neurochemical basis, since dopamine and noradrenaline are important in neural transmission (4). In support of this, Burnet and Wilson (5) have reported a significantly lower titer of noradrenaline in *y* males compared with that in wild-type males and suggest that the behavioral effects of the locus could arise if yellow gene expression in type 2 mutants causes abnormal catecholamine balance in different regions of the nervous system.

We present evidence here which indicates that the yellow locus encodes a 1.9-kilobase (kb) RNA and that the gypsy element does not cause the mutant phenotype of the y^2 allele by affecting termination of transcription, but rather alters its expression by a mechanism similar to that of the transcriptional interference phenomenon (10).

MATERIALS AND METHODS

Description of *Drosophila* stocks. Flies were raised at 22°C and 70% relative humidity. The stocks $y^2\ cv\ v\ f^1$ and $y^{25}\ f^1\ w^{34e}$ were obtained from the *Drosophila* Stock Center at Bowling Green, Ohio. The stock $y^2\ sc\ v\ f^1\ ct^6$; $su(Hw)^{69k}\ e^s/TM6$, $su(Hw)^f$ was obtained from E. H. Grell (Oak Ridge National Laboratory).

Isolation of nucleic acids and gel electrophoresis. Plasmid DNA was isolated by standard procedures (16). DNA from adult flies was isolated by homogenization in 0.1 M NaCl–0.2

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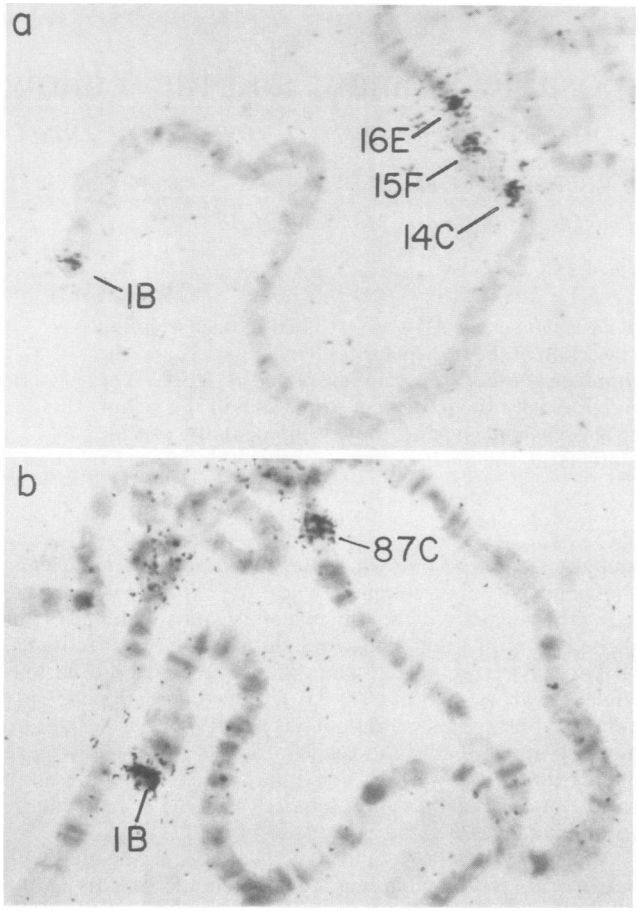

FIG. 1. In situ hybridizations to polytene chromosomes. (a) DNA from a cloned gypsy element was labeled with ^{125}I-dCTP by nick translation and hybridized to polytene chromosomes from salivary glands of third-instar y^2 larvae. (b) A lambda clone (λy1), obtained by probing a y^2 library with the gypsy element, was labeled as above and hybridized to wild-type polytene chromosomes. Hybridization grains can be observed at 1B, the chromosomal location of the yellow locus.

M sucrose–10 mM EDTA–0.5% Triton X-100–30 mM Tris hydrochloride (pH 8.0) followed by filtration through Nitex no. 15 to eliminate cuticular debris. The nuclei were pelleted by centrifugation, suspended in 0.35 M NaCl–10 mM EDTA–10 mM Tris hydrochloride (pH 8.0), and then lysed with 1% *N*-lauroyl sarcosine. The DNA was then phenol extracted and precipitated with ethanol. RNA from different stages of development was prepared by lysing the tissues in a Dounce homogenizer in 4 M guanidine isothiocyanate–0.2% *N*-lauroyl sarcosine–150 mM mercaptoethanol–12.5 mM EDTA–50 mM Tris hydrochloride (pH 7.5). After addition of an equal volume of 100 mM sodium acetate (pH 5.0), the RNA was extracted with phenol-chloroform at 65°C and then kept 10 min on ice. After three cycles of this treatment, the RNA was finally precipitated with 2 volumes of ethanol. Poly(A)-containing RNA was selected by chromatography on oligo(dT)-cellulose. DNA was electrophoresed on 1% agarose gels; RNA was electrophoresed on 1% agarose–formaldehyde gels (8). After electrophoresis, the nucleic acids were transferred to BioTrans (ICN Pharmaceuticals Inc., Irvine, Calif.) in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and hybridized with ^{32}P-labeled DNA in a solution containing 50% formamide, 5× SSC, 10 mM phosphate (pH 6.7), 10% dextran sulfate, and 0.1% sodium dodecyl sulfate. The filters were then washed twice in 2× SSC–0.1% sodium dodecyl sulfate at room temperature and twice in 0.1% SSC–0.1% sodium dodecyl sulfate at 50°C.

DNA enzymology, construction of DNA libraries, and in situ hybridizations. Digestion of DNA with restriction enzymes, ligation of DNA fragments, labeling of DNA by nick translation, and screening of lambda libraries were carried out by standard procedures (16). The genomic DNA library was constructed by partial digestion of DNA from y^2 flies with *Mbo*I followed by cloning of the isolated restriction fragments into the *Bam*HI site of the λ vector EMBL 3 (13). Synthesis of strand-specific RNA probes with SP6 vectors was carried out by using SP6 polymerase and [^{32}P]UTP and [^{32}P]GTP as radioactive precursors (9, 18). In situ hybridizations to polytene chromosomes were carried out by the method of Pardue and Gall (23). The hybridization probe was plasmid DNA labeled by nick translation with ^{125}I-dCTP (2,200 Ci/mmol; New England Nuclear Corp., Boston, Mass.) as a radioactive precursor.

RESULTS

Cloning and characterization of the yellow locus. To understand the molecular basis of suppression in *D. melanogaster*, we decided to investigate the mechanisms underlying this phenomenon using the yellow locus as a model system. The strategy we undertook to clone this gene(s) was based on the fact that the y^2 allele is caused by the insertion of the transposable element gypsy at or near the yellow locus (19). Figure 1a shows an in situ hybridization of the gypsy element to polytene chromosomes from y^2 larvae. Hybridization can be observed at subdivision 1B, the chromosomal location of the yellow locus, indicating that this particular yellow allele is caused by the insertion of the gypsy element. DNA sequences defining this locus were then isolated by probing a λ library made with DNA from y^2 flies with the gypsy element. One of the λ clones obtained, designated λy1, gave positive hybridization at subdivision 1B of wild-type polytene chromosomes, indicating that this clone contained DNA sequences from the yellow locus (Fig. 1b). Using a DNA fragment adjacent to the gypsy element in this clone as a hybridization probe, we cloned 16 kb of wild-type DNA around the yellow locus by probing a Canton-*S* genomic λ library. Figure 2 shows a restriction map of this region, indicating the insertion point of the gypsy element in y^2. The location of the molecular defects associated with several other yellow alleles was determined by Southern analysis of genomic DNA from these mutants (data not shown). The other three mutant alleles tested, y^{2S}, y^{25}, and y^{c4}, are caused by the insertion of DNA sequences within the same 0.5-kb *Hin*dIII-*Bam*HI fragment. The approximate position on the DNA of an unidentified insertion sequence responsible for only the mutant phenotype of y^{2S} is also shown in Fig. 2. The fact that these various mutations are clustered within a small region is a preliminary indication of the location of the yellow locus within these sequences. This locus has also been cloned by Campuzano et al. (6), during their study of the achaete-scute gene complex.

Transcriptional analysis of the yellow locus. Different yellow alleles affect the normal pigmentation of the larval and adult body cuticle and its derivative structures. To explain the phenotypic effect of *y* mutants, we expect the yellow-encoded RNA(s) to be expressed both during larval and

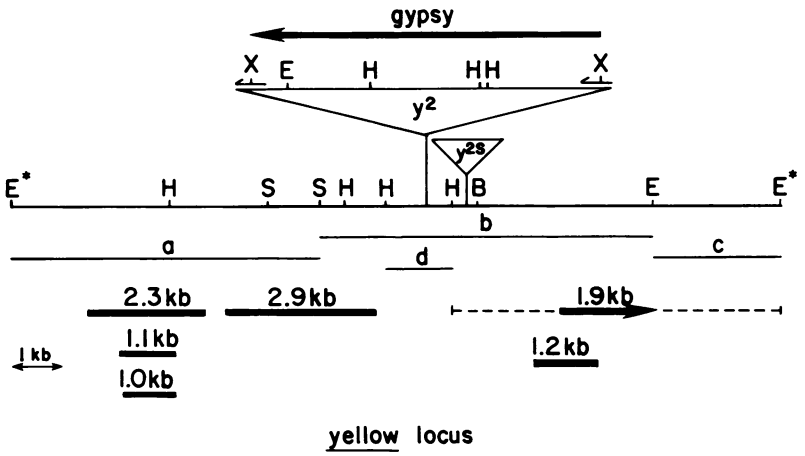

FIG. 2. DNA restriction map of the yellow locus. Indicated on the map are the location of the gypsy element and unidentified DNA sequences responsible for the mutant phenotypes in the y^2 and y^{2S} alleles, respectively. The thin lines below the map (labelled a, b, c, and d) designate the size and location of various hybridization probes used in this study. The thick lines in the lower part of the figure indicate the approximate location and the length of the different transcription units detected in the region. The arrows indicate the direction of transcription of the RNA in the cases when it was determined. The symbols used to designate restriction sites are: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; X, *Xho*I. The symbol E* indicates an artificial *Eco*RI site present in the lambda clone but not in the *Drosophila* genome.

pupal stages, the time of development when the color of these structures is determined. To identify the yellow transcript(s), we analyzed the various RNAs encoded within the DNA region shown in Fig. 2 by probing Northern blots with different cloned restriction fragments. The 6.0-kb *Eco*RI-*Sal*I fragment (probe a), located on the left of the restriction map, encodes four RNAs whose location has not been mapped in detail; the 2.3-kb transcript is expressed only in adult flies, whereas the 1.1-kb RNA is expressed in embryos and early first-instar larvae, and the 1.0-kb transcript accumulates in first- and second-instar larvae (data not shown). The rightmost region of this restriction fragment encodes an additional 2.9-kb RNA which is expressed throughout the development of the fly, although at lower levels in third-instar larval and early pupal stages. This RNA partially overlaps the 6.1-kb *Sal*I-*Eco*RI fragment (probe b), located in the middle of the restriction map in Fig. 2, which encodes two additional transcripts. The 1.2-kb RNA is expressed in all but the embryonic and adult stages (data not shown); this 1.2-kb RNA accumulates in very low amounts, and its presence is not reproducibly detectable in different experiments. The approximate location of these transcripts on the DNA map, based on data obtained from Northern experiments, is shown in Fig. 2. Both the pattern of temporal expression of these transcripts and the fact that their accumulation is not affected by mutations at the *y* locus suggest that these are not yellow-encoded RNAs.

The DNA sequences where the molecular defects of various yellow mutations are located encode a 1.9-kb RNA. Its direction of transcription was determined by using strand-specific RNA probes synthesized utilizing the 6.1-kb *Sal*I-*Eco*RI (probe b) fragment cloned into SP6 vectors (18) and is indicated in Fig. 2. This RNA can be detected by Northern analysis by using both the 2.8-kb *Sal*I-*Bam*HI fragment and the 3.3-kb *Bam*HI-*Eco*RI fragment that constitutes probe b but not with the 1.3-kb *Hin*dIII fragment (probe d) or sequences located from the *Sal*I site to the insertion site of the gypsy element. These results suggest that the 5′ end of this transcript is at least 0.5 kb away from the insertion point of the gypsy element. The limits of hybridization of the 1.9-kb RNA are shown in Fig. 2. The pattern of temporal expression of this RNA coincides with the expected developmental expression of the yellow locus as deduced from the analysis of mutant phenotypes in various *y* alleles. This RNA accumulates in first- and second-instar larvae, is not expressed in late larvae-early pupae stages and accumulates again approximately 48 h after puparium formation (Fig. 3A). In addition, the levels of this transcript are affected by mutations at the yellow locus in two different alleles that we have studied. The y^2 allele affects mainly the color of adult cuticular structures, while y^{2S} has a marked larval phenotype in addition to the adult one, with structures such as the mouth parts of the larvae showing a golden brown color instead of the black wild-type color. We studied the developmental expression of the 1.9-kb RNA in these two mutants using Northern blots of poly(A)-containing RNA from different stages of development. The y^2 allele accumulates very low levels of the 1.9-kb RNA in late pupae; the expression of this transcript in larval stages is nevertheless normal, in agreement with the absence of a larval phenotype (Fig. 3B). The allele y^{2S}, which has a visible larval phenotype in addition to its adult phenotype, shows reduced levels of the 1.9-kb transcript in the larval stages and no detectable amounts of this RNA in late pupae (Fig. 3C). This is apparent when the relative intensity of the 1.9- and 2.9-kb RNAs is compared in wild-type (Fig. 3A) and y^{2S} flies. These results support the idea that the 1.9-kb transcript is a yellow-encoded RNA. Furthermore, mutations at the *su(Hw)* locus, which revert the phenotype of y^2, also cause the amount of the 1.9-kb transcript to return to the normal wild-type levels (Fig. 3D). The levels of this RNA are also abnormal in the y^4 and *y*, ac^1 mutations (6).

To directly compare the amount of yellow RNA that accumulates in wild-type and mutant flies, we scanned the autoradiograms shown in Fig. 3 to determine the intensity of the band corresponding to the 1.9-kb RNA. The same nylon filters were then hybridized with a *Drosophila ras* gene whose expression is constant throughout the development of the fly (20), and the intensity of the *ras*-encoded RNA band was similarly measured after scanning of the autoradiograms. The amount of yellow RNA was then normalized relative to that of the *ras* transcript present in the

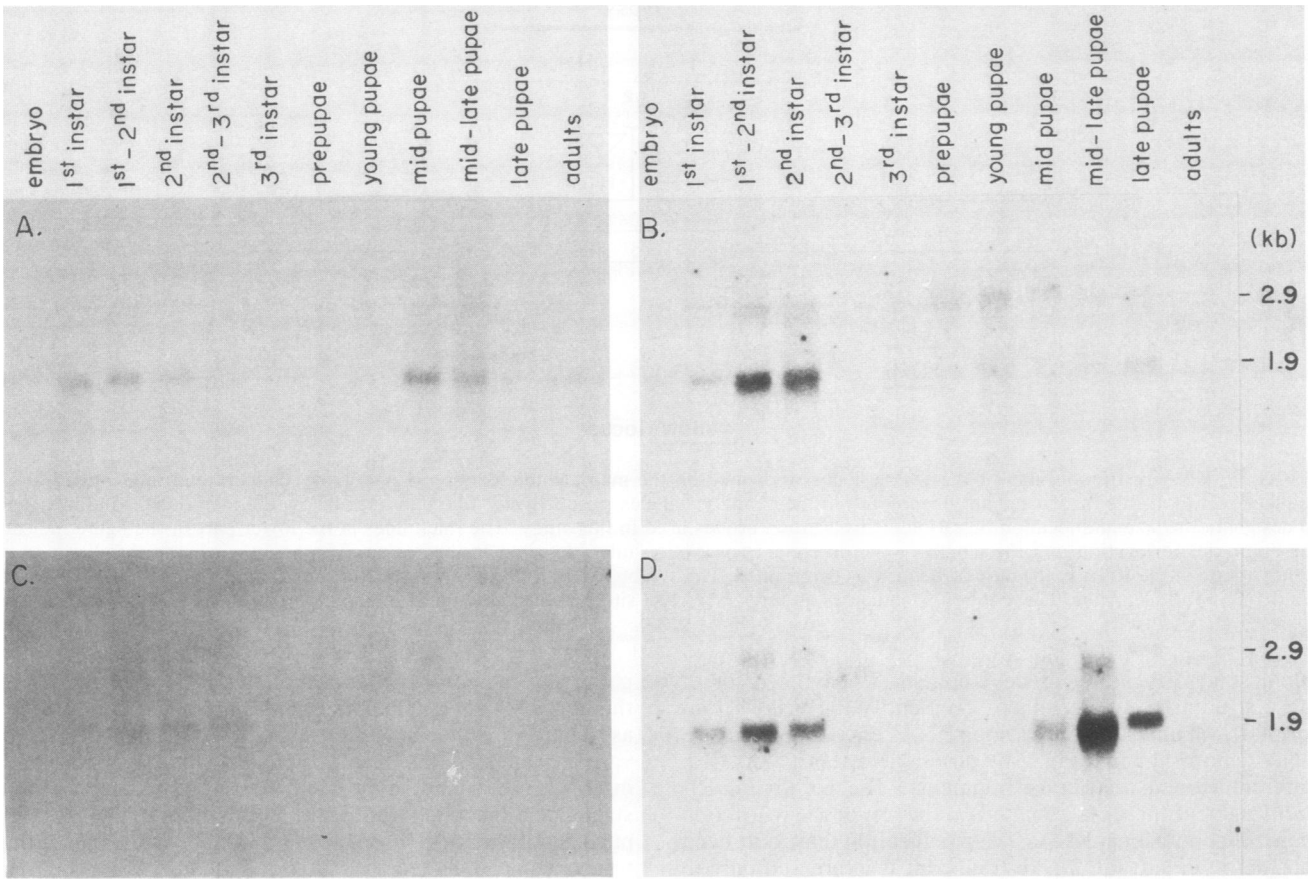

FIG. 3. Developmental expression of RNAs encoded by the yellow locus in wild-type and mutant stocks. RNA (10 μg) obtained from different developmental stages of wild-type and various mutant flies was electrophoresed on a 1% agarose–formaldehyde gel, blotted to BioTrans, and hybridyzed with ^{32}P-labeled DNA (probe b in Fig. 2). (A) Canton-*S*; (B) y^2; (C) y^{2S}; (D) y^2; *su(Hw)*^{69k}*/TM6*, *su(Hw)*^{f}.

same sample. The values thus obtained are diagrammed in Fig. 4.

Temporal transcription of the gypsy element in y^2 flies. The gypsy transposable element gives rise to a major 6.5-kb RNA; this transcript probably starts at the promoter element located in one of the LTRs and extends all the way to the termination signals placed in the second LTR. The transcription of the 6.5-kb RNA in wild-type flies is not constant during development but rather is modulated in a temporal-specific fashion during different stages of the *Drosophila* life cycle, reaching maximal expression in the midstages of pupal life (24). The pattern of transcription of the gypsy element in the mutant y^2 is the same as that in wild-type flies, as deduced from Northern analysis of poly(A)-containing RNA from different developmental stages of these mutant flies (Fig. 5). An additional transcript 1.4 kb long can also be detected in low abundance. It is interesting to note that the expression of gypsy RNA reaches a maximum in 3- to 4-day-old pupae, the time of pupal development when the expression of the yellow gene is highest to attain proper coloration of the adult body structures. This correlation is best observed in the diagram of Fig. 4, where the amount of gypsy RNA that accumulates at different developmental stages was calculated by scanning of the autoradiograms of Fig. 5 and is expressed per copy of euchromatic gypsy and relative to the amount of *ras* RNA present in the same stage.

We similarly analyzed the expression of the gypsy element in y^2; *su(Hw)* flies. The pattern of developmental expression of gypsy in this stock parallels that observed in wild-type and y^2 flies (data not shown), but the levels of the gypsy 6.5-kb RNA, expressed per euchromatic copy of the transposable element present in the genome, decrease by a factor of five with respect to the amount of this RNA that accumulates in y^2 flies (Fig. 4). This effect could nevertheless be due to a position effect on gypsy transcription, since the chromosomal location of the gypsy element varies among the various stocks analyzed (Table 1), rather than to an effect of the *su(Hw)* locus itself on the transcription of gypsy. To address this issue, we compared the levels of gypsy RNA in homozygous and heterozygous *su(Hw)* adult female flies. Since the heterozygous stock contains a different number of gypsy elements but in the same chromosomal positions as the homozygous stock (Table 1), we can directly compare the accumulation of gypsy RNA in both stocks. Poly(A)-containing RNA was prepared from y^2/y^2; +/+, y^2/y^2; *su(Hw)/su(Hw)*, and y^2/y^2; *su(Hw)*/+ adult females and analyzed by Northern blots. The autoradiogram was scanned, and the levels of gypsy 6.5-kb RNA were normalized relative to those of the *ras* 1.6-kb transcript. The amount of gypsy RNA expressed per copy of this transposable element present in the heterozygous stock had a value of 500 expressed in the arbitrary units established above. In the same conditions, homozygous *su(Hw)* females contained 20 units of

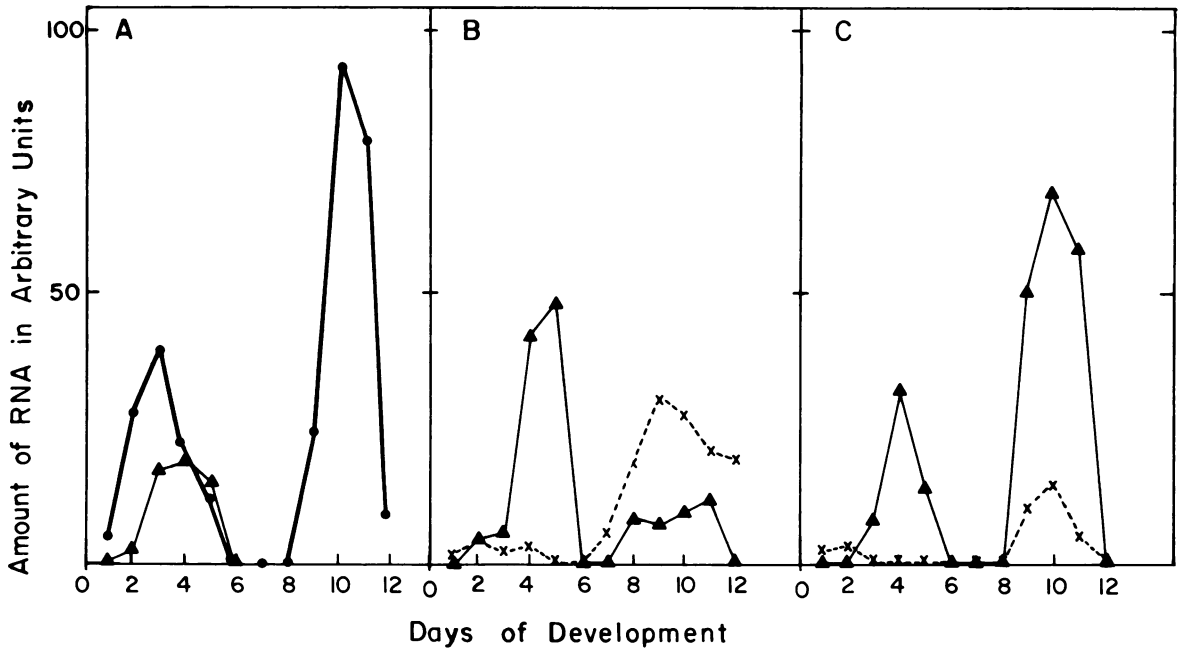

FIG. 4. Quantitation of gypsy and yellow-encoded RNAs. The autoradiograms of Fig. 3 and 5 were scanned to determine the intensity of the bands corresponding to the yellow 1.9-kb and the gypsy 6.5-kb RNAs. The same filters were then dehybridized and rehybridized with a *Drosophila ras* gene, and the intensity of the band corresponding to the *ras* 1.6-kb RNA was similarly determined. The amount of yellow- and gypsy-encoded RNA in each developmental stage was then determined relative to that of the *ras* RNA in the same sample. The results are expressed in arbitrary units. (A) Yellow-encoded RNA in wild-type (●) and y^{25} (▲) flies. (B) Yellow (▲) and gypsy (×) RNAs in y^2 flies. (C) Yellow (▲) and gypsy (×) RNAs in y^2; *su(Hw)* flies.

gypsy transcript, and homozygous y^2 females gave a value of 100 (Table 1). These results suggest that homozygous mutations at the *su(Hw)* locus have an effect on gypsy transcription, causing the accumulation of this RNA to decrease by a factor of 25. This effect was observed with the two alleles tested, $su(Hw)^{69k}/su(Hw)^f$ and $su(Hw)^{f3}$.

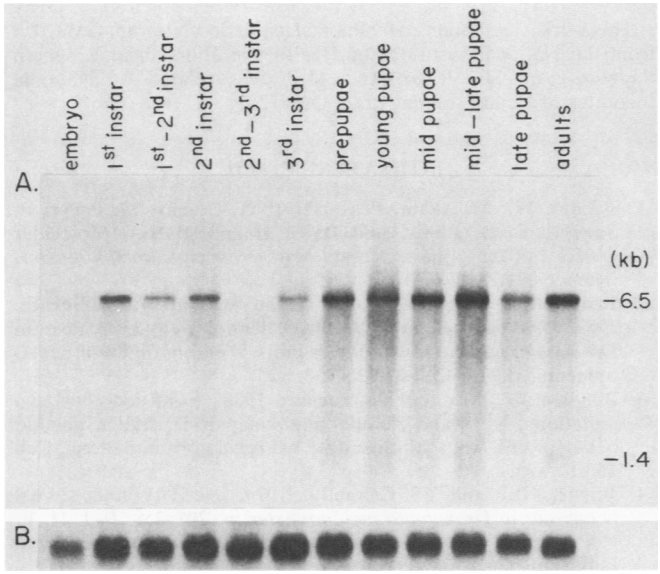

FIG. 5. Analysis of gypsy-encoded transcripts in the y^2 mutant. (A) Poly(A)-containing RNA (10 μg) from different developmental stages of y^2 flies was electrophoresed on a 1% formaldehyde–agarose gel and transferred to BioTrans. The filter was then hybridyzed with a cloned *Xho*I fragment of the gypsy element (Fig. 2). (B) The same filter after dehybridization and then hybridization with a cloned *Drosophila ras* gene to control for the amount of RNA loaded in each sample.

DISCUSSION

We cloned the yellow locus and identified a 1.9-kb RNA encoded by it. This transcript accumulates at the times of development when the *y* gene(s) is predicted to be active, and its expression is quantitatively affected by mutations at the *y* locus. The y^{25} allele affects both the larval and pupal expression of this transcript, indicating that the RNA band observed in both developmental stages in wild-type flies corresponds to the same yellow-encoded transcript. It is interesting to note that the y^2 allele, caused by the insertion of the gypsy element, affects only the expression of the 1.9-kb RNA in the pupal stages, whereas the accumulation of this RNA is normal during the larval period of development. An explanation for this result could be the existence of two independent promoters which govern the synthesis of the 1.9-kb transcript during the larval and pupal stages, respectively; a similar case has been described for the *Drosophila* alcohol dehydrogenase gene (2). The phenotypic effect of the gypsy insertion in y^2 could then be explained if this insertion has taken place in regulatory sequences controlling the pupal but not the larval expression of the yellow RNA. Nevertheless, we do not think this is the case, since revertants of y^2 have a wild-type phenotype, whereas revertants of gypsy-induced mutations in general still contain one of the gypsy LTRs at the insertion site (1). If the mutational role of gypsy is due to a passive insertional effect in DNA sequences involved in transcriptional control, one would expect the y^2 revertants would still have the LTR inserted in

TABLE 1. Quantitation of gypsy RNA in various mutant stocks

Stock	Chromosomal location of gypsy[a]								RNA/no. of gypsy
y^2	1B				14C	15F	16E		100
y^2	1B				14C	15F	16E		
y^2; *su(Hw)*	1B, 1B	7B	13A		14C	15F	16E	3rd	20
y^2; *su(Hw)*	1B, 1B	7B	13A		14C	15F	16E	3rd	
y^2; *su(Hw)*	1B, 1B	7B	13A		14C	15F	16E	3rd	500
y^2; +	1B				14C	15F	16E		

[a] Chromosomal location of the gypsy insertions in the various fly stocks analyzed determined by in situ hybridization of ^{125}I-labeled gypsy DNA to polytene chromosomes from these stocks.

these sequences, impairing their regulatory role on transcription and thus causing a mutant phenotype. Based on these observations, we propose a mechanism that explains both the active role of gypsy in causing the mutation and the phenomenon of suppression in terms of known biological properties of retroviruses and retrovirus-like elements.

It is clear from the pattern of RNA expression in the mutant y^2 that the insertion of gypsy does not cause premature termination of transcription at the LTRs, since the gypsy element appears to be inserted at least 0.5 kb upstream of the yellow RNA transcription initiation site and the RNAs are transcribed in opposite directions; accordingly, the observed phenotype in the y^2 allele is a decrease in the levels of RNA rather than the appearance of new size transcripts. A similar effect of gypsy on the transcription pattern of the gene it mutates has been observed in the forked locus (24). We thus suggest that the y^2 gypsy element causes the yellow mutant phenotype by affecting the rate of initiation of yellow transcripts. It has been shown that the LTR of an avian retroviral provirus is unable to act as an efficient promoter of transcription when a transcriptionally active LTR is present upstream (10). Furthermore, Emerman and Temin (11) have found that selection for the expression of a gene under the control of an LTR promoter suppresses the transcription of another gene located either 5′ or 3′ to it. This type of interference between adjacent promoters has also been observed in the white locus of *D. melanogaster* in which the transcription is not under the control of a typical LTR (3). An analysis of the results summarized in Fig. 4 shows that the mutagenic effect of the gypsy element can be explained by the same scheme. The mutant phenotype of y^2 is caused by a decrease of transcription of the yellow-encoded RNA in 2- to 4-day-old pupae. This is the same time of development when transcription of the gypsy element reaches a maximum, approximately 10 times higher than in any other developmental stage. Thus, the low levels of transcription of the yellow locus in pupae could be due to an epigenetic mechanism involving transcriptional interference as a consequence of the activation of the gypsy promoter. This explanation also accounts for the fact that the insertion of gypsy in the y^2 allele does not affect the expression during the larval stages, since the transcription of gypsy during the larval period is very low (Fig. 4B and 5A).

In addition, our hypothesis explains the reversion of the yellow phenotype by mutations at the *su(Hw)* locus. The transcription of the gypsy element in an *su(Hw)* background diminishes considerably during the pupal stages (Fig. 4C). These experiments were carried out with nonisogenic stocks which contained different numbers of both euchromatic and heterochromatic copies of the gypsy element inserted in different chromosomal locations (Table 1). By analyzing stocks which contain only one euchromatic copy of gypsy, we determined that the heterochromatic copies do not contribute appreciably to the steady-state levels of gypsy RNA (data not shown). One could then argue that the difference in RNA levels per copy of gypsy is due to a position effect on transcription of the euchromatic copies of gypsy. For example, the gypsy elements located at 1B, 7B, 13A, and the third chromosome in the y^2; *su(Hw)* stock might be transcribed at very low levels, such that, when the total amount of RNA is divided by the number of copies of gypsy, we are considering copies of the element that do not contribute to the accumulation of RNA, thus artificially lowering the amount of transcript expressed per copy of the transposon. This argument cannot explain the result obtained with heterozygous flies, since the only difference between these flies and the y^2 stock is the existence of one copy of the gypsy element at 1B, 7B, 13A, and the third chromosome, which we have just assumed are not appreciably contributing to the accumulation of gypsy RNA. This suggests that the effect observed in the y^2; *su(Hw)* stock on the gypsy RNA levels can only be explained if the *su(Hw)* locus acts as a recessive modifier of gypsy transcription. One would expect the accumulation of gypsy RNA in the heterozygous flies to be similar to that in the y^2 stock; the fact that this is not the case suggests that the transcription of the gypsy element might be dependent on its location in the genome and that the results obtained are not a quantitative indication of the effect of mutations at the *su(Hw)* locus on gypsy transcription. Nevertheless, the qualitative changes can only be explained by assuming an effect of the suppressor locus on the expression of the transposon. As a consequence of this effect, the decrease in the expression of gypsy during the pupal stages of development allows the transcription of the yellow RNA to return to the normal wild-type level. The *su(Hw)* locus is then involved in controlling gypsy transcription, a situation similar to that of the *SPT3* locus and the Ty element in yeasts (27).

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