Insertion of a *copia* Element 5' to the *Drosophila melanogaster* Alcohol Dehydrogenase Gene (*adh*) Is Associated With Altered Developmental and Tissue-Specific Patterns of Expression

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ABSTRACT

The *Drosophila melanogaster* alcohol dehydrogenase gene (*adh*) is under the control of two separate promoters (proximal and distal) which are preferentially utilized at the larval and adult life stages, respectively. A variant alcohol dehydrogenase allele (RI-42) isolated from a natural population contains a *copia* retroviral-like transposable element inserted 240 bp upstream from the distal (adult) *adh* transcriptional start site. Levels of *adh* transcripts in the RI-42 variant are reduced in tissues and at life stages where *copia* is actively expressed and are affected in *trans*- by mutant alleles at the *suppressor-of-white-apricot* (*su(w^a)*) and *suppressor-of-forked* (*su(f)*) loci. These suppressor genes have no effect on *adh* expression in wild-type *Drosophila*.

THE largest class of eukaryotic transposable elements share a number of structural and functional properties with mammalian retroviruses (BOEKE *et al.* 1985; FINK, BOEKE and GARFINKEL 1986; FINNEGAN and FAWCETT 1986). For example, retroviral-like transposable elements are bordered on their 5' and 3' ends by long terminal direct repeats (LTRs) (RUBIN 1983); they typically contain long open reading frames having sequence similarly with the retroviral *gag*, *pol* and *env* genes (MARLOR, PARKHURST and CORCES 1986; MOUNT and RUBIN 1985; EMORI *et al.* 1985) and genomic length transcripts of at least some retroviral-like transposable elements can be isolated from viral-like particles (VLPs) which are associated with reverse transcriptase activity (SHIBA and SAIGO 1983; BECKER *et al.* 1987).

The mutational potential of retroviral-like elements is well documented (LAMBERT, McDONALD and WEINSTEIN 1988; SHAPIRO 1983). Many spontaneous mutations have been associated with the insertion of retroviral-like elements (KUBLI 1986), and the effect of these insertions can be complex (McDONALD *et al.* 1987). Many retrotransposon insertion mutations in yeast (*e.g.*, ERREDE *et al.* 1980), *Drosophila* (*e.g.*, MOUNT, GREEN and RUBIN 1988; CHANG *et al.* 1986; PARKHURST and CORCES 1985, 1986) and mammals (VARMUS and SWANSTROM 1985), are accompanied by altered *trans*-regulatory controls as occurs with transposable-element-mediated suppressor and enhancer mutations in *Drosophila* (CHANG *et al.* 1986).

The phenotype of a number of retroviral or *copia*-like element insertion mutations in *Drosophila* can be suppressed or enhanced by the action of alleles mapping to other loci (PARKHURST and CORCES 1986;

MOUNT, GREEN and RUBIN 1988; RUTLEDGE *et al.* 1988). Since nearly all of the mutations which are subject to the action of *Drosophila* suppressor and enhancer genes are the product of *copia*-like element insertion events, it has been hypothesized that suppressor and enhancer loci may, in fact, be *trans*-regulators of *copia*-like element expression (see KUBLI 1986).

The molecular mechanisms which produce the complex phenotypes often associated with mutations resulting from *copia*-like element insertions are not fully understood; however, based upon information which is currently available, a number of hypotheses have been proposed. For example, the fact that not all mutants resulting from the insertion of the same *copia*-like element are subject to the action of the same set of suppressor or enhancer alleles suggests that such variables as the position of an element within a mutant gene or its activity may constitute significant limiting factors in the action of suppressor and enhancer alleles (KUBLI 1986; CHANG *et al.* 1986). Likewise, the fact that mutant phenotypes associated with *copia*-like element insertion mutations are frequently expressed only at specific developmental stages, suggests that these phenotypes are dependent upon the regulated activity of the inserted element (PARKHURST and CORCES 1986).

In this paper, we molecularly characterize a variant *adh* (*Drosophila* alcohol dehydrogenase) allele previously shown to contain a *copia* element inserted upstream of the *adh* gene and to be associated with exceptionally low levels of enzymatic activity (MARONI and LAURIE-AHLBERG 1983). Our results demonstrate that the insertion is 240 bp upstream of the distal

(adult) cap site and is in the same transcriptional orientation as the adjacent *adh* gene. A significant reduction in the level of *adh* transcripts is seen in the mutant which is inversely correlated with developmental and tissue-specific patterns of *copia* expression. The reduction in the level of *adh* transcripts is partially suppressed by the action of mutant alleles at the *suppressor-of-forked* (*su(f)*) and *suppressor-of-white-apricot* (*su(w^a)*) loci. Our results support the hypothesis that the position and regulated activity of a retroviral-like element inserted into a locus can significantly influence the character of the mutant phenotype.

MATERIALS AND METHODS

Drosophila strains: The strains carrying *X* chromosomes with the mutant suppressor and suppressed alleles (*i.e.*, *v*, *su(f)*, *f* and *su(w^a)*, *w^a*) were obtained from the Mid-American *Drosophila* Stock Center. S-1 is a homozygous line carrying a wild-type *adh-slow* allele (ANDERSON and McDONALD 1983). The RI-42 strain was established from a fly isolated from a Rhode Island population and was provided by C. LAURIE (MARONI and LAURIE-AHLBERG 1983).

The strains containing the suppressor alleles introduced into the RI-42 or S-1 (wild type) genetic backgrounds were produced by standard backcrossing techniques. Briefly, ♂♂ carrying the mutant suppressor allele (*su(w^a)* or *su(f)*) were crossed to RI-42 (or S-1) ♀♀s. F₁ ♀♀s, heterozygous for the mutant and wild-type suppressor alleles, were backcrossed to the parental RI-42 (or S-1) stock. F₂ ♀♀s were single-pair-mated to the parental RI-42 (or S-1) ♂♂s. F₃ ♀♀s heterozygous for the mutant suppressor allele (*i.e.*, progeny ♀♀s taken from vials containing ♂♂ progeny showing the mutant suppressor phenotype) were again backcrossed to parental RI-42 (or S-1) ♂♂s. This backcrossing and selection procedure was continued for four generations at which point the genetic background was >95% derived from the parental RI-42 (or S-1) strains. Single pair matings were then used to establish stocks homozygous for the mutant suppressor allele and the RI-42 (or S-1) *adh* allele. Presence of the suppressor alleles in the derived stocks was determined by visual comparison of the derived strains with the appropriate mutant lines (*i.e.*, *w^a* vs. *su(w^a)*, *w^a*, and *f* vs. *v*, *su(f)*, *f*). The *adh* genotype of the derived strains was determined by Southern analyses using an *adh* probe (see below).

All fly strains were cultured at 22° with constant humidity and regular 12-hr light cycles and fed on a diet of molasses, corn meal, and brewer's yeast in agar. Developmental staging was achieved by seeding bottles with 3–5-day adults for 18–24 hr, followed by removal of the adults and culture of the bottles at appropriate intervals. Larvae were collected by aqueous elution from food and pupae by scraping from sides of bottles.

RNA isolation: All RNA isolations were done using the hot phenol method (MANIATIS, FRITSCH and SAMBROOK 1982). Total RNA prepared this way was used for Northern blots directly or used to select polyadenylated (poly(A⁺)) RNA for oligo(dT)-cellulose chromatography.

Northern gel analysis: Nucleic acids were fractionated in agarose gels and transferred to nitrocellulose essentially as described in MANIATIS, FRITSCH and SAMBROOK (1982). Formaldehyde was used to denature RNA. Hybridizations of blots were carried out at 42° in 50% formamide, 5× SSC, 1× Denhardt's solution, 50 mM sodium phosphate, 10% dextran sulfate and 100 µg/ml sonicated salmon sperm DNA

(THOMAS 1980). Probes were made from the appropriate purified DNA fragments and radioactively labeled using random primer methods (FEINBERG and VOGELSTEIN 1983).

Cloning: A genomic library of RI-42 was prepared in the phage vector, EMBL 4 (FRISCHHAUF *et al.* 1983). DNA isolated from the *Drosophila* strain RI-42 (200 µg) was digested to completion with the restriction enzyme, *Bgl*III. The digested DNA was fractionated in a sucrose gradient. Collected fractions were analyzed by gel electrophoresis, those containing DNA approximately 16 kb long were purified for ligation. EMBL 4 DNA was digested with *Bam*HI and phosphatased with calf intestinal phosphatase (CIP). EMBL 4 prepared this way was ligated to the *Drosophila* fragments and packaged *in vitro*. Recombinant bacteriophage were screened for *copia-adh* DNA sequences using cloned *adh* DNA sequences (sACI) as probe. Positive plaques were purified and DNA isolated.

Sequencing: DNA sequences were determined essentially as described by SANGER, NICKLEN and COULSON (1977). Gene-Scribe-Z vectors from USB Corp. were used as recommended by supplier. Subcloning of DNA fragments was done by routine methods as described in MANIATIS, FRITSCH and SAMBROOK (1982).

In situ hybridizations: Specimens were embedded in OCT compound and quick frozen by immersion into liquid nitrogen and subsequently stored at –20°. Serial sections 10 µm in thickness were collected on subbed slides (SHIVERS, SCHACHTER and PFAFF 1986) and dipped briefly in liquid nitrogen and placed at –70° until used. Sections were fixed in 4% paraformaldehyde in 1× PBS for 5 min at room temperature. The fixative was removed by washing twice in 1× Denhardt's solution, 10 mM DTT, 10% dextran sulfate, 50% formamide, and tRNA 250 mg/ml.

Prehybridization solution was washed off in 1× PBS and replaced with hybridization solution (same as prehybridization) containing radioactive ³⁵S-probe (1–2 × 10⁶ cpm/50 µl hybridization solution). An aliquot of 50–100 µl hybridization solution was applied per slide and then covered by a small piece of Parafilm. Probes were prepared using standard reaction conditions as suggested by supplier of the riboprobe transcription system (Promega Catalog, 1986). The probe RNA was reduced in size by treatment with 0.1 M NaOH at 37° for 30 min followed by neutralization with HCl and precipitation in ethanol.

Hybridization was performed in a moist chamber at 37° for 18–24 hr. Following hybridization, the slides were washed in 2× SSC at room temperature to remove the Parafilm cover and subsequently in 0.2× SSC at 37° for 2 hr. After washing, the sections were dehydrated in 70% and 95% alcohols and air-dried. For emulsion radiography, the slides were dipped in Kodak NTB3 emulsion diluted 1:1 with water and exposed 2–4 days in a light-proof dry slide box at 4°. After developing in Kodak D-19 developer, the sections were stained, dehydrated, cleared, and coverslipped (LAWRENCE and SINGER 1985; SHIVERS, SCHACHTER and PFAFF 1986).

Quantitative analysis of tissue-specific expression: Quantitative analysis of *adh* expression in nurse cells vs. fat body cells was accomplished by digitizing images of hybridized microscopic sections containing both cell types within the same field (image) using an image processing system supplied by GW Hannaway and Associates (Boulder, Colorado), consisting of an Imaging Technology (Woban, Massachusetts) board set resident in a Digital Equipment Corporation Vax Station II computer. For intensities of objects within the same video image to be comparable, it is necessary for the intensity of all objects within the image to fall within the linear portion of the intensity-response curve of the

entire video system. The maximum and minimum values of all objects within the tissue sections imaged in our analysis were determined to lie within the linear range of the system (camera + recorder + digitizer) prior to quantitation. Thus our pixel intensities should accurately reflect levels of RNA within the tissue sections examined.

The ratios of *adh* RNA levels in nurse cells (cytoplasm) *vs.* fat body cells (cytoplasm) were computed for three randomly chosen cytoplasmic regions of each cell type contained within each of two independently prepared and imaged tissue sections for each of the two genotypes being compared (RI-42 *vs.* wild type). A nested ANOVA was performed on the complete data set to determine if nurse cell/fat body *adh* RNA ratios were significantly different between RI-42 and wild-type *Drosophila*.

RESULTS

The RI-42 *adh* variant contains a *cop*ia element inserted 240 bp upstream from the distal (adult) *adh* transcriptional start site: A genomic library of the *adh* RI-42 variant was screened with a probe for the *Drosophila* alcohol dehydrogenase gene (*adh*) (sAC1) (GOLDBERG 1980). Of four plaques giving a positive signal in the initial screen, one (λ RI-42A) was arbitrarily chosen for further characterization. That λ RI-42A contained the region 5' to the *adh* gene previously shown to contain an inserted *cop*ia element (MARONI and LAURIE-AHLBERG 1983) was verified by probing Southern blots with *adh* (sAC1) and *cop*ia (cDm5002) (DUNSMUIR *et al.* 1980) specific probes. The restriction patterns of the *cop*ia and *adh* genes in λ RI-42A are identical to those of previously cloned *cop*ia and *adh* sequences (DUNSMUIR *et al.* 1980; GOLDBERG 1980) and indicate that the *cop*ia insert is in the same transcriptional orientation as the adjacent *adh* gene (Figure 1A).

Nucleotide sequence analysis of the 3' long terminal repeat (LTR) of the *cop*ia insertion and the genomic region flanking the distal *adh* promoter region shows that *cop*ia is inserted 240 bp upstream from the start of the distal (adult) promoter in a region previously identified as being important to *adh* expression (POSAKONY, FISCHER and MANIATIS 1985). This position of the *cop*ia insertion in RI-42 is in good agreement with a previous estimate (250 bp) based upon restriction analysis (AQUADRO *et al.* 1986).

***Adh* transcript levels are reduced in the RI-42 variant over development:** The *adh* gene is differentially regulated during *Drosophila* development (URSPRUNG, SOFER and BURROUGHS 1970; SAVAKIS and ASHBURNER 1985). Expression of *adh* is highest in the 2nd to 3rd instar larvae and in adults >1 day post-eclosion. To determine the effect of the *cop*ia insertion on patterns of *adh* expression in the RI-42 variant, RNA was collected from various life stages and compared quantitatively and qualitatively with matched samples collected from a *Drosophila* strain homozygous for a wild-type *adh-slow* allele (S-1) (MCDONALD, SANTOS and ANDERSON 1980; ANDERSON and MC-

DONALD 1983). As a control against unequal loading, each blot was also hybridized with a *Drosophila* β -*tubulin* gene probe (BIALOGAN, FAULKENBURG and RENKAWITZ-POHL 1985).

Figure 2A is a qualitative comparison of *adh* expression during development of RI-42 and wild-type *Drosophila*. The relative abundance of *adh* transcripts is 2- to 10-fold lower in the RI-42 variant compared to wild-type flies at all life stages except embryos where the level of *adh* transcripts in RI-42 is indistinguishable from wild type. Like wild-type *Drosophila*, *adh* expression in the RI-42 variant peaks in 1st to 2nd instar larvae and again in early adults. However, in contrast to the wild-type pattern of expression, levels of *adh* transcripts in the RI-42 variant became very low in adults >1–2 days post-eclosion.

Reduced levels of *adh* transcripts in the RI-42 variant are most pronounced at developmental stages and in tissues where *cop*ia elements are actively expressed: If the *cop*ia element inserted upstream of the RI-42 *adh* gene is transcriptionally active, it might be expected that its effect on *adh* expression would be most pronounced at developmental periods and in tissues where *cop*ia elements are most actively expressed. Figure 2 presents Northern blots contrasting the temporal patterns of *cop*ia and *adh* expression in RI-42 and wild-type *Drosophila*. Consistent with previous studies, we find that *cop*ia transcript levels are developmentally regulated (PARKHURST and CORCES 1987). *Cop*ia transcripts are relatively rare in embryos and become more abundant throughout larval development reaching a peak in late 3rd instar larvae. The level of *cop*ia transcripts drops during pupal stages and increases again in adults >1–2 days post-eclosion. Comparison of the developmental patterns of *cop*ia and *adh* expression in RI-42 and wild-type *Drosophila* indicates that the most dramatic reductions in levels of *adh* transcripts in the RI-42 variant occur at those developmental periods (*i.e.*, 1st to 2nd instar larvae and adults >2 days post-eclosion) when *cop*ia is abundantly expressed. At those life stages where *cop*ia expression is reduced (*i.e.*, embryos and young adults), *adh* expression in the RI-42 variant approaches wild-type levels (Figure 2).

To determine whether reduced levels of *adh* transcripts in the RI-42 variant are limited to tissues in which *cop*ia is expressed, we compared the tissue-specific pattern of *adh* and *cop*ia expression in RI-42 and wild-type *Drosophila* by *in situ* hybridization (MCDONALD *et al.* 1988; D. STRAND, M. BROWN and J. F. MCDONALD, in preparation). Frozen sections of wild-type and RI-42 adult females were hybridized with RNA probes complementary to *adh* or *cop*ia transcripts. The results (Figure 3) demonstrate that, relative to wild type, *adh* transcripts in the RI-42 variant are substantially reduced in tissues where *cop*ia

FIGURE 1.—A, Structure of the RI-42 *adh* allele and its transcripts. Exons of the *adh* gene are represented by shaded boxes. The *copia* element is indicated by the stippled box flanked by two open boxes with arrows above to denote the LTRs. The two *adh* transcripts are represented below. Restriction sites are: (E) *EcoRI*; (H) *HindIII*; (Pv) *PvuII*; (Hp) *HpaI*; (M) *MboI*; (S) *SalI*; (B) *BamHI*. B, Sequence of 3' LTR of the *copia* insertion and 5' flanking region of the RI-42 *adh* allele. The sequence of the LTR of the *copia* element inserted upstream of the RI-42 *adh* allele is identical to that of two previously isolated *copia* elements (MOUNT and RUBIN 1985; EMORI *et al.* 1985) and differs by two bases (indicated in parentheses) from the LTR of the *copia* element contained in Dm5002 (RUBIN *et al.* 1980).

FIGURE 2.—Northern blot analysis of *copia* and *adh* RNA expressed during development in wild-type (S-1) and RI-42 *Drosophila*. A, Ten micrograms of total RNA was loaded in each lane and hybridized with a *Drosophila adh* probe (437 bp *HindIII-PvuII* fragment from sACI, GOLDBERG 1980). A *Drosophila* β -tubulin probe (BIALOGAN, FAULKENBURG and RENKAWITZ-POHL 1985) was co-hybridized as a control against loading error. B, The RI-42 blot shown in (A) was rehybridized with a *copia* probe (Dm5002, DUNSMUIR *et al.* 1980).

is transcriptionally active (e.g., fat body) but not in tissues where *copia* is not expressed (e.g., oocytes). The relative levels of *adh* RNA in nurse cells *vs.* fat body was determined by quantitative video image analysis. The mean value ($\pm$ SD) of the ratio of *adh* RNA levels

present in nurse cells *vs.* fat body cells of the RI-42 variant was 1.39 ± 0.11 , *i.e.*, there is about 30% less *adh* RNA in fat body than in nurse cells in the RI-42 variant. In contrast the nurse cell *vs.* fat body ratio in wild type was 0.96 ± 0.03 , *i.e.*, about equal levels of *adh* RNA are present in fat body and nurse cells in wild-type *Drosophila*. The results of ANOVA demonstrated that the variation in nurse cell to fat body *adh* RNA ratios between the RI-42 variant and wild type was highly significant relative to error variation (between replicate slides of same genotype) ($P < 0.001$). These results indicate that the reduced expression of *adh* in the RI-42 variant is most pronounced at developmental stages and in tissues where *copia* elements are most actively expressed.

Levels of RI-42 *adh* transcripts are elevated in flies homozygous for mutant *suppressor-of-white apricot* (*su(w^a)*) or *suppressor-of-forked* (*su(f)*) alleles: The effect of *copia*-like element insertion mutations in *Drosophila* may be, at least partially, suppressed or enhanced by a variety of recessively inherited, *trans*-acting alleles (MODELELL, BENDER and MESELSON 1983; PARKHURST and CORCES 1985; 1986; ZACHAR *et al.* 1985; RUTLEDGE *et al.* 1988). Mutant alleles at the *su(w^a)* and *su(f)* loci have been reported to modify expression of the *white-apricot* (*w^a*) allele in which a *copia* element has been inserted (LEVIS, O'HARE and RUBIN 1984; O'HARE *et al.* 1984; ZACHAR *et al.* 1985). To assess the effect of these modifier loci on RI-42 *adh* expression, we constructed four *Drosophila* strains in which mutant *su(w^a)* and *su(f)*

FIGURE 3.—*In situ* hybridization to frozen thin sections of female wild-type and RI-42 adults using anti-sense RNA probes specific for either *copia* (A) or *adh* (B and C). Hybridization with the corresponding sense probes to adjacent sections of the same animal demonstrated the absence of nonspecific signal (data not shown). A, A section through an adult female abdomen which has been hybridized to a *copia* probe. Note that *copia* transcripts are abundant in fat body cells (fb) but not present in oocytes (nc = nurse cells in oocytes; yr = yolk region of oocytes). This pattern of *copia* expression is the same in wild-type (S-1) and RI-42 *Drosophila*. B, A section through a wild-type (S-1) adult female abdomen which has been hybridized to an *adh* probe. Note that levels of *adh* transcripts are equally abundant in fat body cells. C, A section through a RI-42 adult female abdomen which has been hybridized to an *adh* probe. Note that the ratio of *adh* transcripts in fat body (fb), a tissue in which *copia* is expressed, relative to oocytes (nc = nurse cells; yr = yolk region), a tissue in which *copia* is not expressed, is substantially lower in the RI-42 variant than in wild-type *Drosophila* (see B).

FIGURE 4.—Northern blot hybridization of RNA prepared from adult *Drosophila* homozygous for (A) the S-1 *adh*-slow allele or (B) the RI-42 *adh copia* insertion variant and either functional or nonfunctional *suppressor-of-white-apricot* (*su(w^a)*) and *suppressor-of-forked* (*su(f)*) alleles. Lane designations: S-1, wild-type *adh* allele (S-1) and functional *su(w^a)* and *su(f)* alleles; S-1 *su(w^a)*, wild-type *adh* allele and nonfunctional *su(w^a)* allele; S-1 *su(f)*, wild-type *adh* allele and nonfunctional *su(f)* allele.

alleles were introduced into the RI-42 or wild-type *adh* genetic background.

Northern blots of *adh* transcripts isolated from these strains and compared to controls show that RI-42 *adh* expression was significantly elevated in flies homozygous for either mutant suppressor allele (Figure 4B). Mutant alleles at the *su(w^a)* and *su(f)* loci exerted no effect on wild-type *adh* expression nor did they affect levels of β -tubulin RNA which was used as a control against unequal loading (Figure 4A). Based upon these results, we conclude that loss-of-function at either the *su(w^a)* or *su(f)* loci results in partial suppression of the quantitative aspect of the mutant RI-42 *adh* phenotype. The suppressor effect of *su(f)* allele on RI-42 *adh* expression is in contrast to its effect on *w^a* where it enhances the mutant phenotype (e.g., RUTLEDGE *et al.* 1988).

DISCUSSION

The aberrant pattern of *adh* expression associated with the RI-42 variant is most likely the result of insertion of a transcriptionally active *copia* element: Although we cannot formally dismiss the possibility that the cause of the novel pattern of *adh* expression which characterizes the RI-42 variant is independent of the inserted *copia* element, we believe there are compelling reasons for concluding otherwise. Restriction analysis of the RI-42 *adh* gene and Northern analysis of its transcripts provide no evidence of structural abnormalities. In addition, the fact that wild-type or nearly wild-type levels of *adh* transcripts are present at some developmental stages (e.g., embryos and young adults) and in some tissues (e.g., young

oocytes) in the RI-42 variant makes it unlikely that the aberrant pattern of expression is solely due to defective *cis*-regulatory sequences or to the physical displacement of upstream *adh* control sequences (POSAKONY, FISCHER and MANIATIS 1985) by a transcriptionally inactive *copia* insert. The fact that RI-42 *adh* expression is preferentially reduced at life stages and in tissues where *copia* elements are actively expressed strongly suggests that the *copia* element in the RI-42 variant is transcriptionally active and that its regulated pattern of expression plays an active role in determining the mutant *adh* phenotype.

The quantitative pattern of *adh* expression in the RI-42 variant is consistent with a model of promoter interference: Our results demonstrate that patterns of *adh* expression are quantitatively disrupted in the RI-42 variant at life stages and in tissues where *copia* expression is partially (young adults) or totally (eggs, embryos) repressed, *adh* expression in the RI-42 variant approaches wild-type levels. These facts are consistent with a model of promoter interference whereby the expression of the upstream *copia* element actively interferes with expression of the adjacent *adh* gene.

A similar model of promoter interference has previously been invoked to account for the stage-dependent effect of several *gypsy* element insertion mutations in *Drosophila* (PARKHURST and CORCES 1985, 1986). One of these mutants, *yellow²* (*y²*), is similar in structure to the RI-42 variant in that the *gypsy* element has inserted 5' to the *y* gene's coding region. Another *gypsy* mutant *forked¹* (*f¹*), contains a *gypsy* element inserted into an intron of the *forked* (*f*) gene, and the levels of both *y²* and *f¹* transcripts are reduced at a life stage (pupae) in which *gypsy* elements are transcriptionally active.

In addition, the model has been corroborated by results of studies on the effect of two putative *trans*-regulators of *gypsy* expression, *su(Hw)* and *su(f)*. The *su(Hw)* gene is thought to encode a transcriptional activator of *gypsy* expression. Double mutants homozygous for either the *y²* or *f¹* allele and carrying null alleles at the *su(Hw)* locus display substantially reduced levels of *gypsy* expression but increased levels of expression from the two mutant genes, *y²* and *f¹*. This negative correlation between *gypsy* expression and that of two *gypsy* insertion mutants is consistent with the model of promoter interference.

Loss-of-function mutations at another putative *trans*-regulatory locus of *gypsy* expression, *su(f)*, results in a significant elevation in levels of *gypsy* transcripts. *Drosophila* homozygous for mutant *su(f)* and *f¹* alleles also display elevated levels of *f¹* transcripts. Thus, the absence of either a putative activator or repressor of *gypsy* expression, influences the *f¹* inser-

tion mutant in a similar way. This suggests that it is not the transcriptional status of the inserted *gypsy* element which interferes with expression of the mutant gene, but rather the physical presence or absence of a regulatory molecule in the vicinity of the *gypsy* insert. The effect of mutant alleles at the *su(w^a)* and *su(f)* loci on *adh* expression in the RI-42 variant has led to a similar molecular interpretation of our results.

Both the *su(w^a)* and *su(f)* gene have been identified as putative *trans*-regulators of the *copia* insertion mutation, *white apricot* (*w^a*) (ZACHAR *et al.* 1985). Loss-of-function mutations at the *su(w^a)* locus suppress the mutant *w^a* phenotype resulting in an eye color which approaches wild type. Although the mode of action of *su(w^a)* on *copia* is unknown, homozygosity for mutant alleles at the *su(w^a)* locus does not seem to affect *copia* transcript levels (P. BINGHAM and J. BIRCHLER, personal communication, and our unpublished observations). Mutations at the *su(f)* locus enhance the *w^a* phenotype. As stated above, *su(f)* is hypothesized to encode a transcriptional repressor of *gypsy* expression. Hence, *su(f)* may also play a regulatory function with regard to *copia* expression, although its precise role remains to be delineated.

The fact that loss-of-function mutations at either the *su(w^a)* or *su(f)* loci result in a significant increase in levels of RI-42 *adh* transcripts is consistent with a transcriptional interference model whereby the presence of *trans*-regulatory molecules in or around the upstream *copia* insert in the RI-42 variant interferes with transcription of the adjacent *adh* gene.

Position of a *copia* insertion may influence its mutagenic effect: Comparison of the RI-42 variant with a previously described *copia* insertion mutant, *w^a*, suggests that the site of a *copia*-like element insertion may be important in determining its effect on the mutant gene's expression. The *w^a* mutation is due to an insertion of *copia* into an intron of the *w* locus (BINGHAM, LEVIS and RUBIN 1981). This insertion results in premature termination of *w* transcripts in the *copia* LTR. In the RI-42 variant, insertion of a *copia* element upstream of the *adh* gene is associated with a significant decrease in the abundance of *adh* transcripts at those developmental stages and in tissues where *copia* elements are abundantly expressed.

The effect of a *copia*-like element inserted in a gene's *cis*-regulatory controls would presumably be dependent upon the position of the insert. For instance, disruption of normal patterns of *adh* promoter usage in the RI-42 variant would likely not have occurred if *copia* had inserted downstream of the *adh* gene's coding sequence. Likewise, premature termination of *w* transcripts in *w^a* mutants, would not likely have occurred if *copia* had inserted upstream of the *w* gene's transcriptional initiation site. However, comparison of other properties of the RI-42 *adh* and *w^a*

genes reveals additional position-dependent effects which might not have been so easy to predict, *i.e.*, the differential response of these two *copia* insertion mutants to loss-of-function alleles at the *su(w^a)* or *su(f)* loci. Loss-of-function at the *su(w^a)* locus suppresses the mutant *w^a* phenotype. In contrast, loss-of-function alleles at either *su(w^a)* or *su(f)* result is in an increase in levels of *adh* expression (*i.e.*, partial suppression of the mutant phenotype). As to why *su(f)* should act as a suppressor of the *gypsy* insertion mutants *y²* and *f¹* as well as the *copia* insertion variant RI-42 but act as an enhancer of the *w^a* mutant is unknown. These and other unexpected properties of *copia*-like element insertion mutants hint at the complexities which are associated with this class of mutations.

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LITERATURE CITED

- ANDERSON, S. M., and J. F. McDONALD, 1983 Biochemical and molecular analysis of naturally occurring *adh* variants in *D. melanogaster*. *Proc. Natl. Acad. Sci. USA* **80**: 4798-4802.
- AQUADRO, C., S. DESSE, M. BLAND, C. LANGLEY and C. LAURIE-AHLBERG, 1986 Molecular population genetics of the alcohol dehydrogenase gene region of *Drosophila melanogaster*. *Genetics* **114**: 1165-1190.
- BECKER, J.-L., F. BARRE-SINOSSI, D. DORMONT, M. BEST-BEL-POMME and J.-C. CHERMANN, 1987 Characterization of the purified RNA-dependent DNA polymerase isolated from *Drosophila*. *Cell. Mol. Biol.* **33**: 225-235.
- BIALOGAN, S., D. FAULKENBURG and D. RENKAWITZ-POHL, 1985 Characterization and developmental expression of β -tubulin genes in *Drosophila melanogaster*. *EMBO J.* **3**: 2543-2548.
- BINGHAM, P., R. LEVIS and G. RUBIN, 1981 Cloning of DNA sequences from the *white* locus of *D. melanogaster* by a novel and general method. *Cell* **25**: 693-704.
- BOEKE, J. D., D. J. GARFINKEL, C. A. STYLES and G. R. FINK, 1985 Ty elements transpose through an RNA intermediate. *Cell* **40**: 491-500.
- CHANG, D.-Y., B. WISELY, S.-M. HUANG and R. A. VOELKER, 1986 Molecular cloning of *suppressor-of-sable*, a *Drosophila melanogaster* transposon-mediated suppressor. *Mol. Cell. Biol.* **6**: 1520-1528.
- DUNSMUIR, P., W. J. BROREIN, M. A. SIMON and G. M. RUBIN, 1980 Insertion of the *Drosophila* transposable element *copia* generates a 5 base pair duplication. *Cell* **21**: 575-579.
- EMORI, Y., T. SHIBA, S. KANAYA, S. INOUE, S. YUKI and K. SAIGO, 1985 The nucleotide sequences of *copia* and *copia*-related RNA in *Drosophila* virus-like particles. *Nature* **315**: 773-776.
- ERREDE, B., T. CARDILLO, F. SHERMAN, E. DUBOIS, J. DESCHAMPS and J. WIAME, 1980 Mating signals control expression of mutations resulting from insertion of transposable repetitive elements adjacent to diverse yeast genes. *Cell* **25**: 427-436.
- FEINBERG, A., and M. VOGELSTEIN, 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6-13.
- FINK, G., J. BOEKE and D. GARFINKEL, 1986 The mechanisms and consequences of retrotransposition. *Trends Genet.* **2**: 118-123.

- FINNEGAN, D., and D. FAWCETT, 1986 Transposable elements in *Drosophila melanogaster*. *Oxf. Surv. Eukaryotic Genes* **3**: 1-62.
- FRISCHHAUF, A., D. GEHRACH, M. POUSTKA and R. MURRAY, 1983 Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* **170**: 827-842.
- GOLDBERG, D. A., 1980 Isolation and partial characterization of the *Drosophila alcohol dehydrogenase* gene. *Proc. Natl. Acad. Sci. USA* **77**: 5794-5798.
- KUBLI, E., 1986 Molecular mechanisms of suppression in *Drosophila*. *Trends Genet.* **2**: 204-208.
- LAMBERT, M. E., J. F. McDONALD and I. B. WEINSTEIN (Editors), 1988 *Eukaryotic Transposable Elements as Mutagenic Agents*. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- LAWRENCE, J., and R. SINGER, 1985 Quantitative analysis of *in situ* hybridization methods for the detection of *actin* gene expression. *Nucleic Acids Res.* **13**: 1777-1799.
- LEVIS, R., K. O'HARE and G. RUBIN, 1984 Effects of transposable element insertions on RNA encoded by the *white* gene of *Drosophila*. *Cell* **38**: 471-481.
- MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- MARLOR, R. L., S. M. PARKHURST and V. G. CORCES, 1986 The *Drosophila melanogaster* Gypsy transposable element encodes putative gene products homologous to retroviral proteins. *Mol. Cell. Biol.* **6**: 1129-1134.
- MARONI, G., and C. LAURIE-AHLBERG, 1983 Genetic control of *adh* expression in *Drosophila melanogaster*. *Genetics* **105**: 921-933.
- MCDONALD, J., M. SANTOS and S. ANDERSON, 1980 Biochemical differences between products of the *adh* locus in *Drosophila*. *Genetics* **95**: 1013-1022.
- MCDONALD, J. F., D. J. STRAND, M. E. LAMBERT and I. B. WEINSTEIN, 1987 Responsive genome: evidence and evolutionary implications, pp. 239-263 in *Development as an Evolutionary Process*, edited by R. RAUFF and E. RAUFF. Alan R. Liss, New York.
- MCDONALD, J., D. STRAND, M. BROWN, S. PASKIEWITZ, A. CSINK and S. VOSS, 1988 Evidence of host-mediated regulation of retroviral element expression at the post-transcriptional level, pp. 219-234 in *Eukaryotic Transposable Elements as Mutagenic Agents*, edited by M. LAMBERT, J. McDONALD and I. B. WEINSTEIN. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- MODELELL, J., W. BENDER and M. MESELSON, 1983 *Drosophila melanogaster* suppressible by the *suppressor-of-hairy-wing* are insertions of a 7.3 kilobase mobile element. *Proc. Natl. Acad. Sci. USA* **80**: 1678-1682.
- MOUNT, S., and G. RUBIN, 1985 Complete nucleotide sequence of the *Drosophila* transposable element *copia*: homology between *copia* and retroviral proteins. *Mol. Cell. Biol.* **5**: 1630-1638.
- MOUNT, S., M. GREEN and G. RUBIN, 1988 Partial revertants of the transposable element-associated suppressible allele *white-apricot* in *Drosophila melanogaster*: structures and responsiveness to genetic modifiers. *Genetics* **18**: 221-234.
- O'HARE, K., C. MURPHY, R. LEVIS and G. RUBIN, 1984 DNA sequences of the *white* locus of *Drosophila melanogaster*. *J. Mol. Biol.* **180**: 437-455.
- PARKHURST, S., and V. G. CORCES, 1985 *Forked*, *Gypsy*, and suppressors in *Drosophila*. *Cell* **41**: 429-437.
- PARKHURST, S., and V. G. CORCES, 1986 Interactions among the Gypsy transposable element and the *yellow* and the *suppressor-of-hairy-wing* loci in *D. melanogaster*. *Mol. Cell. Biol.* **6**: 47-52.
- PARKHURST, S., and V. G. CORCES, 1987 Developmental expression of *D. melanogaster* retrovirus-like transposable elements. *EMBO J.* **6**: 419-425.
- POSAKONY, J. W., J. A. FISCHER and T. MANIATIS, 1985 Identification of DNA sequences required for the regulation of *Drosophila alcohol dehydrogenase* gene expression. Cold Spring Harbor Symp. Quant. Biol. **50**: 515-520.
- RUBIN, G., 1983 Dispersed repetitive DNAs in *Drosophila*, pp. 329-361 in *Mobile Genetic Elements*, edited by J. SHAPIRO. Academic Press, New York.
- RUBIN, G., W. BROREIN, P. DUNSMUIR, A. FLAVELL, R. LEVIS, E. STROBEL, J. TOOLE and E. YOUNG, 1980 *Copia*-like transposable elements in the *Drosophila* genome. Cold Spring Harbor Symp. Quant. Biol. **45**: 619-628.
- RUTLEDGE, B., M. MORTIN, E. SCHWARZ, D. THIERRY-MIEG and M. MESELSON, 1988 Genetic interactions of modifier genes and modifiable alleles in *Drosophila melanogaster*. *Genetics* **119**: 391-397.
- SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.
- SAVAKIS, C., and M. ASHBURNER, 1985 A simple gene with a complex pattern of transcription: the *alcohol dehydrogenase* gene in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **50**: 505-514.
- SHAPIRO, J. (Editor), 1983 *Molecular Genetic Elements*. Academic Press, New York.
- SHIMBA, T., and K. SAIGO, 1983 Retrovirus-like particles containing RNA homologous to the transposable element *copia* in *D. melanogaster*. *Nature* **302**: 119-124.
- SHIVERS, B., M. SCHACHTER and L. PFAFF, 1986 *In situ* hybridization for the study of gene expression in the brain. *Methods Enzymol.* **124**: 497-510.
- THOMAS, P., 1980 Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **77**: 5201-5205.
- URSPRUNG, H., W. H. SOFER and N. BURROUGHS, 1970 Ontogeny and tissue distribution of *alcohol dehydrogenase* in *Drosophila melanogaster*. *Wilhelm Roux' Archiv. Dev. Biol.* **164**: 201-208.
- VARMUS, H., and R. SWANSTROM, 1985 Replication of retroviruses, pp. 389-512 in *Molecular Biology of Tumor Viruses: RNA Tumor Viruses*, Ed. 2, edited by R. A. WEISS *et al.* Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- ZACHAR, A., D. DAVISON, D. GARZA and P. BINGHAM, 1985 A detailed developmental and structural study of the transcriptional effects of insertion of the *copia* transposon into the *white* locus of *D. melanogaster*. *Genetics* **111**: 495-515.

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