

An Env-like protein encoded by a *Drosophila* retroelement: evidence that *gypsy* is an infectious retrovirus

Sun U. Song,¹ Tatiana Gerasimova,¹ Maryellen Kurkulos,^{1,2} Jef D. Boeke,^{2,3} and Victor G. Corces^{1,3}

¹Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218 USA; ²Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore - Maryland 21205 USA

The *gypsy* element of *Drosophila* differs from most LTR retrotransposons in containing a third open reading frame that resembles retroviral *env* genes. The protein encoded by ORF3 is glycosylated and processed, like all retroviral envelope proteins. The protein is expressed at high levels in fly strains in which *gypsy* elements are active. In these strains the protein is found primarily in viral particles. When larvae of fly strains in which *gypsy* is normally inactive are exposed to sucrose gradient fractions containing these particles, a high level of *gypsy* insertion activity is observed in their progeny. Thus, *gypsy* has the expected properties of an insect retrovirus.

[Key Words: *Drosophila*; mutagenesis; transposable element; insect retrovirus]

Received May 6, 1994; revised version accepted July 11, 1994.

The *gypsy* element of *Drosophila melanogaster* has been classified traditionally as a long terminal repeat (LTR) retrotransposon; however, it is one of a small group of LTR retrotransposons from insects that are unusual in that they contain three open reading frames (ORFs). In these retroelements, the first two ORFs correspond to retroviral *gag* and *pol*, whereas ORF3 is of unknown function but corresponds in size and genomic location to retroviral *env* (Fig. 1). All elements with three ORFs described so far are from insects, either *Drosophila* or the lepidopteran *Trichoplusia ni*. Recent results have shown that in two elements encoding an ORF3, *gypsy* and *tom*, a subgenomic mRNA similar in structure to retroviral *env* mRNAs can be found (Pélisson et al. 1994; Tanda et al. 1994). In the case of *gypsy*, this transcript is observed only in certain strains in which *gypsy* transpositional activity is high (Pélisson et al. 1994). The primary sequences of the encoded ORF3 proteins of these elements are quite variable and show no obvious similarity to retroviral Env proteins. However, retroviral Env proteins are themselves very variable in primary sequence. Like retroviral Env proteins, the proteins encoded by "retrotransposon" ORF3s contain a putative transmembrane domain near their carboxyl terminus, multiple putative N-glycosylation sites, and putative protease cleavage sites (resembling the cleavage sites in a variety of retroviral Env proteins) at conserved positions (Fig. 1). These features of *gypsy* and the other ORF3-containing insect retrotransposons have prompted the suggestion that these elements may represent endogenous insect retro-

viruses (Boeke 1988; Boeke and Corces 1989; Coffin 1993).

To address the question of whether *gypsy* represents a virus or a transposon more directly, we have turned to the use of *Drosophila* strains that show genetic instabilities associated with high-frequency insertion by *gypsy* elements. These strains are characterized by a large number of full-length *gypsy* elements in the euchromatin, appearance of *gypsy* insertion mutations at high frequency, and the presence of both large amounts of *gypsy* full-length RNA and spliced ORF3 mRNA (Pélisson et al. 1994; N. Prud'homme, M. Gans, M. Masson, C. Terzian, and A. Bucheton, in prep.).

Mobilization of *gypsy* can be detected by a sensitive assay using the female-sterile mutation *ovo*^{D1}. This dominant mutation has no effect on males but prevents development of the female germ line. *gypsy* insertion into the *ovo*^{D1} allele results in its conversion into a recessive *ovo* allele (Mével-Ninio et al. 1989). Thus, heterozygous *ovo*^{D1}/+ females are sterile, whereas females in which *gypsy* insertions into *ovo*^{D1} occurred during germ-line development are able to produce progeny. When *ovo*^{D1} males are crossed to females of *gypsy*-active backgrounds, a high frequency of *ovo*^{D1} reversion is observed; many of these reversion events are caused by *gypsy* or *cop* insertion (Mével-Ninio et al. 1989). No such reversion is observed when *ovo*^{D1} males are crossed to females of the SS line, in which *gypsy* is inactive (Kim et al. 1994). The characteristic of high-frequency *gypsy* transposition is conferred by the *flamenco* gene on the X chromosome; females of the *y v f mal flam* strain must be homozygous for the X chromosome for high-fre-

³Corresponding authors.

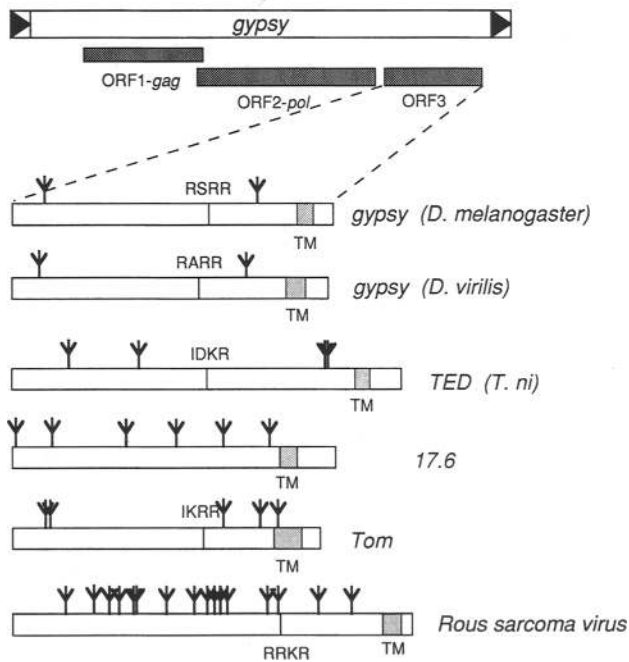


Figure 1. ORF3 of certain insect retroelements encodes a protein resembling retroviral *env*. Overall structure of *gypsy* DNA (Marlor et al. 1986) is indicated at top. ORF3s have been described from a handful of insect LTR retroelements; the conserved aspects of the structures of their predicted proteins are indicated to scale in the lower panel. (Forks) Putative N-linked glycosylation sites [only those amino-terminal to the proposed transmembrane (TM) domains are indicated]; (stippled boxes) TMs; (vertical lines with tetrapeptide motifs) putative dibasic cleavage sites. All elements are from *D. melanogaster* except as indicated. Note that all features are well-conserved between the *D. melanogaster* and *D. virilis* *gypsy* elements, despite their 20% amino acid sequence divergence (Mizrokhi and Mazo 1990). TM domains were predicted by Kyte–Doolittle hydropathy plots using DNA Strider 1.2 (Marck 1988).

quency *gypsy* insertion to occur in their progeny (N. Prud'homme, M. Gans, M. Masson, C. Terzian, and A. Bucheton, in prep.). Thus, there is a maternal effect of the *flam* gene that determines *gypsy* mobilization. This suggests that a critical *flam*-controlled step must occur in the ovaries of the mother to determine whether *gypsy* insertion will occur in the subsequent generation. ORF3 protein is expressed in the ovarian follicle cells of these strains (Pélisson et al. 1994), suggesting that virus assembly might be occurring in this tissue. Preliminary support for the idea of an infectious *gypsy* virus came from the studies of Kim et al. (1994), who have shown that microinjection of crude egg plasm from active strains into inactive strain embryos (or feeding of pupal extracts to larvae) results in detection of subsequent *gypsy* insertions in the progeny of the recipients using the *ovo*^{D1} reversion assay. Because these experiments made use of extremely crude preparations, we have examined whether an infectious particle with the characteristics of a retrovirus is present in the ovaries of *flam* females and

might be responsible for the subsequent *gypsy* insertions. We find expression of *gypsy* ORF3 protein products in the ovaries of these flies and show that these proteins have Env-like characteristics. Furthermore, we provide direct evidence for infectious *gypsy* virus particles in these tissues.

Results

Env-like proteins encoded by *gypsy* ORF3 are expressed in ovaries of *flam* females

gypsy encodes a subgenomic mRNA for ORF3 of 2.2 kb; the sequence of this mRNA predicts a protein of 54 kD (Pélisson et al. 1994). The ORF3 protein contains a putative signal peptidase cleavage site as well as a dibasic cleavage site resembling those in retroviral Env protein precursors. Cleavage at these sites would result in a surface protein of 32 kD and a trans-membrane protein of 20 kD (Fig. 1); these values do not take into account unpredictable variations that may result from glycosylation. To investigate the nature of *gypsy* ORF3 proteins, monoclonal antibodies were raised against a *trpE*–ORF3 fusion protein (see Materials and methods). Two monoclonal antibodies (mAb), 7B3 and 8E7, were isolated, which reacted specifically with the ORF3 portion of the fusion protein. These antibodies were used to probe immunoblots of SDS–polyacrylamide gels on which various ovary extracts or in vitro translation products were separated. Both antibodies react specifically with the ORF3 portion of fusion proteins (Materials and methods), as well as an in vitro translation product expressed from a T7–ORF3 expression construct (Fig. 2C). The in vitro translated protein migrates with an apparent molecular mass of 60 kD.

Using either antibody, ORF3-specific protein bands of 66 and 54 kD are observed in the *y v f mal flam* (referred to from this point on simply as *flam*) ovary extracts (Fig. 2A). In addition, antibody 7B3 reacts with a protein of 28 kD. These protein bands are absent in *Ore-R* and *flam*/+ heterozygotes and, thus, represent native *gypsy* ORF3 proteins. The presence of multiple ORF3-specific species suggests that post-translational modifications and proteolytic cleavages may have occurred.

gypsy insertion activity, as measured by the *ovo*^{D1} reversion assay, is known to be influenced by the age at which the females are mated to the *ovo*^{D1} males (Pélisson et al. 1994; N. Prud'homme, M. Gans, M. Masson, C. Terzian, and A. Bucheton, in prep.). The expression of ORF3 protein peaks at the same age (i.e., in 1- to 3-day-old females) as does *gypsy* activity in the *ovo*^{D1} reversion assay, and is absent in strains heterozygous for the *flam* X chromosome (Fig. 2B). Thus, the pattern of expression of ORF3 mRNA and protein parallels that of *gypsy* insertion activity.

If ORF3 encodes an envelope protein, it is expected to be a glycoprotein, as is the case for retroviral Env proteins (see Fig. 1). This hypothesis was tested by immunoblotting of ovary extracts with and without treatment with endoglycosidase F (EndoF) and comparison to in

Song et al.

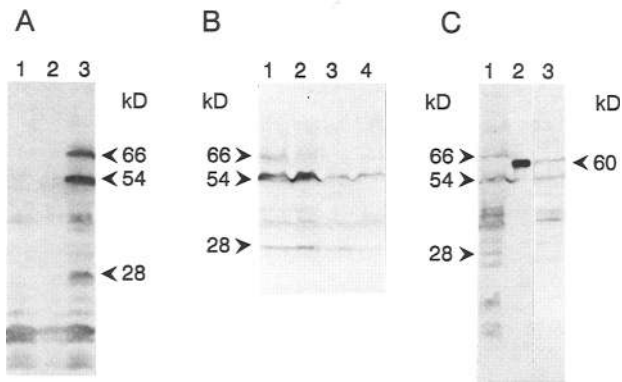


Figure 2. ORF3 glycoprotein is expressed and processed in *flam* strains. Ovary extracts were run on PAGE and immunoblotted with antibody 7B3. *flam*-Specific bands are indicated with an arrowhead. (A) Specific expression of ORF3 in *flam* strains. Approximately 70 μ g of protein was run per lane. (Lane 1) Ore-R (*flam*⁺); (lane 2) *flam*/+ heterozygote; (lane 3) *flam* homozygote; 12% PAGE. (B) Time course of ORF3 protein expression. Ovaries were extracted from flies of various ages: (Lane 1) 2–3 days; (lane 2) 4–5 days; (lane 3) 6–7 days; (lane 4) 8–9 days; 15% PAGE. (C) ORF3 protein is glycosylated. Ovary extracts were run without pretreatment (lane 1) or after EndoF treatment (lane 3); also included in lane 2 is in vitro-translated ORF3 protein; 12% PAGE.

vitro-synthesized ORF3 protein. As can be seen from Figure 2C, EndoF treatment reduces the apparent molecular mass of the 66-kD species such that it comigrates with ORF3 protein translated in vitro.

ORF3 proteins are associated with virus-like particles

Extracts of 3- to 5-day-old *flam* and control Oregon R (Ore-R) (*flam*⁺) females were prepared and subjected to sucrose density gradient analysis. Figure 3 shows the result of reverse transcriptase (RT) assays using poly(A)-oligo(dT) primer template and immunoblotting analyses done on the gradient fractions. In both strains three peaks of RT activity were observed, but the second peak is larger in the *flam* extract. *gypsy*-specific ORF3 proteins were observed primarily in the *flam* gradient where they were readily detected with both mAbs in the second peak, in which the proteins were abundant. A small amount of protein cross-reacting with ORF3 antibodies was seen in the first peak of both strains.

Peak 2 fractions from both the *flam* and Ore-R gradients were concentrated and examined by immunoelectron microscopy using mAbs 7B3 and 8E7 and colloidal gold-labeled goat anti-mouse IgG. Virus-like particles of irregular shape and \sim 100 nm in diameter were observed

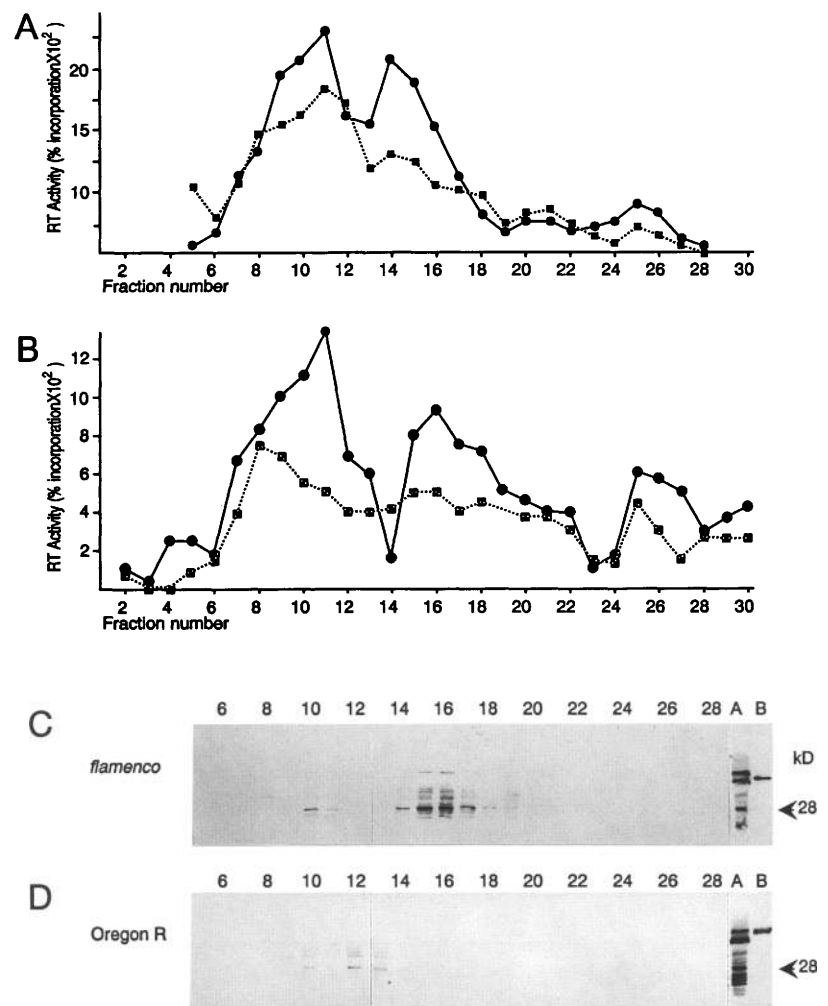


Figure 3. Sucrose density gradient analysis of *gypsy* particles. RT profiles from two independent viral particle preparation experiments (gradient experiments 2 and 1, respectively) are presented (A,B). (●) *flam*; (■) Ore-R. The input extracts contained equal amounts of protein. The indicated fractions of gradient 2 were immunoblotted with the 7B3 antibody (C,D); similar results were obtained with the 8E7 antibody (not shown). Lanes A and B in these panels contain ovary extract from *flam* flies and in vitro-translated ORF3 protein, respectively. Samples A and B were frozen and thawed once between the time gels C and D were run; this may account for the larger amount of apparent degradation products in lane A of panel D.

and decorated by the gold particles; no such particles were observed in the control fractions (Fig. 4). There are also clusters of gold particles associated with what appear to be small fragments of membrane in the *flam* gradient fractions only. These may represent envelope proteins sloughed off of *gypsy* particles.

gypsy-specific endogenous RT products

Endogenous RT reactions, in which only triphosphates (one of which was radioactive) but no primers or templates are supplied, were carried out with material from the three peaks. The products of these reactions were then deproteinized and used as hybridization probes against DNA blots containing *Drosophila gypsy*, *copia*, *412*, and *17.6* element DNAs (Fig. 5). All three peaks contained some products that hybridized to *gypsy*. The material from the second peak hybridized only to *gypsy*, whereas the material from peaks 1 and 3 hybridized to *copia*, *412*, and *17.6* (not shown). We conclude that peak 2 contains particles containing both *gypsy* RNA and RT, and relatively little other retroelement particles. Peak 1 may represent soluble material or partially disrupted particles with little specificity, and peak 3 (which is the position at which nonenveloped and, hence, much denser yeast Ty1 particles band on this type of gradient) probably contains a variety of nonenveloped particles.

Exposure of larvae of gypsy-inactive strains to particulate fractions results in ovo^{D1} reversion

An experiment was designed to detect infectious *gypsy* viruses in the gradient fractions containing ORF3 pro-

tein. This experiment takes advantage of the sensitivity of *ovo^{D1}* to insertion by *gypsy*. These experiments made use of the SS strain, which although it is a *flam* strain, does not support high-level *gypsy* mobility because it lacks active *gypsy* sequences. We attempted to compensate for the latter defect by introducing *gypsy* virus particles by feeding. The SS strain was raised in the presence of material derived from the second peak throughout the larval period of development; 25 μ l of a concentrated peak gradient fraction was mixed gently into the food every 2 days. Although this is sometimes referred to as a "feeding" experiment, we note that these larvae were also exposed to the gradient material externally. Females emerging from these vials were crossed to *ovo^{D1}* males. Approximately 1000 individual female progeny from this cross were crossed to Ore-R males and monitored for fertility. Approximately 1.5% of these females were fertile, indicating reversion of *ovo^{D1}*, whereas no such revertants were observed in unfed control flies or flies fed the Ore-R gradient fraction (Table 1). These results are highly significant statistically (fed vs. unfed, $P=0.0001$; *flam* vs. Ore-R, $P=0.091$).

Certain ovo^{D1} reversions are caused by gypsy insertion

The *ovo^{D1}* revertants and their progeny were analyzed by a PCR method as well as by in situ hybridization with *gypsy* and *copia* probes. Sixteen revertants were obtained in total from the feeding experiment; 14 of these gave rise to multiple progeny and could be used to establish new lines (Table 2). For PCR analysis, DNA was prepared from ~ 30 F₂ progeny from each line to be tested and amplification was carried out with oligonucleotide prim-

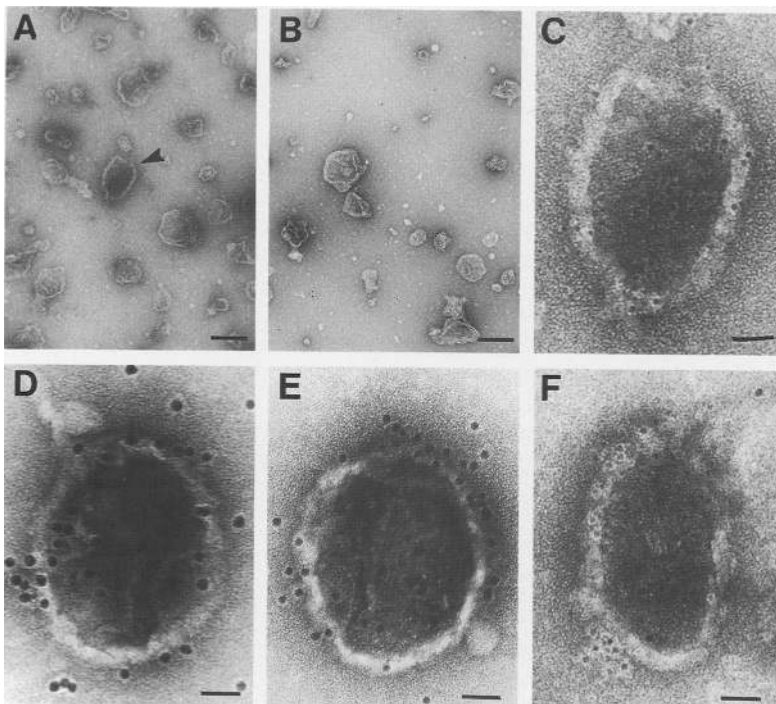
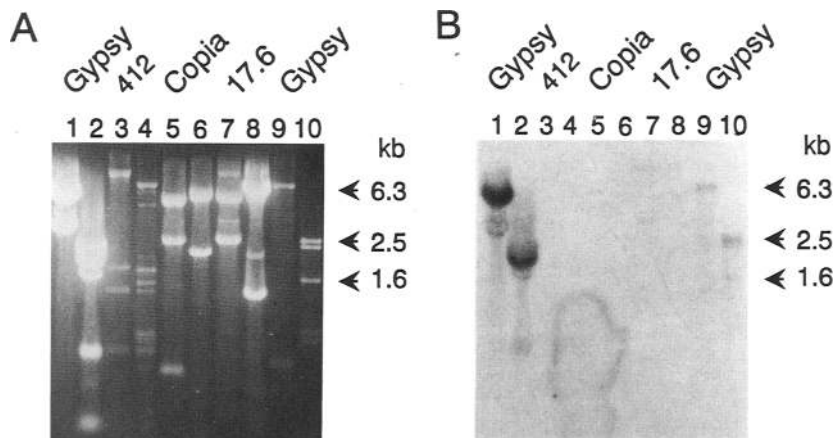


Figure 4. Immunoelectron microscopy. Fractions 14–19, concentrated as described from gradient experiment 1, were examined by negative staining and immunoelectron microscopy. (A, C–F) the *flam* gradient; (B) the Ore-R gradient. A, B, C, and F were stained with 5-nm gold particles; D and E were stained with 10-nm gold particles. Bars, 100 nm (A, B); 20 nm (C–F).

Figure 5. *gypsy*-specific RT products. (A) Plasmids containing the indicated *Drosophila* retrotransposons were cleaved with different restriction enzymes and the fragments were separated electrophoretically. The DNA was then blotted to nitrocellulose. (B) The product of an endogenous reaction using material from fractions 15–17 of the *flam* gradient 1 were separated from unincorporated nucleotides by Sephadex G50 chromatography and used as a hybridization probe.



ers derived from the *ovo* locus or the *gypsy* terminus (Fig. 6). As expected, all samples gave rise to a wild-type *ovo* band, indicating at least one unrearranged *ovo* locus. Four of the revertants gave rise to PCR products whose size was consistent with that of previously isolated *gypsy* insertions in *ovo* (Mével-Ninio et al. 1989). Three of these PCR products, as well as the wild-type *ovo* PCR product, were sequenced and were shown to represent *gypsy* insertions into *ovo* (Fig. 6). All three sequenced insertions were different, and none of these insertion sites were exact matches to the previously proposed *gypsy* target site consensus sequence; instead, they conformed to the more general consensus sequence YRYRYR [(Y) pyrimidine; (R) purine].

The remaining revertants were analyzed by in situ hybridization with *gypsy* and *copia* probes. Three additional lines were shown to contain *gypsy* insertions in 4E, the cytological location of *ovo*. The remaining seven lines all showed hybridization of the *copia* probe to 4E (Fig. 7). Insertion of *copia* into *ovo* is not completely unexpected because it was observed in a previous study in which the *y v f mal flam* strain was crossed to *ovo*^{D1} (Mével-Ninio et al. 1989). Thus, all 14 lines tested contained an insertion of either a *gypsy* or a *copia* element at the *ovo* locus, at least at the resolution of in situ hybridization. In addition to the *gypsy* elements inserted at 4E, additional sites of new *gypsy* insertion were detected in many of the revertant lines.

Table 1. Feeding of particulate fractions results in *ovo*^{D1} reversion

Treatment ^a	F ₀ females studied	Total F ₁ females tested	Fertile F ₁ females	Reversion frequency (%) ^b
<i>flam</i> fraction	31	1048	16	1.53
Ore-R fraction	18	451	0	<0.2
Control unfed	33	1194	0	<0.1

^aSS larvae were fed (or not fed) with the indicated fraction.

^bThe literature value for reversion of *ovo*^{D1} is 0.01% (Mével-Ninio et al. 1989).

Unusual properties of *ovo*^{D1} revertant progeny

The F₂ progeny of *ovo*^{D1} revertants show a number of unusual properties (Table 2). The phenotype of the F₂ males is expected to be 50% white-eyed and 50% red-eyed because of the heterozygous *white* (*w*) mutation expected to be present in the SS mother. However, the observed result was that within any one line, males were either all white eyed (6 lines; Table 2) or all red eyed (8 lines). This behavior differs from that of crosses in which *gypsy* was introduced directly by crossing *flam* females × *ovo*^{D1} males. In this case, individual revertant lines gave rise to a mixture of *yellow*⁺ and *yellow* male progeny.

The unusual segregation of the *w* marker in the male progeny of these crosses is difficult to explain. There are two classes of such lines: those that give only white-eyed male progeny and those that give rise to only red-eyed male progeny. In principle, the former class could be explained by a mitotic recombination event that resulted in homozygosity of the X chromosome, resulting in homozygosity for *ovo*⁺ and *w*. However, this cannot explain our results because some of these lines show evidence of *gypsy* or *copia* insertion at 4E, which would not have been selected for were the *ovo* reversion already accounted for by homozygosity. Sex ratios of the progeny were not unusual in any of these lines; however, recessive lethality was associated with three of the X chromosomes that we studied (data not shown).

Several of the lines derived by feeding of particles showed both somatic and germ-line instabilities in the F₂ generation and beyond. Apparent somatic mutations with phenotypes corresponding to *w* and *Lobe* were observed. Also, new mutations were observed in the subsequent generations with a variety of mutant phenotypes (Table 2).

gypsy insertions in SS strain after exposure of larvae to particulate fractions

In a second feeding experiment, SS larvae that were fed particulate fractions were simply mated to unexposed SS flies. The progeny larvae from this cross were examined

Table 2. Characteristics of fertile derivatives of fed larvae

Revertant	1	2	3	4	5 ^a	6	7	8	9	10	11	12	13	14	15	16 ^a
PCR ^b	-	-	-	-	N.D.	-	-	+	+	-	+	-	-	-	+	N.D.
In situ	<i>copia</i>	<i>copia</i>	<i>copia</i>	<i>gypsy</i>	N.D.	<i>copia</i>	<i>gypsy</i>	N.D.	<i>gypsy</i>	<i>copia</i>	N.D.	<i>copia</i>	<i>copia</i>	<i>gypsy</i>	N.D.	N.D.
Germ-line mutations ^c	held-up bristle			<i>y</i>		wing	<i>y</i>	bristle	wings apart		singed			small wings		
Somatic mutations ^d	white		white					loz	loz		lobed eye			loz		

^aLines 5 and 16 had only one or two progeny and were not studied further.

^bPresence (+) of band with PCR with either primer pairs P1 and P2 or P3 and P2 (see Fig. 6) or absence (-) of either band. (N.D.) Not determined.

^cHeritable mutant phenotypes resembling known mutations are indicated. (bristle) Thin (or thick) and short bristles; (wing) crumpled wings; (loz) lozenge-like; (held-up) held-up wings.

^dIndividual somatic mutations affecting eye color (white) or structure (lobed), identified phenotypically.

directly by in situ hybridization with a *gypsy* probe. Because the SS strain contains only two *gypsy* elements in its euchromatin, and these together with the few elements in the heterochromatin are inactive (Kim et al. 1994), new *gypsy* copies present in these progeny must have derived from the donor (*flam*) strain. More than half of the larvae examined had new euchromatic *gypsy* insertions, demonstrating that a high frequency of *gypsy* insertion is observed even in the absence of any selection for special mutations such as reversion of *ovo*^{D1} (Fig. 7; Table 3). In contrast, no new insertions were seen when 14 unfed SS larvae were examined (fed vs. unfed, $P=0.001$). The new insertions were observed both when fed females were crossed to unfed males and vice versa, suggesting that both male and female germ lines can be infected. Among the new *gypsy* insertions observed were two to five insertions into the 4E region of the X chromosome, providing strong evidence that this region of

the X chromosome represents an especially hot spot for *gypsy* insertion. No new *copia* insertions were observed in this experiment, suggesting that simple feeding of particulate fractions is not sufficient to effect the activation of *copia* transposition.

Antibody inhibits *gypsy* activity

In a separate feeding experiment, we fed particulate fractions that had been preincubated with purified mAbs: either the *gypsy* ORF3-specific antibodies or a control antibody raised against Ty1 integrase protein. The assay described above was used to monitor *gypsy* insertion activity in the SS strain. The number of new insertions was reduced about fourfold in the flies that were fed with particles preincubated with anti-ORF3 relative to the flies fed particles preincubated with anti-Ty1 integrase (Table 4; anti-ORF3 vs. Ty1, $P=0.019$). In addition, by

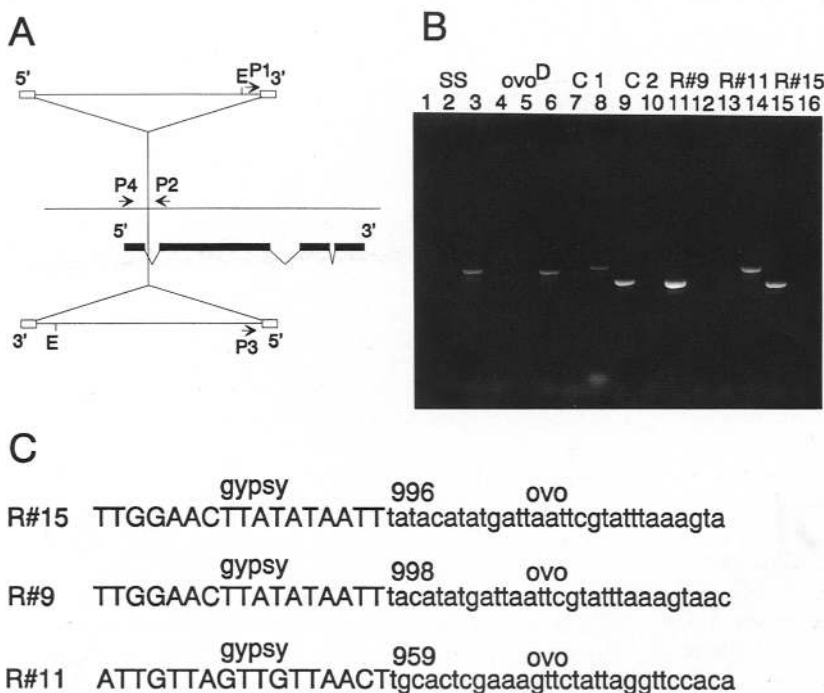


Figure 6. PCR and sequence analysis. (A) The structure of the *ovo* locus (Mével-Ninio et al. 1991) is presented, indicating the position of some of the *gypsy* insertions analyzed in this study. (Solid bars) Putative exons; (V shapes) introns; (open bars) LTRs; (arrows) PCR primers used. (B) PCR products. DNA from the indicated strains was used as a template for PCR using the following primer pairs: [P] (Lanes 1,4,7,9,11,13,15) P1 and P2; (lanes 2,5,8,10,12,14,16) P3 and P2; (lanes 3,6) P4 and P2. Revertant strains C1 and C2 were obtained by crossing the *flam* strain directly to the *ovo*^{D1} strain; revertants R9, R11, and R15 were obtained by feeding virus particles. (C) Junction sequences determined. The *gypsy* terminal sequences are indicated in upper case and the flanking *ovo* sequences in lower case.

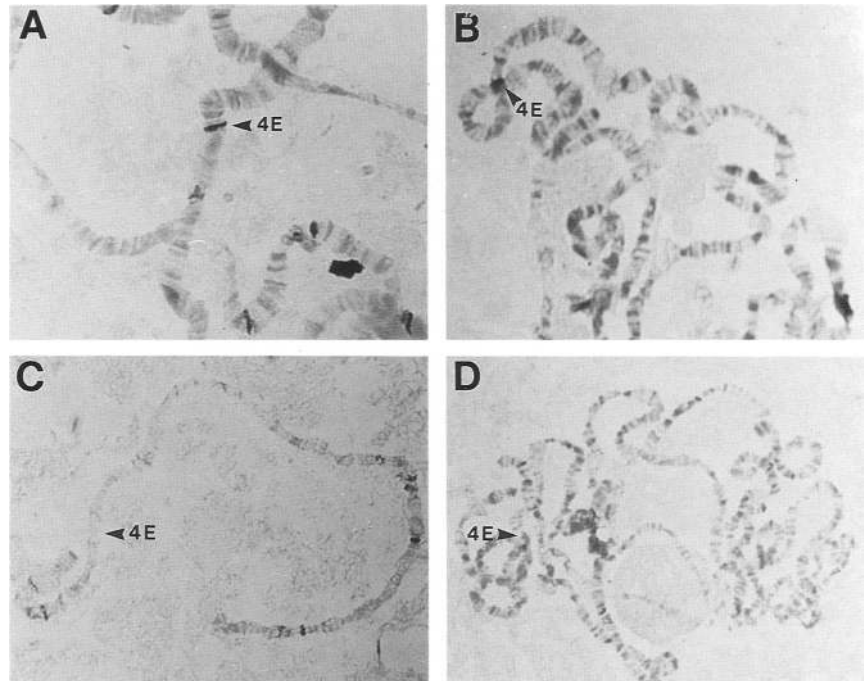


Figure 7. In situ hybridization. (A) *copia* in situ hybridization on *ovo^{D1}* revertant 2; (B) *gypsy* in situ hybridization on SS feedant 5–7; (C) *copia* in situ hybridization on the SS strain; (D) *gypsy* in situ hybridization on SS. See Table 3 for additional data on SS feedants.

the *ovo^{D1}* reversion assay, there was also a fourfold inhibition of *ovo^{D1}* reversion conferred by pretreatment with anti-ORF3 antibody (Table 4; anti-ORF3 vs. Ty1, $P=0.045$). Thus, the anti-ORF3 antibodies appear to exert a partially protective effect against the *gypsy* virus particles.

Discussion

Evidence that ORF3 is env-like

Our experiments show that ORF3-containing fractions also contain a *gypsy*-specific RT activity, which is capable of producing *gypsy*-specific cDNA species. These results suggest that *gypsy* RNA and *gypsy* primer tRNA (or partially synthesized *gypsy* cDNAs) cosediment with RT in the sucrose gradient. Furthermore, we observe virus-like particles by immunoelectron microscopy of the same fractions using an anti-ORF3 antibody. Neither the activity nor the virus-like particles (VLPs) are observed in a control gradient prepared from a *flam⁺* strain. These observations provide strong evidence for a mature *gypsy* virus particle that contains ORF3 protein. The peak position of these *gypsy* particles in the sucrose gradients (fraction 16) is very different from that of control Ty1 particles from yeast, which peak at fraction 25 under these conditions. This may be because the *gypsy* particles contain a membranous envelope that would greatly reduce their density relative to Ty1 particles, which lack an envelope. In support of this notion, a second peak of *gypsy*-specific RT activity is found at fractions 25–28; these fractions lack ORF3 protein completely. *copia* and 412 RTs are also found in these fractions; neither *copia* nor 412 have an ORF3 and, thus, are more like yeast Ty1.

We suspect that this peak of *gypsy* activity represents either partially assembled *gypsy* particles or partially degraded particles that lack an envelope. A previous study

Table 3. New *gypsy* and *copia* copies in derivatives of SS strain that were fed gradient fractions

Line	New <i>gypsy</i> copies (no.)	New <i>copia</i> copies (no.)
1–2	0	
2–1	1	0
2–2	0	0
3–1	0	
4–1	1	
5–1	2	0
5–2	1	
5–3	3 (including 4E insertion)	
5–6	0	
5–7	2 (including 4E insertion)	0
8–1	0	0
8–2	0	
8–3	2 (including 4E insertion)	
8–4	2 (including 4E insertion)	0
8–5	4	
8–6	0	0
8–7	3 (including 4E insertion)	0
8–8	0	
10–1	2 (including 4E insertion)	0

New *gypsy* and *copia* copies were detected by in situ hybridization. Progeny, e.g., 2–1 and 2–2, are siblings. For lines 1–2 and 10–1, a fed male was crossed to an unfed female. For all other lines, a fed female was crossed to an unfed male. The 4E insertions in 8–3, 8–4, and 8–7 may not be independent. In a control experiment, 14 unfed SS larvae were examined and 0/14 had new copies of *gypsy*.

Table 4. Antibody inhibition

	Antibody	
	anti-ORF3 ^a	anti-Tyl integrase ^b
Feeding of SS strains, assay by in situ hybridization		
number of new <i>gypsy</i> copies observed/ number of strains checked by in situ (%)	6/33 (18)	22/33 (67)
<i>ovo</i> ^{D1} reversion assay ^c		
number of fertile F ₁ females/ F ₁ females tested (%)	2/1030 (0.19)	9/930 (0.97)

^aMixture of 45 µg of 7B3 and 45 µg of 8E7.

^b90 µg of 8B11 (Eichinger and Boeke 1988).

^cPerformed as described for Table 1.

reported tentative evidence for *gypsy* virus (or virus-like) particle production in tissue culture cells. A variety of virion types were found in extracellular fluid and were associated with some *gypsy* RNA, but no evidence was presented to identify definitively the *gypsy* particles (Symonin et al. 1993). It would be interesting to test the supernatants of such tissue culture cell lines for *gypsy* insertion activity by the *ovo*^{D1} reversion assay.

We have shown that ORF3 protein is N-glycosylated by treatment of protein extracts with EndoF and observed a shift in the electrophoretic mobility of the ORF3 protein to a mobility very similar to that of in vitro-translated ORF3 protein. Because retroviral Env proteins are also N-glycosylated, this result provides further support for the conclusion that ORF3 has an *env*-like function. N-linked glycosylation signals are conserved in the ORF3s of *gypsy* (both *D. melanogaster* and *D. virilis*), 17.6, 297, *tom*, and TED. Furthermore, the TED retroelement ORF3 protein has also been shown to be glycosylated in insect cells (P. Friesen, pers. comm.). Thus N-glycosylation is apparently a conserved feature of this class of retroelement ORF3 proteins. Furthermore, the ORF3 protein appears to be cleaved into at least two smaller products that may correspond to retroviral surface and transmembrane proteins.

Infectivity

Supporting evidence for a virus intermediate in the *gypsy* insertion process comes from experiments in which the fractions containing the virus particles were introduced into the SS strain, which lacks active *gypsy* copies. These particles were introduced into the flies by exposing larvae to the fractions. Whether the *gypsy* particles enter the larvae by ingestion, by adsorption to external larval surfaces, or by some other larval surface is not clear. However, such exposure resulted in a high frequency of *gypsy* insertion into the *ovo*^{D1} locus, as monitored by the reversion assay. Similar results to these were obtained by Kim et al. (1994), who microinjected whole embryo extracts of *flam* strains into SS embryos and exposed SS larvae to whole pupal extracts of *flam* strains. In our experiments, additional copies of *gypsy*

were observed to have been inserted in these *ovo*^{D1} revertant lines as well, suggesting high *gypsy* activity in the established lines, and their progeny showed evidence of both germ-line and somatic mutations at high frequencies. Kim et al. (1994) made no mention of either additional insertions or continuing genetic instability in their *ovo*^{D1} revertant lines; therefore, it is possible that by using purified particle fractions, much higher levels of *gypsy* are introduced than when the virus is transmitted by a cross.

In subsequent experiments in which SS larvae were exposed to particles, no selection for *ovo*^{D1} reversion was carried out, and the larvae exposed to particles were simply crossed to unexposed SS flies. The F₁ progeny of these flies also had a very high frequency of unselected *gypsy* insertions. The latter experiment is important because it shows that there is no need for special selections to observe high-frequency *gypsy* infection/insertion. Remarkably, a large fraction of these flies carried a *gypsy* insertion at 4E, the cytological location of *ovo*.

By preincubating the particles with anti-ORF3 antibodies before feeding, we observed a nearly fourfold reduction in the number of new insertion events resulting from feeding, consistent with ORF3 protein being required for infection and subsequent insertion of *gypsy*.

A hybrid dysgenesis-like phenomenon

The progeny of the *ovo*^{D1} revertants showed a number of unexpected and, in some cases, difficult-to-explain properties. Many of these properties are reminiscent of the behavior of the progeny of hybrid dysgenic crosses. The first is the appearance of visible mutants in subsequent generations, as if genetic instability mediated by these viruses or other entities in the fractions to which the flies were exposed can be maintained over several generations. This is somewhat surprising because both *ovo*^{D1} strain and Ore-R fail to give rise to *ovo*⁺ revertants at high frequency (N. Prud'homme, M. Gans, M. Masson, C. Terzian, and A. Bucheton, in prep.). If a *flam* mutation is necessary for *gypsy* mobilization, it is not obvious why additional *gypsy* insertions are seen in subsequent generations. Either the requirement for *flam*

phenotype can be overridden by the very high *gypsy* virus load obtained by these flies, or perhaps preformed *gypsy* viruses can be physically transmitted to subsequent generations. The latter could also explain, at least theoretically, the appearance of somatic mutation in the subsequent generations.

A number of the *ovo*^{D1} revertant lines showed evidence for *cop*ia insertions at band 4E, the known cytogenetic location of *ovo*. None of the parental strains in this experiment (i.e., *ovo*^{D1}, Ore-R, SS, or *flam*) carries a *cop*ia insertion at this position. Although we have not proven that the *cop*ia insertions are directly responsible for this class of *ovo*^{D1} reversion event, it does seem likely that this is the case. There are numerous additional examples among those we have examined by *in situ* hybridization of *cop*ia inserted at positions not corresponding to those of any of the parental lines. This result suggests (but does not prove) that *cop*ia transposition is somehow elevated in these lines. There are many reports in the literature suggesting that hybrid dysgenic lines show mobility of elements other than the transposon responsible for the dysgenesis (e.g., in P-M dysgenic strains; Gerasimova et al. 1984a,b; Lewis and Brookfield 1987), but these conclusions remain controversial and do not appear to represent a completely general phenomenon (Woodruff et al. 1987; Eggleston et al. 1988; Engels 1989), and the mechanism of mobility in these cases (i.e., transposition or homology dependent) is certainly unproven. In the case of *cop*ia mobilization by injection of *gypsy* viruses, two possible mechanisms could be imagined: (1) *cop*ia retrotransposition could be mobilized *in trans* by *gypsy* elements, that is, *trans*-capsidation, or (2) high-frequency *gypsy* infection/integration causes a DNA damage response to which *cop*ia (and perhaps other transposable elements) are responsive. The first hypothesis seems unlikely given that *cop*ia and *gypsy* are molecular archetypes of the two major subclasses of the LTR retrotransposons and, hence, are about as dissimilar in sequence and structure as two LTR retrotransposons can be. Furthermore, much experimentation has shown that molecular hybrids between even rather closely related LTR retrotransposons are inactive for transposition. There is evidence that LTR retrotransposons in a variety of host organisms respond to global cellular regulatory pathways, including DNA damage pathways (Strand and McDonald 1985; Rolfe et al. 1986; McEntee and Bradshaw 1988). Thus, the latter explanation seems more plausible.

A number of reports in the literature implicate high-frequency *gypsy* insertion as correlated with genetic instabilities reminiscent of hybrid dysgenesis. These include the strains *Uc* (unstable chromosome), *tuh* (tumorous head), *flam* (used in this study), and MS (mutator strain). The *Uc* strain is characterized by a high frequency of chromosome rearrangements, new *gypsy*-induced germ line and somatic insertions, and the gradual appearance of sterility (Lim 1980; Lim et al. 1983; B.H. Judd, pers. comm.). Another stock that gave rise to many *gypsy*-induced insertion mutations bears a *tuh*^{1,3} mutation; this stock is also unstable with regard to the high-

frequency *gypsy* insertion phenotype (Kuhn 1970; D. Kuhn, pers. comm.). The *y v f mal flam* strain is known to give a high rate of *gypsy*-induced *ovo*^{D1} reversion, as well as giving a high frequency of *cut* mutations (Mével-Ninio et al. 1989). The MS strain is characterized by a high rate of *gypsy*- and *hobo*-induced mutations, especially *cut* and *forked* mutations, and also shows somatic insertions (Kim et al. 1990, 1994; Kim and Belyaeva 1991).

Conclusions

Kim et al. (1994) have performed experiments in which crude embryo or pupal extracts derived from the MSN1 strain (an SS strain carrying a *gypsy* element derived from MS) were microinjected into embryos or fed to larvae of SS strains, respectively. Using the *ovo*^{D1} reversion assay, they provided evidence that the MSN1 strain extracts contained a *trans*-acting factor that stimulated *gypsy* insertion. Although these results strongly support the notion that *gypsy* is an infectious retrovirus, they failed to prove it. In the experiments by Kim et al., as well as in our *ovo*^{D1} reversion experiment, it is formally possible that what is being transmitted to the progeny is not a viral particle but a positive activator of transposition. Although the recipient SS strain used in these experiments lacks active *gypsy* copies, the *ovo*^{D1} strain to which it was crossed may well contain active *gypsy* elements, and these experiments cannot rule out the possibility that these *ovo*^{D1} strain-derived elements were activated *in trans* by the material from the MSN1 (or *flam*) donor strains. However, in our second feeding experiment, in which only the inactive SS strain was used, we have ruled out any role of the *gypsy* elements in the *ovo*^{D1} strain. Final proof of *gypsy* infectivity will require the mobilization of genetically marked *gypsy* elements.

Could *gypsy* be both a retrotransposon and a retrovirus? The fact that the *flam* mutation specifically affects ORF3 expression raises the possibility that *gypsy* is a facultative retrovirus, expressing an infectious form only under special circumstances, and that under separate conditions it might transpose by an intracellular pathway that requires only *gag* and *pol*. However, at this point this must remain only a speculation, for the existing data are equally consistent with all *gypsy* "transposition" being mediated entirely by an intercellular infection process. The finding that *gypsy* is probably an infectious retrovirus of *Drosophila* raises many new questions that will be the subject of future investigations. The availability of a genetically tractable system with infectious retroviruses will be especially useful for tackling such problems. What is the receptor for the ORF3 protein, and what is the route by which virus particles enter the larva, and eventually the germ line of the progeny? What is the host range of the *gypsy* virus? Its presence in sibling species of *D. melanogaster* (Mizrokhi and Mazo 1990) suggests that at least the genus *Drosophila* will be susceptible. The development of marked *gypsy* elements bearing phenotypic markers will be a powerful tool for answering such questions, and also

should allow the development of a new microinjection-independent germ-line transformation strategy for *Drosophila*.

Materials and methods

Strains and genetic crosses

The strains MG#3 (*flam/FM3*) [N. Prud'homme, M. Gans, M. Masson, C. Terzian, and A. Bucheton, in prep.], *ovo^{D1} v* [Mével-Ninio et al. 1989], and SS (*w flam*) [Kim et al. 1990] were provided by A. Bucheton (CNRS, Gif-sur Yvette, France). These strains are maintained on standard *Drosophila* medium; all genetic experiments were carried out at 25°C.

Generation of mAbs

The mAbs 7B3 and 8E7 were generated by using the following protocol. The 1612 bp *StyI-XhoI gypsy* fragment was inserted into pATH3 [Koerner et al. 1991] for TrpE-ORF3 fusion protein production. Cells containing the construction were induced with β -indole acrylic acid and an 89-kD TrpE-ORF3 fusion protein was isolated. BALB/c mice (5–6 months old) were immunized with the fusion protein. Mice received an initial injection of 100 mg of protein emulsified 1:1 with Freund's complete adjuvant. After 2 weeks, the mice were given three boosts of 100 mg of protein in Freund's incomplete adjuvant at 2-week intervals. Six days after the final boost, serum samples were tested by immunoblotting. Mice giving good serum responses were boosted with 100 mg of protein with Freund's incomplete adjuvant 4 days before the fusion. Spleen cells were fused in the presence of PEG 4000 (GIBCO) to sp2/0 myeloma cells, using standard protocols [Harlow and Lane 1988]. Hybridoma supernatants were screened 1–2 weeks later on immunoblot strips containing either TrpE protein or the fusion protein. Antibodies 7B3 and 8E7 react only with the fusion protein. Positive preclones were cloned by limiting dilution. Ascites were produced as described [Harlow and Lane 1988].

Immunoblotting

Ovaries from 3- to 5-day-old female flies were isolated in buffer [0.1 M NaCl, 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA, 0.001 M PMSF] and transferred into SPS lysis buffer [2.5% SDS, 60 mM Tris-HCl (pH 7.4), 0.005% bromophenol blue, 10% glycerol] for homogenization. After homogenization, proteins were boiled for 10 min, spun for 5 min, and stored at –20°C. Gradient fractions (100 μ l) were precipitated by 10% TCA and resuspended in sample buffer. Samples for immunoblotting were prepared as above.

Proteins were run on 12% or 15% polyacrylamide gels and electroblotted onto nitrocellulose. Immunoblots were blocked for 0.5–1 hr with 5% powdered milk in PBST [150 mM NaCl, 10 mM phosphate (pH 7.0), 0.3% Tween 20]. Primary antibodies (hybridoma culture supernatant diluted 1:10) were added in 1% milk in PBST and incubated for 1.5 hr at room temperature. Blots were washed for 1 hr in PBST and incubated with peroxidase-conjugated secondary antibodies (Sigma) for 1.5 hr in PBST at a dilution of 1:10,000. The washing procedure was repeated, and the blots were subjected to ECL Western blotting protocols (Amersham).

Homogenization of flies and sucrose density gradient analysis

Two grams of 3- to 5-day-old female flies were homogenized in

buffer [0.1 M NaCl, 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA, 0.001 M PMSF] using a Dounce homogenizer at 4°C. This homogenate was spun down at 10K rpm for 10 min. The supernatant was transferred to a new 15-ml tube and spun down again at 10K rpm for 5 min to get rid of debris. The total protein content of *flam* and control extracts was assayed by OD₂₈₀ and found to be identical. The final supernatants (~4.5 ml) were loaded onto 20–70% linear sucrose gradients prepared and fractionated as described [Braiterman et al. 1994]. In gradient experiment 1, no protease inhibitor cocktails were added, whereas in experiment 2, 1 \times PIC1 and PIC2 were added to the extracts as described [Braiterman et al. 1994]. Five-microliter aliquots of the fractions were used for exogenous and endogenous RT assays, and 100- μ l aliquots were precipitated by TCA and used for immunoblot analysis.

RT assays

Exogenous RT assays using a poly(A)-oligo(dT) primer template were performed in 42 mM Tris-HCl (pH 8.2), 16.7 mM DTT, 50 mM NaCl, 5 mM MgCl₂, 0.5 mM MnCl₂, 0.042% NP-40, 8.3 μ M TTP, 4.17 μ g/ml of oligo(dT), and 8.33 μ g/ml of poly(A). [α -³²P]TTP (0.25 μ Ci) was added per 25 μ l of reaction cocktail and 5 μ l of fraction, and incubated at room temperature for 1 hr. Incorporation was assayed as described [Goff et al. 1981]. The washed DEAE paper was analyzed directly with ImageQuant software using a Molecular Dynamics PhosphorImager. The endogenous RT reaction uses internal primer templates; it was performed in an assay cocktail containing 50 mM Tris-HCl (pH 8), 20 mM DTT, 60 mM NaCl, 6 mM MnCl₂, 5 mM MgCl₂, 0.1% NP-40, 2 mM dATP, and 600 mM each of dGTP, dTTP, and dCTP. In 65 μ l of the cocktail, 5- μ l aliquots of sucrose gradient fractions and 100 μ Ci of [α -³²P]dATP were added and incubated for 3 hr at room temperature. After incubation, 10 ml of 1 mM dATP was added and incubated for an additional 30 min. The final product was phenol/chloroform-extracted and precipitated in ethanol. DNA purified on a Sephadex G50 column was used as a probe for the DNA blotting analysis.

Electron microscopy

Aliquots (100 μ l) of fractions 14–20 were pooled together and run on 20–70% step sucrose gradients to concentrate the virus particles. Centrifugation was for 1.5 hr at 25,000 rpm at 4°C in a Beckman SW28 rotor. Thirty microliters of concentrated virus particles were adsorbed to a collodion grid for 5 min at room temperature. The grids were rinsed on three drops of PBS for 1 min each and then incubated on a drop of a mixture of 7B3 and 8E7 (as ascites fluid at a dilution of 1:200 in PBS) for 30 min at 37°C and then for 30 min at room temperature. After the primary antibody incubations, the grids were washed on three drops of PBS and incubated on a drop of gold-labeled goat anti-mouse IgG (Amersham) at a concentration of 1:30. After secondary antibody incubation, the grids were rinsed on two drops of PBS and then on two drops of H₂O. The grids were fixed in 2.5% glutaraldehyde for 5 min at room temperature and then rinsed in H₂O three times for 1 min each. After washing, the grids were stained negatively in a solution of 1% uranyl acetate and 0.2% tylose on two drops for 45 sec each. Electron microscopy was performed on a Zeiss transmission electron microscope (model TEM 10A).

DNA manipulations

Genomic DNA was isolated from 20–30 adult flies using the following method. Flies were homogenized in 400 μ l of buffer

Song et al.

[0.1 M Tris-HCl (pH 9.0), 0.1 M EDTA, 1% SDS, 1% DEPC] with a plastic rod in an Eppendorf tube. This homogenate was incubated for 30 min at 70°C, and then 54 ml of 8 M potassium acetate was added and left on ice for 30 min. After spinning for 15 min at 4°C, the supernatant was transferred carefully to a fresh Eppendorf tube. DNA was then precipitated by adding a 0.5-volume of isopropanol at room temperature and spun down for 5 min. The DNA pellet was washed carefully with 70% ethanol, respun, dried, and resuspended in 30–50 ml of H₂O. About 20 ng of genomic DNA was used as template for PCR. PCR was done as follows: 94°C for 1 min and 20 sec, 65°C for 2 min, and 72°C for 3 min for 40 cycles. HotTub polymerase and reaction buffers (Amersham) were used with 200 μM dXTPs, 1 μM each primer, and 1% glycerol included in the reaction.

DNA cycle sequencing using oligonucleotide primer JB715, *Taq* polymerase, and fluorescent dideoxy terminators was carried out directly on PCR products isolated from agarose gels.

The oligonucleotides used were P1, 5'-CAACATGACCGAG-GACGGTCATAAAC-3'; P2, 5'-CTCCCGCTCTGCGGGCTT-CTCTT-3'; P3, 5'-CTTTGCCGAAAATATGCAATG-3'; P4, 5'-CGGCTTTTTTCAGCGGCTAACC-3'; and JB715, 5'-TAAA-ACGGAGGTGGCGG-3'.

For *in situ* hybridization, biotinylated probes of *gypsy* and *copia* DNA were used and hybridizations were performed as described (Lim 1993).

Exposure of larvae to gradient fractions

For infectivity experiments in which larvae were exposed to particles, particles were concentrated by step gradient centrifugation. Particles were concentrated from fractions 14–18 by pooling the fractions and pelleting the particles through 20 ml of 20% sucrose at 25,000 rpm in an SW28 rotor for 90 min onto a 20-ml 70% sucrose cushion. The concentrated particles were collected as a single 400-μl interface fraction. For feeding experiments, 25 μl of this concentrate or unconcentrated fraction 16 was added to standard fly food by gentle mixing every other day for 10 days.

For the antibody inhibition experiment, the indicated antibodies were purified from ascites on protein G using a mAb-TrapGII kit (Pharmacia) before use. Concentrated particles (125 μl) were preincubated with antibodies (90 μg) overnight at 4°C and fed as described above.

Methods for obtaining new lines from feeding experiments

SS flies were fed sucrose gradient fractions containing particles five times every 2 days. Females emerging from these vials were crossed with *ovo*^{D1 v} males (for reversion experiments, see Table 2) or to unfed SS males for direct observation of polytene chromosomes of progeny larvae without selection (Tables 3 and 4). The F₁ female progeny from the former cross were mated to Ore-R males to check their fertility. The F₂ progeny from this cross were subjected to PCR and *in situ* hybridization. Individual progeny F₂ females were also crossed with *FM4* males. Individuals from this cross were mated again with *FM4* males to test for lethals in the *ovo*^{D1} revertant chromosome (a lethal would produce *FM4* males only). These manipulations are diagrammed in Figure 8.

Deglycosidation with endoglycosidase F

Ovary protein preparation was made as explained above in Western analysis. These proteins were denatured in denaturing buffer (0.5% SDS, 1% β-mercaptoethanol) by boiling for 10 min at 100°C. This protein extract was spun down, and the super-

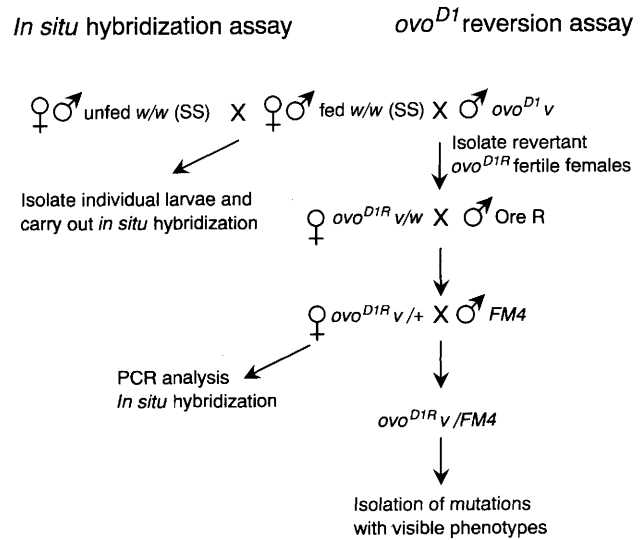


Figure 8. Scheme for *in situ* hybridization assay and for generating *ovo*^{D1} revertant lines. *ovo*^{D1R} indicates *ovo*^{D1} revertant.

natant was taken to a new tube. After adding deglycosidation reaction buffer [50 mM Na phosphate (pH 7.5), 1% NP40], 10 units of PNGase F (peptide/N-glycosidase F; New England Biolabs) enzyme was added. Incubation was for 5 hr at 37°C.

In vitro transcription/translation

A 1.5-kb DNA fragment, corresponding to the spliced ORF3 mRNA, was amplified using primers 1475 (5'-AGTTAAGTTA-GAAAAGCATGTTCCACCTCATGATGTTTCATACCCTTG-3') and 1463 (5'-ACGAAGCAATACATTGTTAGTTGT-3'). These PCR fragments were cloned directly into the TA cloning vector (pCRII, Invitrogen Corp.). The orientation of the insert was determined by restriction enzyme digestion and sequencing. The plasmid pTA-env has *gypsy* ORF3 under the control of the bacteriophage T7 promoter. Coupled *in vitro* T7 transcription-translation was done with the TnT-coupled reticulocyte lysate system (Promega) by following their standard protocol. For the production of nonradiolabeled protein, both amino acid mixture (-Met) and amino acid mixture (-Leu) were used in the reaction.

Statistical analyses

P values were calculated by a contingency table analysis using Statview II (Abacus Concepts). The reported values are continuity-corrected *P* values.

Acknowledgments

We are especially grateful to Alain Pélisson, Alexander Kim, Nicole Prud'homme, and Alain Bucheton for generously providing *Drosophila* strains as well as information about their experiments before publication. We thank Mike Dellannoy for assistance with immunoelectron microscopy. This work was supported by a Human Frontiers Grant to J.D.B. and National Institutes of Health grant GM35463 and American Cancer Society grant DB-7F to V.G.C.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby

marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Boeke, J.D. 1988. Retrotransposons. In *RNA genetics*. Volume II, *retroviruses, viroids, and RNA recombination* (ed. E. Domingo, J.J. Holland, and P. Ahlquist) pp. 59–103. CRC Press, Boca Raton, FL.
- Boeke, J.D. and V.G. Corces. 1989. Transcription and reverse transcription in retrotransposons. *Annu. Rev. Microbiol.* **43**: 403–433.
- Braiterman, L.T., G.M. Monokian, D.J. Eichinger, S.L. Merbs, A. Gabriel, and J.D. Boeke. 1994. In-frame linker insertion mutagenesis of yeast transposon Ty1: Phenotypic analysis. *Gene* **139**: 19–26.
- Coffin, J.M. 1993. Reverse transcriptase and evolution. In *Reverse transcriptase*. (ed. S. Goff and A. Skalka) pp. 445–479. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Eggleston, W.B., D.M. Johnson-Schlitz, and W.R. Engels. 1988. P-M hybrid dysgenesis does not mobilize other transposable element families in *D. melanogaster*. *Nature* **331**: 368–371.
- Eichinger, D.J. and J.D. Boeke. 1988. The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: Cell-free Ty1 transposition. *Cell* **54**: 955–966.
- Engels, W.R. 1989. P elements in *Drosophila melanogaster*. In *Mobile DNA* (ed. D.E. Berg and M.M. Howe), pp. 437–484. American Society for Microbiology, Washington, D.C.
- Gerasimova, T.I., L.V. Matyuyina, Y.V. Ilyin, and G.P. Georgiev. 1984a. Simultaneous transposition of different mobile elements: Relation to multiple mutagenesis in *Drosophila melanogaster*. *Mol. & Gen. Genet.* **194**: 517–522.
- Gerasimova, T.I., L.J. Mizrokhi, and G.P. Georgiev. 1984b. Transposition bursts in genetically unstable *Drosophila melanogaster*. *Nature* **309**: 714–716.
- Goff, S., P. Traktman, and D. Baltimore. 1981. Isolation and properties of Moloney murine leukemia virus mutants: Use of a rapid assay for release of virion reverse transcriptase. *J. Virol.* **38**: 239–248.
- Harlow, E. and D. Lane. 1988. *Antibodies: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Kim, A.I. and E.S. Belyaeva. 1991. Transposition of mobile elements *gypsy* (*mdg4*) and *hobo* in germ-line and somatic cells of a genetically unstable mutator strain of *Drosophila melanogaster*. *Mol. & Gen. Genet.* **229**: 437–444.
- Kim, A.I., E.S. Belyaeva, and M.M. Aslanian. 1990. Autonomous transposition of *gypsy* mobile elements and genetic instability in *Drosophila melanogaster*. *Mol. & Gen. Genet.* **224**: 303–308.
- Kim, A.I., C. Terzian, P. Santamaria, A. Pélisson, N. Prud'homme, and A. Bucheton. 1994. Retroviruses in invertebrates: The *gypsy* retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **91**: 1285–1289.
- Koerner, T.J., J.E. Hill, A.M. Myers, and A. Tzagoloff. 1991. High expression vectors with multiple cloning sites for construction of *trpE* fusion genes: pATH vectors. *Methods Enzymol.* **33**: 477–490.
- Kuhn, D. 1970. Another case of mass mutation. *Drosophila Inf. Serv.* **45**: 127.
- Lewis, A.P. and J.F.Y. Brookfield. 1987. Movement of *Drosophila melanogaster* transposable elements other than P elements in a P-M hybrid dysgenic cross. *Mol. & Gen. Genet.* **208**: 506–510.
- Lim, J.K. 1980. Site-specific intrachromosomal rearrangements in *Drosophila melanogaster*: Cytogenetic evidence for transposable elements. *Cold Spring Harbor Symp. Quant. Biol.* **45**: 553–560.
- . 1993. In situ hybridization with biotinylated DNA. *Drosophila Inf. Serv.* **72**: 73–77.
- Lim, J.K., M.J. Simmons, J.D. Raymond, N.M. Cox, R.F. Doll, and T.P. Culbert. 1983. Homologue destabilization by a putative transposable element in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **80**: 6624–6627.
- Marck, C. 1988. "DNA Strider": A "C" program for the fast analysis of DNA and protein sequences on the Apple Macintosh family of computers. *Nucleic Acids Res.* **16**: 1829–1836.
- 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.
- McEntee, K. and V. Bradshaw. 1988. Effects of DNA damage on transcription and transposition of Ty retrotransposons in yeast. In *Banbury Report 30. Eukaryotic transposable elements as mutagenic agents* (ed. M.E. Lambert, J.F. McDonald, and I.B. Weinstein), pp. 245–253, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Mével-Ninio, M., C.M. Mariol, and M. Gans. 1989. Mobilization of the *gypsy* and *copia* retrotransposon in *Drosophila melanogaster* induces reversion of the *ovo^D* dominant female-sterile mutations: Molecular analysis of revertant alleles. *EMBO J.* **8**: 1549–1558.
- Mével-Ninio, M., R. Terracol, and F.C. Kafatos. 1991. The *ovo* gene of *Drosophila* encodes a zinc finger protein required for female germ line development. *EMBO J.* **10**: 2259–2266.
- Mizrokhi, L.J. and A.M. Mazo. 1990. Cloning and analysis of the mobile element *gypsy* from *D. virilis*. *Nucleic Acids Res.* **19**: 913–916.
- Pélisson, A., S.U. Song, N. Prud'homme, P. Smith, A. Bucheton, and V.G. Corces. 1994. *gypsy* transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the *Drosophila flamenco* gene. *EMBO J.* (in press).
- Rolfe, M., A. Spanos, and G. Banks. 1986. Induction of yeast Ty element transcription by ultraviolet light. *Nature* **319**: 339–340.
- Strand, D.J. and J.F. McDonald. 1985. *copia* is transcriptionally responsive to environmental stress. *Nucleic Acids Res.* **13**: 4401–4410.
- Syomin, B.V., V. Konstantin, K.V. Kandror, A.B. Semakin, V.L. Tsuprun, and A.S. Stepanov. 1993. Presence of the *gypsy* (*mdg4*) retrotransposon in extracellular virus-like particles. *FEBS Lett.* **323**: 285–288.
- Tanda, S., J.L. Mullor, and V.G. Corces. 1994. The *Drosophila tom* retrotransposon encodes an envelope protein. *Mol. Cell. Biol.* **14**: 5392–5401.
- Woodruff, R.C., J.L. Blount, and J.N. Thompson Jr. 1987. Hybrid dysgenesis in *D. melanogaster* is not a general release mechanism for DNA transpositions. *Science* **237**: 1206–1208.



An env-like protein encoded by a *Drosophila* retroelement: evidence that gypsy is an infectious retrovirus.

S U Song, T Gerasimova, M Kurkulos, et al.

Genes Dev. 1994, **8**:

Access the most recent version at doi:[10.1101/gad.8.17.2046](https://doi.org/10.1101/gad.8.17.2046)

References

This article cites 26 articles, 6 of which can be accessed free at:
<http://genesdev.cshlp.org/content/8/17/2046.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

The advertisement features a dark background with a colorful, abstract image of what appears to be a DNA double helix or a similar molecular structure. On the left, the text 'Dharmacon Reagents' is displayed in white, with a smaller line below it: 'Custom synthesis, RNAi, and CRISPR solutions'. In the center, the words 'Infinite Reliability' are written in a large, white, sans-serif font. To the right of this text is a small white box containing the word 'More'. On the far right, the word 'horizon' is written in a white, lowercase, sans-serif font, with 'a PerkinElmer company' in a smaller font below it.