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Retroviruses in invertebrates: The *gypsy* retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*

ALEXANDER KIM*, CHRISTOPHE TERZIAN, PEDRO SANTAMARIA, ALAIN PÉLISSON, NICOLE PRUD'HOMME, AND ALAIN BUCHETON†

Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France

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ABSTRACT **Retroviruses are commonly considered to be restricted to vertebrates. However, the genome of many eukaryotes contains mobile sequences known as retrotransposons with long terminal repeats (LTR retrotransposons) or viral retrotransposons, showing similarities with integrated proviruses of retroviruses, such as *Ty* elements in *Saccharomyces cerevisiae*, *copia*-like elements in *Drosophila*, and endogenous proviruses in vertebrates. The *gypsy* element of *Drosophila melanogaster* has LTRs and contains three open reading frames, one of which encodes potential products similar to gag-specific protease, reverse transcriptase, and endonuclease. It is more similar to typical retroviruses than to LTR retrotransposons. We report here experiments showing that *gypsy* can be transmitted by microinjecting egg plasm from embryos of a strain containing actively transposing *gypsy* elements into embryos of a strain originally devoid of transposing elements. Horizontal transfer is also observed when individuals of the "empty" stock are raised on medium containing ground pupae of the stock possessing transposing elements. These results suggest that *gypsy* is an infectious retrovirus and provide evidence that retroviruses also occur in invertebrates.**

Retroviruses are widespread among species and have been found in all vertebrates that have been examined for their presence (1). They show long terminal repeats (LTRs) and typically contain three genes, *gag, pol*, and *env* (2), the latter being responsible for infective properties. They are usually considered to be restricted to vertebrates (1, 2).

However, transposable elements structurally related to proviruses of retroviruses appear to be more widely distributed. The genome of many eukaryotes including vertebrate and nonvertebrate animals, plants, and fungi contain mobile sequences known as viral or LTR retrotransposons, such as *Ty* elements in yeast (3), *copia*-like elements in *Drosophila* (4), and endogenous proviruses in vertebrates (2). They are characterized by the presence of typical LTRs. Many of them contain only two large open reading frames (ORFs), the potential products of which show similarities with gag and pol polypeptides of retroviruses. They are unlikely to be infectious because they are devoid of a third ORF encoding products similar to env (Fig. 1). For instance, *Ty1* elements of *Saccharomyces cerevisiae* encode products similar to gag(TYA) and pol(TYB) but not to env (Fig. 1).

Gypsy, also known as *mdg4*, is a 7.5-kb retrotransposon of *Drosophila melanogaster* that has LTRs of 482 nucleotides (5, 8, 9) and contains three ORFs, one of which encodes potential products similar to gag-specific protease, reverse transcriptase, and endonuclease (5). It therefore belongs to a particular class of LTR retrotransposons that includes other *Drosophila* transposable elements such as *297* (10) and *17.6* (11), which are more similar to retroviruses than are other retrotransposons. They potentially encode a third product, which might be a functional equivalent of retroviral env polypeptides (Fig. 1). In addition, sequence and organization of ORF2 in these elements are much more similar to that of the *pol* gene of retroviruses than to that of the other so-called *copia*-like retrotransposons: in particular the arrangement of the various domains of their *pol* gene is protease–reverse transcriptase–RNase H–integrase as observed in typical retroviruses, whereas *copia*-like elements show a protease–integrase–reverse transcriptase–RNase H arrangement (12–14) (Fig. 1).

All strains of *D. melanogaster* contain 20–30 defective *gypsy* elements located in pericentromeric heterochromatin that do not transpose (8, 15, 16). The last point is illustrated by the fact that most *gypsy* elements are located at similar positions when their distribution in unrelated strains is compared by Southern blot experiments (17). It seems that many stocks possess in addition a few active elements, usually less than five, located in euchromatic regions (8, 15, 16). *Gypsy* is in some way repressed and does not transpose detectably in most of them. A few stocks containing 30–40 *gypsy* elements in euchromatin have been described (15, 16, 18, 19). In one of them, called Mutator Strain (*MS*), this high copy number is associated with high rates of transposition occurring in both the germ-line and somatic cells (19, 20).

Although the structure of *gypsy* is strikingly similar to that of integrated proviruses of retroviruses it is considered as a transposable element, because retroviruses are thought to be restricted to vertebrates, and no infective properties have yet been described. We report experiments showing that it is an infectious retrovirus. A strain in which *gypsy* transposes at a high rate can be used to contaminate another strain originally devoid of transposing *gypsy* elements.

MATERIALS AND METHODS

***Drosophila* Strains.** *SS* (Stable Strain) carries a white (*w*) gene mutation on the X chromosome and is devoid of transposing *gypsy* elements (19–21). It is nevertheless permissive for *gypsy* transposition (see below).

MSN1 is isogenic to *SS* and contains a high copy number of actively transposing *gypsy* elements. It has been obtained by introducing into *SS* a functional *gypsy* from the Mutator Strain (*MS*) previously described (19–21) by *P*-element-mediated transformation. The high copy number of *gypsy* in this stock results from transposition of the unique element originally introduced in the genome using the *P*-element as a vector for transformation (unpublished results). The high frequency of *gypsy* transposition also resulted in mutation of the gene forked (*f*) so that it has an X chromosome carrying the *w* and *f* markers.

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Abbreviations: LTR, long terminal repeat; ORF, open reading frame.

*Permanent address: Department of Genetics, Lomonosov Moscow State University, 119899 Moscow, Russia.
†To whom reprint requests should be addressed.

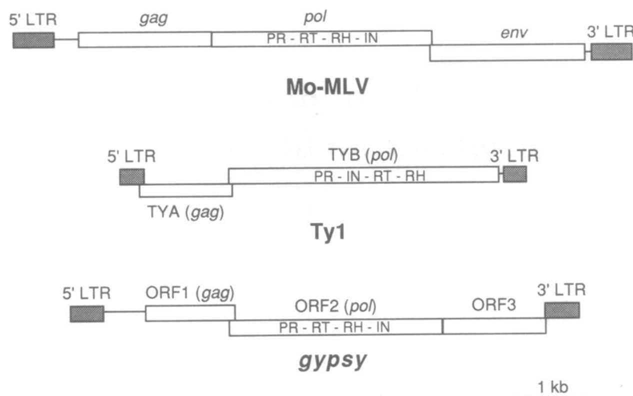

FIG. 1. Comparison of *gypsy* with viral retrotransposons (*Ty1* in yeast) and retroviruses [Moloney murine leukemia virus (Mo-MLV)]. The structures of *gypsy*, Mo-MLV, and *Ty1* are drawn according to the sequences reported, respectively, in refs. 5, 6, and 7. Shaded boxes represent LTRs and open boxes are ORFs. Arrangement of various enzymatic activities that characterize the *pol* gene is indicated: PR, protease; RT, reverse transcriptase; RH, RNase H; IN, integrase.

ovo^{D1} *v* is a stock containing the ovo^{D1} female sterile dominant and vermilion (*v*) recessive mutations on the X chromosome. $ovo^{D1}/+$ females have rudimentary ovaries and are therefore sterile.

The strains are maintained on standard *Drosophila* medium (22). All experiments were carried out at 25°C.

***ovo* "Reversion" Assay.** The *ovo* gene is a hot spot of insertion of *gypsy* (18). Insertion of *gypsy* into ovo^{D1} results in mutation of the dominant toward a recessive allele of the gene. The activity of the element was monitored by this *ovo* reversion assay. Females to be studied (*MSN1* or *SS* subjected or not to the various experiments described below) were individually crossed with males carrying the ovo^{D1} mutation (ovo^{D1} *v*) and the presence of ovaries in their daughters was tested. Males to be studied (*SS* after being submitted to the various treatments described below) were individually crossed with females of the *SS* stock, and their female progeny were crossed en masse with ovo^{D1} *v* males to study *ovo* mutability. Fertile daughters could result from mitotic recombination occurring early in development of the female germ line. These could be detected easily because they produced only white-eyed progeny and were discarded. Only fertile females giving white^{+} progeny were taken into account in all experiments.

Microinjection Experiments. We used a needle with a diameter of 8 μm for microinjections of egg plasm from *MSN1* into *SS* embryos, taking care not to inject cells or nuclei. In addition, the presence of forked in *MSN1* allowed easy detection of events that could result from cell or nucleus transplantation. We have detected no events of this type.

***In Situ* Hybridization to Salivary Gland Chromosomes.** We used plasmid pDm111 (8) containing a full-length *gypsy* as a probe to localize the element on polytene chromosomes of salivary glands of larvae. The probe was labeled by random priming with biotin-11-dUTP. Salivary gland preparations and hybridization were performed as described (23).

DNA Extraction and Analysis. DNAs were extracted from adult flies as reported (24), digested with restriction enzymes, electrophoresed on 0.8% agarose gels, transferred to nylon membranes, and hybridized with probes marked with ^{32}P by random priming. All these procedures were carried out as described (25).

RESULTS

High Rates of Transposition of *gypsy* in Stock *MSN1* Results in High Frequency of Reversion of ovo^{D1}. Demonstrating infectivity of an element like *gypsy* raises several difficulties. It requires a stock in which *gypsy* activity is increased that can be used as a source of particles, as well as an "empty" stock devoid of functional *gypsy* elements but permissive for activity of the element to be used as a recipient strain. A procedure must also be designed to detect the presence of active elements introduced into the empty stock.

The strain *SS* is devoid of transposing *gypsy* (19–21). Nevertheless, it is permissive for *gypsy* transposition. Stocks like *MS* in which *gypsy* transposes at high frequency (see above) have been obtained by introducing into the genome of *SS* an active element by *P*-element-mediated transformation (unpublished results). *MSN1* is such a stock. We used it as a source of elements with which to infect *SS*.

Gypsy activity can be monitored by induced mutability of the *ovo* gene. This gene is located on the X chromosome and is known to be a hot spot of insertion of *gypsy* (18). Females heterozygous for the ovo^{D1} dominant mutation are sterile because they do not develop ovaries. Crosses of females of a stock *y v f mal* in which *gypsy* transposes actively with ovo^{D1} males produce a high frequency of fertile daughters. This results from mutation of the dominant toward a recessive mutant allele of *ovo* due to insertion of *gypsy* early in development of the germ-line stem cells, usually allowing development of one functional ovary (18). This *ovo* reversion assay provides a powerful system with which to assay the activity of *gypsy* in the female germ-line.

We have studied whether transposition of *gypsy* in *MSN1* induces *ovo* reversion. Females of the stock were individually crossed with ovo^{D1} males and their daughters were checked for the presence of ovaries. As shown in Table 1, these crosses produced some fertile daughters. By contrast, *SS* females crossed with ovo^{D1} males produced only sterile daughters (Table 1). These results indicate that, as in the case of the *y v f mal* stock, transposition of *gypsy* in *MSN1* also occurs in the female germ line and induces mutations at the

Table 1. Number of contaminated flies estimated by the *ovo* reversion assay

	MSN1	SS	Exp. 1		Control	Exp. 2a		Exp. 2b	Control	Exp. 3a		Exp. 3b
	♀	♀	♀	♂	C2 ♀	♀	♂	♀	C3 ♀	♀	♂	♀
No. of flies studied	33	400	67	75	104	52	57	111	51	54	33	19
No. of contaminated flies (giving a positive result in the *ovo* reversion assay)	16	0	29	12	0	11	7	3	0	4	1	2
Total no. of daughters analyzed	1255	8500	9849	18,330	11,826	3751	2533	11,822	5627	2073	1848	1488
No. of fertile daughters	31	0	48	45	0	15	7	3	0	4	1	2

Females from stocks *MSN1* and *SS* and females and males resulting from the experiments described in the text were studied for the presence of actively transposing *gypsy* elements using the *ovo* reversion assay as indicated in *Materials and Methods*.

ovo locus. The *ovo* reversion assay can therefore be used to study the activity of *gypsy* in this stock.

***Gypsy* Can Be Transmitted by Egg Plasm Transplantation.** We have performed various experiments to demonstrate that *gypsy* can be infective. In experiment 1, egg plasm from *MSN1* embryos taken after cellularization was microinjected at the posterior pole of *SS* embryos before cellularization. Experiments 2a and 2b were identical to experiment 1, except that egg plasm was microinjected at the posterior end of *SS* embryos at the time of germ band extension—i.e., after cellularization and migration of pole cells (see ref. 26). As a control of experiment 2, egg plasm from *SS* embryos was microinjected into embryos of the same stock at the time of germ band extension (experiment C2). *SS* females resulting from the experiments were individually crossed with ovo^{D1} males and their daughters were checked for the presence of ovaries. Males were individually crossed with *SS* females and their daughters were crossed with ovo^{D1} males en masse to analyze *ovo* mutability as described above (males were not analyzed in experiments 2b and C2).

The results (Table 1) show that females obtained in experiments 1, 2a, and 2b can induce *ovo* reversion. Males give female progeny exhibiting the same property. We kept lines from the progeny of all fertile daughters for further studies that have shown that they result from *gypsy* insertion into the *ovo* gene (see below). No *ovo* reversion was observed after microinjection of egg plasm from *SS* (Table 1, control C2).

Therefore, *gypsy* in some form is present in the egg plasm of *MSN1* embryos and can be introduced by injection into *SS* embryos, which were originally devoid of transposing elements. The fact that in experiments 2a and 2b microinjections were done into *SS* embryos after cellularization indicates that introduction of *gypsy* into germ cells, resulting in *ovo* reversion, is presumably an infectious process.

***Gypsy* Is Infectious.** In two other experiments (experiments 3a and 3b), *SS* individuals were raised during most developmental stages (from hatching larvae to adults) on medium made of standard *Drosophila* food mixed with homogenized *MSN1* pupae in relative amounts of ≈1:5 (vol/vol). As a control (experiment C3), *SS* individuals were raised in the same conditions on standard medium containing homogenized *SS* pupae (relative amounts, 1:5). As in the other experiments, the resulting females were individually crossed with ovo^{D1} males and their daughters were studied for the presence of ovaries. Males of experiment 3a were crossed individually with *SS* females and their female progenies were subjected to the *ovo* reversion assay.

The results in Table 1 show that *SS* females raised in the presence of homogenized *MSN1* pupae can induce *ovo* reversion and that *SS* males raised in the same way give female progeny exhibiting the same property. This result is clearly different from that observed in the control experiment (C3) since all daughters resulting from crosses between *SS* females raised on a medium containing homogenized *SS* pupae, and ovo^{D1} males, have no ovaries (Table 1, control C3). Again, we kept lines from the progeny of all fertile daughters to study them in more detail.

Revertants Result from *gypsy* Insertions into the *ovo* Gene. We carried out *in situ* hybridization experiments of *gypsy* DNA to salivary gland chromosomes of larvae originating from 12 revertant lines obtained in all experiments. Fig. 2 shows the result observed with line R1 obtained in experiment 3a. A *gypsy* element is *de novo* inserted in region 4DE to which the *ovo* gene maps. The same result was obtained with all 12 lines studied. No *gypsy* sequences can be detected at this position in both parental strains (*SS* and ovo^{D1}).

We also analyzed in Southern blot experiments the structure of the *ovo* locus in 23 revertants. A sample of these experiments is given in Fig. 3. The results show that 21 revertants, originating from all experiments, contain a typical *gypsy* inserted in either orientation in the region of the *ovo* gene previously defined as its hot spot of insertion (18) (Fig. 3 *a* and *b*). We also checked that these *gypsy* elements contain the *Hin*dIII and *Xba* I restriction sites that have been reported to be characteristic of functional elements (8, 21). An example of the results is given in Fig. 3*c*. They show that the elements inserted in the *ovo* gene of these revertants possess the diagnostic restriction sites. The other two revertants contain insertions showing some differences compared

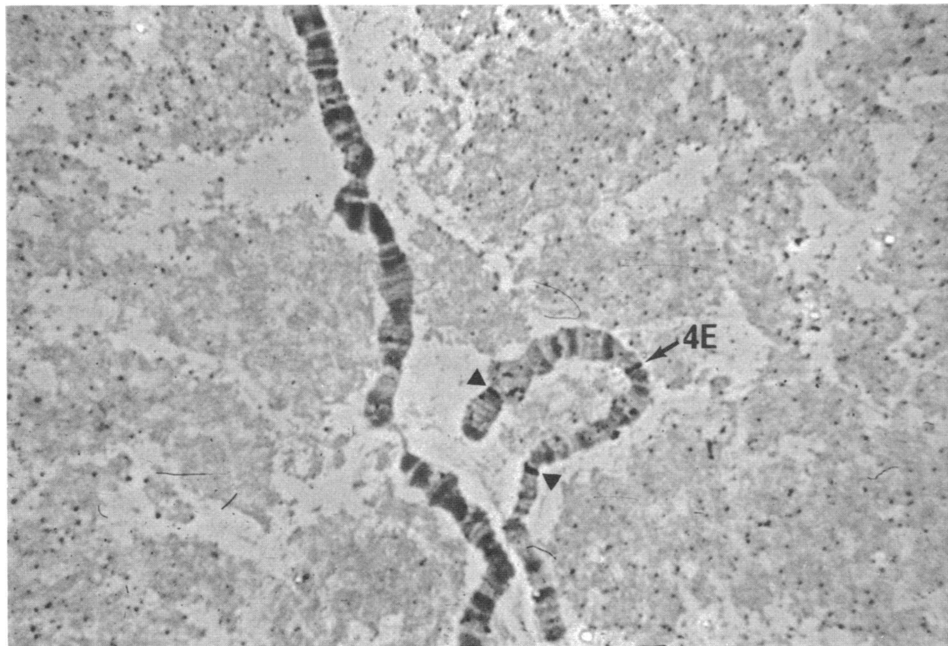

FIG. 2. *De novo* insertion of *gypsy* in the *ovo* locus of revertant line R1. This revertant line was obtained in experiment 3a (see text). Hybridization was performed using clone pDm111 (8), which contains a complete *gypsy* element. Arrow indicates the signal observed in region 4DE where the *ovo* gene maps. Arrowheads show other hybridization sites of *gypsy*.

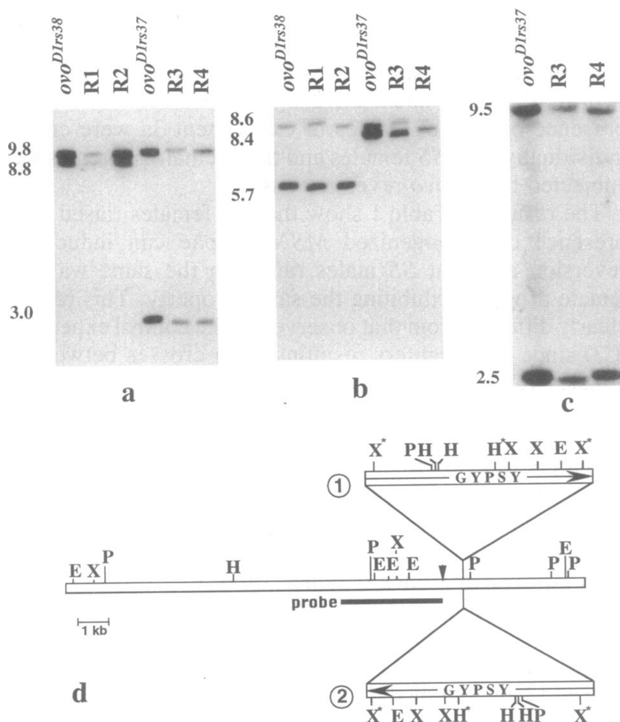

FIG. 3. Structure of the *ovo* locus in revertants. *Eco*RI (*a*) and *Pst* I (*b*) digests of DNAs from revertants R1, R2, R3, and R4 and from two mutations of the *ovo* gene (ovo^{D1rs38} and ovo^{D1rs37}) previously characterized as *gypsy* insertions in orientations 1 and 2, respectively (18) (see *d*). R1 and R2 were obtained in experiment 2a, and R3 and R4 were obtained in experiment 3a (see text). Results show that *gypsy* in the revertants is inserted at the same place as reported (18), in orientations 1 in R1 and R2 and 2 in R3 and R4. The probe used in the experiments was a 3.3-kb fragment shown in *d*. The 5.1-kb wild-type *Eco*RI fragment is replaced in revertants by an 8.8- or 3-kb fragment when *gypsy* is inserted in orientation 1 or 2, respectively. The 3.4-kb wild type *Pst* I fragment is replaced by a 5.7- or 8.4-kb fragment in revertants when *gypsy* is inserted in orientation 1 or 2, respectively. (*c*) *Xba* I digests of DNAs from revertants R3 and R4 obtained in experiment 3a and from ovo^{D1rs37} containing a *gypsy* element inserted in the same orientation (see above). The presence of a 2.5-kb fragment indicates that the *gypsy* elements contain at least one of the *Xba* I restriction sites typical of functional elements (5, 21). (*d*) Schematic structure of the *ovo* locus in the revertants. Orientations 1 and 2 are drawn. Solid bar corresponds to the probe used in the experiment. The region of the locus presented is included between coordinates −10 and +4 previously defined (18). Arrowhead indicates coordinate 0. E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; X, *Xba* I. Asterisks indicate restriction sites characteristic of active *gypsy* elements (5, 21).

to the typical *gypsy*. Therefore most revertants obtained in the experiments result from insertion of *gypsy* into *ovo*.

DISCUSSION

All the data presented here suggest that *gypsy* is a retrovirus that can actually infect flies. As shown especially in experiments 3a and 3b, it can be transmitted from homogenized individuals in which it was transposing actively to individuals originally devoid of transposing elements by contact or feeding.

The potential product of ORF3 (see Fig. 1) does not show significant similarities with other proteins (5) but has structural characteristics of transmembrane polypeptides. We suggest that it is analogous to *env* of retroviruses and is responsible for the infectious properties of the element.

These results show that *gypsy*, like retroviruses, can be transmitted horizontally. Since it is present in the germ line it is also inherited vertically, as are retrotransposons. Consequently, *gypsy* may be viewed as an endogenous retrovirus. In all experiments reported above, only infection of the germ line could be monitored. Therefore, unlike induced endogenous retroviruses, *gypsy* discloses an unusually high ability to infect germ cells. Sharing characteristics of both retrotransposons and retroviruses, it can be used to study the relationships between these two entities.

Gypsy is the only retrovirus identified so far in *Drosophila* and in any invertebrate. It is likely that retroviruses are more frequent in these species than usually believed. As indicated above, the *297* and *17.6 Drosophila* elements contain three ORFs and the organization of their *pol* gene is similar to that of *gypsy*. The same is true for the lepidopteran *TED* element in *Trichoplusia ni* (27). The *412 Drosophila* transposable element possesses only two ORFs, but one of them is unusually long and could result from fusion of the *pol* and *env* genes (28). All these elements share viral characteristics defined above and might well be endogenous retroviruses rather than retrotransposons.

Detailed studies of the *I* factor, a *Drosophila* retrotransposon devoid of terminal repeats, has shown that defective copies have accumulated in pericentromeric heterochromatin, presumably resulting from inactivation of a functional element early during evolution of the species (17, 24, 29, 30). This indicates that nonfunctional copies of transposable elements tend to accumulate in this part of the genome (17, 31). Similarly, the fact that all strains contain defective *gypsy* elements located in pericentromeric heterochromatin suggests that they are vestiges of an invasion of the species that occurred early in the evolutionary history of *D. melanogaster*. The low number of copies of potentially functional *gypsy* elements and their stability in many stocks indicate that host genes repressing activity of the element have been selected during evolution. Mutations of these genes should result in deregulation of the element. Genetic instabilities observed in strains *y v f mal* (18) and *MS* (19, 20) result from high rates of transposition of *gypsy*. Several other stocks like *tuh-3* (32) and *Uc* (33) have exhibited high mutability resulting from *gypsy* movements (16, 34). In addition, like *y v f mal* and *MS*, they contain a high copy number of *gypsy* elements (16). As is the case in *MSN1*, this seems to result from genetic properties of the stocks themselves rather than of the elements. The easiness of genetic and molecular analyses in *Drosophila* should allow characterization of the sequences responsible for these properties and of the mechanisms involved in the control of gypsy. Therefore, this organism appears to be an excellent model system to study the relationships between a retrovirus and the host genome.

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