

tirant, a Newly Discovered Active Endogenous Retrovirus in *Drosophila simulans*

Abdou Akkouche,^a Rita Rebollo,^{a*} Nelly Burlet,^a Caroline Esnault,^{a*} Sonia Martinez,^a Barbara Viginier,^b Christophe Terzian,^b Cristina Vieira,^{a,c} and Marie Fablet^a

Université de Lyon, Université Lyon 1, CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, Villeurbanne, France^a; Université de Lyon, Université Lyon 1, INRA EPHE, UMR754, Rétrovirus et Pathologie Comparée, Lyon, France^b; and Institut Universitaire de France, Paris, France^c

Endogenous retroviruses have the ability to become permanently integrated into the genomes of their host, and they are generally transmitted vertically from parent to progeny. With the exception of *gypsy*, few endogenous retroviruses have been identified in insects. In this study, we describe the *tirant* endogenous retrovirus in a subset of *Drosophila simulans* natural populations. By focusing on the envelope gene, we show that the entire retroviral cycle (transcription, translation, and retrotransposition) can be completed for *tirant* within one population of this species.

Endogenous retroviruses are genomic sequences that are widely dispersed throughout the host genome. These sequences constitute 8% of the human genome and represent the remnants of ancient infections by retroviruses (1). Some of these retroviruses were domesticated and generated cellular neogenes, such as the *syncytin* gene (9, 14). The structure of a canonical endogenous retrovirus consists of three open reading frames (ORFs), which are bordered by long terminal repeats (LTRs). The third ORF encodes the envelope protein (Env), which possesses fusogenic properties and is responsible for the infectious behavior of exogenous retroviruses. In insects, several endogenous retroviruses have been described, and most of them possess a complete retroviral structure. Representative insect endogenous retroviruses (IERVs) or insect ErantiViruses (24) include the following: *gypsy*, *ZAM*, *Idefix*, *tirant*, 17.6, 297, and *nomad* in *Drosophila melanogaster*; *tom* in *Drosophila ananassae*; *Tv1* in *Drosophila virilis*; *TED* in *Trichoplusia ni*; *Oswaldo* in *Drosophila buzzatii*; and *Yoyo* in *Ceratitis capitata* (see reference 19 for a review). With the exception of *nomad* and *Tv1*, each of these displays a complete *env* ORF. Thus far, only the well-known *gypsy* element of *D. melanogaster* has been shown to possess infectious properties (10), as Moloney murine leukemia virus pseudotypes with the *gypsy* Env protein were shown to infect insect cells (25).

The *tirant* LTR retrotransposon of *D. melanogaster* was previously described to share sequence similarity with the fusion proteins of certain baculoviruses (13, 16, 20) (see Fig. 1 for the *tirant* structure). In a phylogenetic study of numerous elements from insects, Terzian et al. (24) proposed that the *tirant* element from *D. melanogaster* (GenBank accession number X93507) belongs to the IERVs. Thus, the *tirant* element was placed into this clade of retroviruses, which uses a tRNA-Ser binding site to prime reverse transcription (RT). In the study described in this report, we examined this ERV family in *Drosophila simulans* using a collection of strains with variable numbers of genomic insertions (7), and we demonstrated that this family was able to produce Env proteins in the ovaries of the host. We found that *tirant* was capable of performing the first step required for infection, i.e., the production of a functional Env protein, which suggests that it could be classified as an active endogenous retrovirus of *D. simulans*. Furthermore, we demonstrated that the complete retrotransposition cycle of *tirant* occurs within a particular natural strain of *D. simulans*,

which indicates that the endogenization of *tirant* in the natural populations of this species is an ongoing process. In addition, this experimental system is of particular interest because our panel of natural strains displayed three distinct states in regard to *tirant* dynamics and thus will be ideal for deciphering the fine regulatory mechanisms: (i) the absence of transcription in most strains, (ii) transcription but the absence of translation, and (iii) transcription and translation both occurring in a single strain.

MATERIALS AND METHODS

***Drosophila* strains.** We analyzed samples of flies collected from several geographically distinct natural populations of *D. simulans*. These populations were maintained in the laboratory at 25°C as isofemale lines or as small-mass cultures with approximately 50 pairs in each generation.

The *D. simulans* strains used were previously described by Fablet et al. (7). They differed in the number of euchromatic copies of *tirant* that could be detected by *in situ* hybridization on polytene chromosomes. These strains and their respective copy numbers of *tirant* were as follows: Chicharo (Portugal, 0 copies), Makindu (Kenya, 5 copies), Zimbabwe (2 copies), Mayotte (Indian Ocean, 2 copies), and Brazzaville (Congo, 0 copies). Each population is known to display specific, unique euchromatic insertion sites, although bands representing heterochromatic sites may appear to be shared between populations in transposon display experiments.

As controls, we used a strain of *Drosophila pseudoobscura*, which is a species devoid of any *tirant* copies (17), that was kindly provided by F. Lemeunier from Gif-sur-Yvette, France, and a *D. melanogaster* isolate of a natural population from Senegal, which was grown in the laboratory and contains 9 euchromatic insertions of *tirant* that can be detected by *in situ* hybridization on polytene chromosomes (2). It should also be noted that

Received 18 December 2011 Accepted 10 January 2012

Published ahead of print 25 January 2012

Address correspondence to Marie Fablet, marie.fablet@univ-lyon1.fr.

* Present address: Rita Rebollo, Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada. Caroline Esnault, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA.

Supplemental material for this article may be found at <http://jvi.asm.org/>.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JVI.07146-11

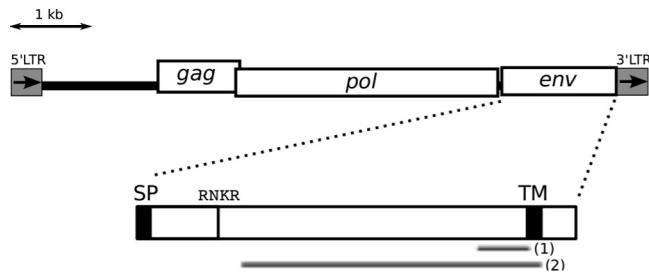


FIG 1 Structure of *tirant env*. SP, putative signal peptide, as predicted previously (4); TM, transmembrane domain, as predicted previously (27); RNKR, peptide sequence of the furin cleavage site, as determined previously (16); fragment (1), RT-qPCR amplicon; fragment (2), probe for *in situ* hybridization of the transcripts.

D. melanogaster populations display much less variability of the *tirant* copy number than *D. simulans* populations (2, 7).

Analysis of genomic copies of *tirant env*. (i) **Screening for *env* full-length ORFs in natural populations of *D. simulans*.** To search for full-length ORFs for *tirant env* in natural populations of *D. simulans*, we used the protein truncation test (PTT) protocol, which consists of PCR amplification of the *env* gene followed by *in vitro* transcription and translation. A T7-coupled reticulocyte lysate system (Promega) was used for the PTT analysis, according to the protocol recommended by the manufacturer (the TNT quick-coupled transcription/translation system and the Transcend nonradioactive translation detection system from Promega). The primers used for *env* amplification were designed from the *tirant* sequence (GenBank accession number AC0054444, positions 50203 to 58729) and consisted of the following sequences: forward (Fw) primer 5' GGA TCC TAA TAC GAC TCA CTA TAG GGA GCC ACC ATG GAA CAA TTC 3' and reverse (Rv) primer 5' CCT GAA TTT TTT TTT GGG TTA GAG GCG 3'. The upstream primer was positioned at the beginning of ORF3, which allowed transcription to begin at the underlined ATG. We also used *D. pseudoobscura*, a species devoid of *tirant* insertions, as a negative control.

(ii) **Analysis of *tirant env* sequence polymorphism.** We isolated *tirant env* sequences from a subset of the pool of natural populations by PCR using the following primers: Fw primer 5' TGT ATG GAA CAA TTC CAT CTG AC 3' and Rv primer 5' TTG TTT AGA GGC GTG GGG GT 3'. These PCR products were then cloned (TOPO TA cloning kit; Invitrogen) and sequenced (Sanger method; GenoScreen, France). The DNA sequences were aligned and clustered using the Seaview software (version 4) (8; <http://pbil.univ-lyon1.fr/software/seaview.html>), and DNA polymorphisms were measured using the DnaSP software (version 5) (21). The alignment of the sequences is provided in the supplemental material.

Analysis of *tirant env* transcription. (i) **Isolation of *tirant* mRNAs and synthesis of cDNA.** Total RNA was extracted from pools of 20 to 30 ovaries from adult females using an RNeasy kit (Qiagen) and subsequently treated with DNase (DNA-free kit; Ambion). One microgram of the total RNA was then converted into cDNA using a ThermoScript Invitrogen kit primed with a mix of oligo(dT)₂₀ and random primers.

(ii) **Quantification of *env* transcripts of *tirant* by qPCR.** The cDNAs were diluted 50 times and then quantified using SYBR green quantitative PCR (qPCR) in a LightCycler apparatus (Roche Diagnostics) with primers specific to the *tirant env* gene (Fw primer 5' ACA CGT TCC CTG AAC AGA CG 3' and Rv primer 5' GAA CGT TAC CAA TCC GAG CA 3') (see Fig. 1 for the position of the amplicon). The transcript quantity was estimated relative to that of the *rp49* gene, for which we used the following primers: Fw primer 5' CGG ATC GAT ATG CTA AGC TGT 3' and Rv primer 5' GCG CTT GTT CGA TCC GTA 3'. The *rp49* gene demonstrated equivalent amounts of transcripts for all of the strains analyzed, which enabled the amount of *env* transcripts to be compared. Measurements were obtained from two independent experiments; i.e., RNAs were ex-

tracted from two different pools of ovaries. For each experiment, the reactions were performed in duplicate, and the standard curves were calculated using serial dilutions of genomic DNA. The primers were tested for all the strains, and an efficiency value greater than 1.9 was maintained.

(iii) **Detection of *tirant env* transcripts by *in situ* hybridization in ovaries.** Ovaries were dissected in PBT (1× phosphate-buffered saline [PBS], 0.2% Tween 20), fixed for 20 to 40 min, washed in PBT, and then incubated in 1× PBS containing 3.7% formaldehyde for 10 min at room temperature. Hybridization (overnight) and washing were performed at 65°C. The *tirant* antisense riboprobe was a 1-kb fragment corresponding to the *env* gene and included a T3 promoter site in the reverse primer (Fw primer 5' ACG GGG TTT GAT TAA CG 3' and Rv primer 5' GGA ATT AAC CCT CAC TAA AGG GAG TGT CCA GGT GTG CTG 3'). The riboprobe was labeled by the *in vitro* transcription of T3 using a digoxigenin (DIG)-RNA labeling mix (Roche). DIG-labeled RNA probes were detected using anti-DIG Fab antibody fragments (1/50 dilution; Roche) and fluorescence amplification (Tyramid signal amplification kit; PerkinElmer). An RNase H control was included to prevent RNA-DNA hybridization in the experiments. Specifically, after the hybridization and washing steps, the ovaries were treated with RNase H (0.3 U/μl) for 1 h at 37°C and then evaluated by immunodetection. To stain the DNA, the ovaries were incubated in PBS containing Sytox green (Invitrogen), and visualization was performed with a Zeiss LSM510 Meta confocal microscope.

Analysis of *tirant* Env protein production by detection of *tirant* Env proteins in ovary preparations using immunochemistry. (i) **Antibodies.** A polyclonal antibody against the *tirant* Env protein was produced via the DNA vaccination of mice. The Env cDNA sequence was synthesized and cloned into a pVax plasmid designed using the ICantibodies technology (developed by In Cell Art for *in vivo* gene expression), and this plasmid was injected into a Swiss mouse.

(ii) **Immunochemistry.** Ovaries were dissected in PBS on ice and fixed with 4% paraformaldehyde in PBS with 0.2% Triton X-100 at room temperature for 20 min. The tissue samples were then washed three times for 2 min each with PBS. After blocking in PBS containing 0.2% Triton X-100 and 0.1% bovine serum albumin (BSA) for 2 h at room temperature, the ovaries were incubated overnight at 4°C with the primary antibody at a 1/50 dilution in PBS containing 0.2% Triton X-100 and 0.1% BSA. After three washes in PBT, the ovaries were incubated with the secondary antibody (1/600 dilution of Alexa 488-conjugated anti-mouse IgG [H+L]; Molecular Probes) for 2 h at room temperature. For the negative controls, the ovaries were incubated with a 1/500 dilution of mouse preimmune serum for the corresponding primary antibody. The DNA was stained with propidium iodide, and confocal images were acquired using an LSM510 microscope (Carl Zeiss).

Detection of *tirant* retrotransposition by transposon display. Total genomic DNA was isolated from individual flies using a standard phenol-chloroform extraction procedure after proteinase K digestion. The transposon display was performed using a modified version of the protocol employed previously (6). Briefly, genomic DNA from individual adult flies was digested with 10 units of MseI for 3 h at 37°C, and the adaptors consisted of the following sequences: 5' AAC AGC TGG ACG ATG AGT CCT GAG A 3' (AdaptMseI+) and 5' TAT CTC AGG AGT GTA 3' (AdaptMseI-). The ligation of the adaptors was performed at 37°C for 1 h with 2.5 units of T4 DNA ligase. During the first round of amplification, 0.625 units of Dream *Taq* polymerase (Fermentas), 0.2 μM deoxynucleoside triphosphate, 0.4 μM the adaptor-specific primer (5' AAC AGC TGG ACG ATG AGT 3'), and 0.2 μM the *tirant*-specific primer (5' GTC TTC CCG GTT GAG TGT 3') were used. During the second amplification round, 0.2 μM the adaptor primer, 0.25 μM the nested *tirant*-specific primer with 5' hexachloro-6-carboxyfluorescein (HEX) fluorescent labeling (5' TAG AGG CGT GGG GGT TTA 3'), and 0.625 units of Dream *Taq* polymerase (Fermentas) were used. The PCR was performed for 35 cycles with amplification steps that lasted for 45 s. Negative controls that used the adaptor-specific primer or the element-specific primer alone were

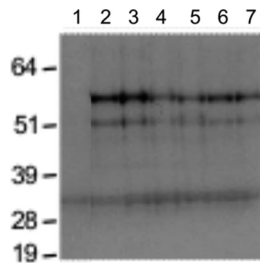


FIG 2 Protein truncation test for *tirant env* in *D. simulans* natural strains. Western blotting of the products from *in vitro* transcription and translation. Lanes: 1, *D. pseudoobscura* negative control; 2, *D. melanogaster* strain from Senegal; 3 to 7, *D. simulans* strains from Mayotte, Brazzaville, Zimbabwe, Makindu, and Chicharo, respectively. The molecular mass ladder (in kDa) is included on the left side of the image. All of the tested *D. simulans* natural populations displayed identical profiles that corresponded to a potentially functional *env* ORF. The fainter, lower 33-kDa band is not *tirant* specific because we also found this band in the *D. pseudoobscura* negative control (a species devoid of *tirant* insertions).

included. One microliter of the PCR products was loaded onto a 3730XL capillary DNA analyzer (Applied Biosystems) with a GS600LIZ standard size marker (Applied Biosystems). The raw data were analyzed using the GeneMapper software (Applied Biosystems).

We measured the number of novel bands that were obtained in the progeny (7 to 10 males and females) of five independent pairs for the Makindu population and four independent pairs for the Mayotte population. The bands were considered novel only when they were absent from both parental samples and were present in no more than one individual in the progeny.

Nucleotide sequence accession numbers. The nucleotide sequences were deposited in the GenBank database with the following accession numbers: JN786085 to JN786091 for Makindu, JN786092 to JN786098 for Zimbabwe, JN786099 to JN786104 for Chicharo, and JQ219965 to JQ219971 for Mayotte.

RESULTS

***D. simulans* strains display potentially functional *env* genes.** We evaluated the integrity of the *tirant env* genes in our strains using a PTT, which consists of the amplification of genomic DNA sequences by PCR, followed by *in vitro* transcription and translation. In this technique, subsequent Western blotting reveals whether the *env* gene contains a full-length ORF. A premature stop codon or internal deletions at the genomic level appear as bands of smaller mass compared with those of the expected full-length protein. Our assay revealed the presence of complete ORFs for *tirant env* in the tested natural strains of *D. simulans*, which demonstrates that these sequences could potentially be functional *in vitro* (Fig. 2). Similarly, the *tirant env* ORFs of *D. melanogaster* were found to be full-length ORFs. Indeed, Western blotting after *in vitro* translation indicated two specific bands that corresponded to approximately 56 kDa and 52 kDa. As the expected product would give a band corresponding to 56.2 kDa, we assumed that the observed signal represented a complete and potentially functional Env protein. The shorter products may correspond to the use of a weaker downstream AUG signal (located 177 bp downstream) or to the presence of a premature stop codon or deletions in certain *tirant* copies that conserve the reading frame.

We isolated and analyzed in detail the sequences of *tirant env* from a subset of these populations (Makindu, Mayotte, Zimbabwe, and Chicharo) (see the supplemental material for sequence alignment). We did not observe length polymorphism in the PCR

products, although the sequencing of a few clones revealed the existence of two sequence variants (Fig. 3). A previous study of *tirant* sequences identified two subfamilies in natural populations of *D. simulans*, based on differences in the sequences of the regulatory regions of the element: the C subfamily is transcriptionally active and found in both euchromatin and heterochromatin, and the S subfamily is silenced and exclusively heterochromatic (7). Both of the variants identified here belonged to the transcriptionally active C subfamily of the *tirant* copies. One of the sequence variants (referred to here as variant Ca) was found in all four tested strains (GenBank accession numbers JN786085 to JN786091 for Makindu, JQ219965 to JQ219969 for Mayotte, JN786092 to JN786095 and JN786097 for Zimbabwe, and JN786103 and JN786104 for Chicharo), and the sequences were highly similar (nucleotide diversity [π] = 0.0079). This variant presumably corresponds to the translated sequence from the PTT assay and is thus a putatively functional sequence. The other variant (referred to here as variant Cb) was found in all the samples except that of the Makindu strain (GenBank accession numbers JQ219970 and JQ219971 for Mayotte, JN786096 and JN786098 for Zimbabwe, and JN786099 to JN786102 for Chicharo), and this variant also displayed high levels of sequence similarity among the strains (π = 0.0059). However, this variant presented a premature stop codon. The calculation of π for the consensus of each variant resulted in a value of 0.0429. The sequencing of the *env* cDNAs that were obtained from the total mRNA in the Mayotte sample revealed that both variants are transcribed.

***tirant* produces *env* transcripts in the ovaries of certain *D. simulans* strains.** We tested four *D. simulans* strains for the presence of *tirant env* transcripts. Our RT-qPCR experiments showed that only two strains, Makindu and Mayotte, had significant levels of *tirant env* transcripts in the ovaries (Fig. 4A). Even the *D. melanogaster* strain, which harbors significantly more insertions within the euchromatin, did not contain *tirant* transcripts in the ovaries.

To visualize the localization of the *tirant* transcripts in the germ line, we performed RNA fluorescent *in situ* hybridization for the ovaries. As expected, no transcripts were detected in the strains that showed no RT-qPCR signal, namely, Zimbabwe and Chicharo, or in the *D. melanogaster* strain (Fig. 4B). We observed that *tirant* transcripts accumulated in the mature egg chambers of the ovaries of the Makindu and Mayotte strains (a mature egg chamber consists of the oocyte and nurse cells, which both constitute the germ line, surrounded by the somatically derived follicle cells) (Fig. 4B). However, the patterns of transcript localization were different between these two strains; *tirant* transcripts were detected in both the follicle and nurse cells for the Makindu strain, whereas these transcripts accumulated substantially in the follicle cells and were absent from the nurse cells for the Mayotte strain. This observation may also explain why the transcript levels, as determined by RT-qPCR, were higher in the Makindu strain than the Mayotte strain.

***tirant env* transcription results in protein production in one *D. simulans* strain.** We evaluated the production of *tirant* Env proteins using immunocytochemistry, which was performed on whole ovaries using the *tirant* Env antibody. We observed staining for *tirant* Env in the ovaries of the Mayotte strain, whereas no clear signal for Env was detected in the other samples (Fig. 4C). Our immunofluorescence analysis revealed the presence of the Env protein at a late stage (stage 9) of oogenesis in the follicle cells of the Mayotte strain.

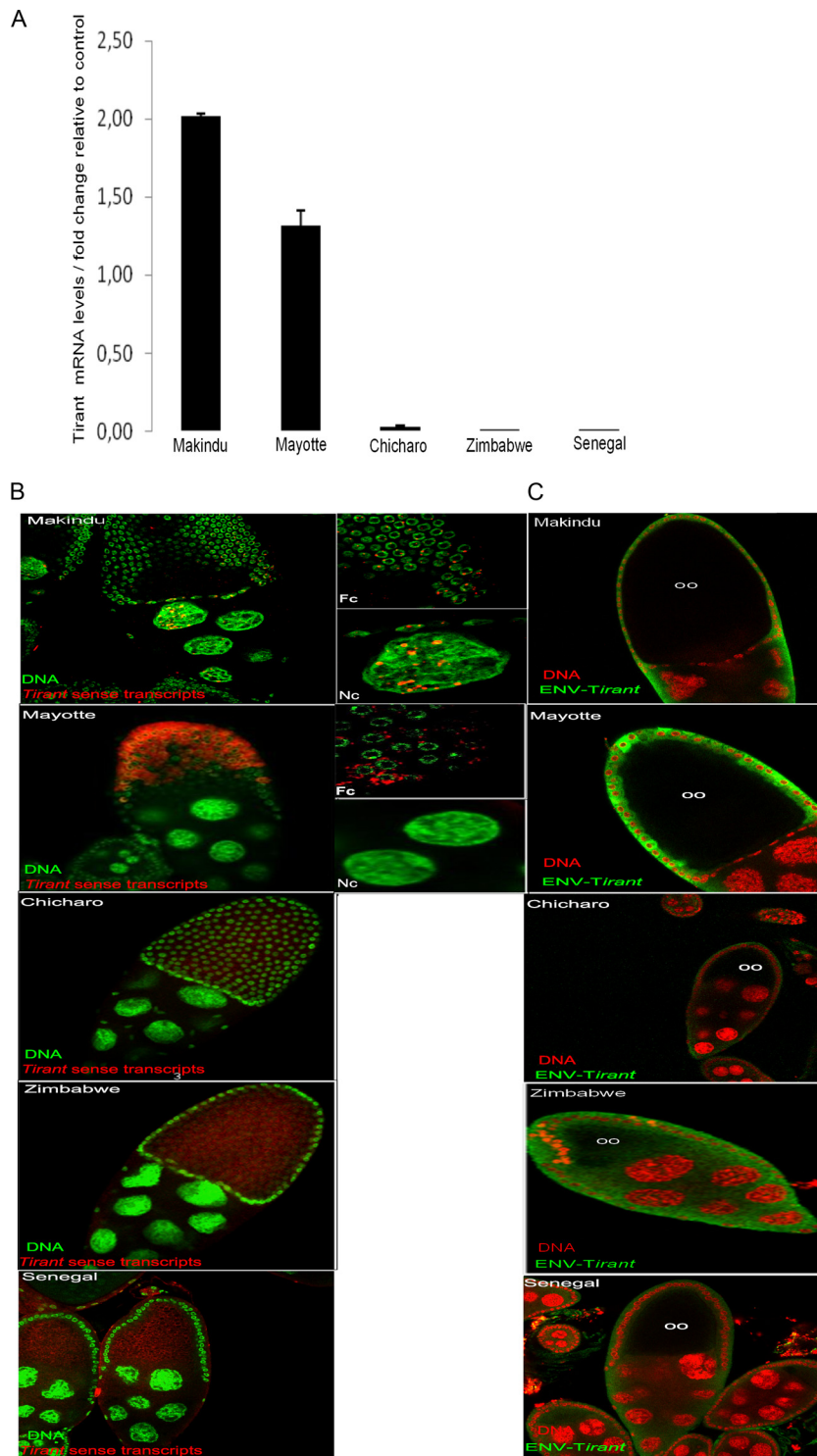


FIG 4 Expression of the *tirant env* gene in the ovaries of natural strains of *D. simulans*. (A) RT-qPCR of the ovaries. The transcript levels were estimated relative to those of the *rp49* gene. Significant levels of *tirant env* transcripts were detected in the Makindu and Mayotte populations. *D. melanogaster* Senegal was used as a control strain. (B) *In situ* hybridization of *tirant* transcripts (red). DNA is labeled in green (Sytox Green). Fc, follicle cells; Nc, nurse cells. (Left) View of ovary chambers (magnification, $\times 40$); (right) details of the left panel. *tirant* transcripts were found in the nurse cells and follicle cells of the Makindu strain and in the follicle cells of the Mayotte strain. No staining was observed in the other strains. (C) Detection of *tirant* Env protein by immunochemistry (green) in the Mayotte strain. DNA is stained in red (propidium iodide). oo, oocyte. The green halos in the other strains do not represent Env staining but rather correspond to autofluorescence, as they were also observed following staining with preimmune serum.

TABLE 1 Transposon display results

Strain	Pair no.	No. of male/no. of female progeny	No. of male/no. of female novel bands	No. of male/no. of female progeny without novel bands
Makindu	1	10/9	0/0	10/9
	2	10/9	0/0	10/9
	3	9/10	1/1	9/9
	4	9/10	3/1	8/9
	5	10/10	4/1	8/9
Mayotte	1	8/10	3/4	6/8
	2	10/10	0/2	10/8
	3	10/7	8/10	7/4
	4	10/10	7/15	8/3

Furthermore, we found that this IERV was not active in *D. melanogaster*, which suggests that the same endogenous retrovirus can display different behaviors in different but closely related species. This is not the first report to describe such differences between *D. melanogaster* and *D. simulans*. For example, one recent study revealed that the respective contents of the transposable elements in these species are quite different; *D. melanogaster* harbors mainly complete copies, whereas *D. simulans* displays many deleted and degenerated copies of elements within the same family (12).

These findings indicate that each strain of *D. simulans* studied possesses at least one potentially complete functional *tirant env* gene in its genome. A detailed sequence analysis revealed that each of these populations had highly similar sequences for *tirant env*. Moreover, another variant of *env* that contains a premature stop codon was also found in most of the studied populations (in all except Makindu).

In this study, we found one *D. simulans* strain in which *tirant env* was both transcribed and translated. In this strain, the staining for Env proteins was observed in the follicle cells of the ovaries during the later stages of development. Other IERVs have also been shown to display activity in the ovaries of certain strains that are referred to as “permissive” or “unstable.” In these strains, for example, Tanda et al. (22) found that *tom* RNAs accumulate in the germarium, in nurse cells (germ line cells), and in the oocyte nucleus. *idefix* was shown to be expressed in very specific early follicle cells (somatic cells) within the ovaries of unstable strains (23). *gypsy*, which is the only infectious IERV identified to date, was shown to produce Env proteins that accumulated in the follicle cells of permissive females at stage 10 of oogenesis (18). Leblanc et al. (11) studied *ZAM*, which is the IERV most closely related to *tirant*, and found *ZAM* Env proteins in the follicle cells surround-

ing the posterior portion of the oocytes in *D. melanogaster* ovaries at stages 9 and 10.

For *gypsy* and *ZAM*, the somatic expression of the *env* genes was thus demonstrated. However, only a transfer from the soma to the oocyte (germ line cell) can result in an increase in the copy number of these IERVs in the following generation. This mechanism was demonstrated for *gypsy* (5) and *ZAM* (3), and it likely involves the yolk protein traffic machinery that operates between the follicle cells and the oocyte (3). In the Mayotte strain, *tirant* transcripts are located in the follicle cells, which is also the case for *gypsy* and *ZAM*. Our transposon display results, together with the more recent *in situ* hybridization experiments on polytene chromosomes, show that *tirant* does transpose in this strain. Therefore, we may assume that the infection of an oocyte by *tirant* viral particles occurs and that these particles are produced in the surrounding follicle cells as they are for *gypsy* and *ZAM*.

The *tirant* element: a model for the study of ERV proliferation in natural populations. As described for other endogenous retroviruses of *Drosophila*, such as *ZAM* and *gypsy*, most strains are classified as “restrictive” or “stable,” which means that the activity and copy number of the endogenous retroviruses are limited. In addition, there are certain “permissive” or “unstable” strains for which this inhibition is no longer detected. Our study assessed the original features of *tirant* in a collection of natural populations, and we detected the following three categories of strains that differed according to their *tirant* dynamics: (i) the Chicharo category had zero (or very few, as in the case of Zimbabwe) euchromatic copies of *tirant* and no detectable production of transcripts; (ii) the Mayotte category, which displays the qualities of a permissive strain, possessed *tirant* copies that were able to undergo the entire retrotransposition cycle; and (iii) the Makindu category had an intermediate state, where *tirant* was transcribed but not translated. Most natural populations worldwide behave similarly to those within the Chicharo category and present no detectable *tirant* activity. Our results indicate that these strains have potentially functional *tirant env* genes, which are, however, silenced.

Therefore, at least in the Mayotte strain, *tirant* is able to complete the retroviral cycle and can therefore be considered an active endogenous retrovirus of *D. simulans*. However, because we do not have the sequences of all the *tirant* entire copies, we cannot exclude the possibility that the expression of the other ORFs may originate from different copies and that the retroviral cycle is achieved through complementation. It remains unclear why *tirant* could not be translated in the Makindu strain. However, we can postulate that a posttranscriptional control of *tirant* occurs in the Makindu strain, which is not the case in the Mayotte strain. Our

TABLE 2 Results obtained with the different strains

Population	No. of euchromatic copies ^a	mRNA level ^b	<i>In situ</i> localization of mRNAs	PTT result	Env protein
Makindu	5	1.53	Nurse cells +, follicle cells +	Complete ORF	ND ^c
Mayotte	2	1	Follicle cells + + +	Complete ORF	Follicle cells
Zimbabwe	2	0.02	ND	Complete ORF	ND
Chicharo	0	4.10 ⁻⁴	ND	Complete ORF	ND
Senegal (<i>D. melanogaster</i>)	9	3.10 ⁻⁵	ND	Complete ORF	ND

^a As previously determined by *in situ* hybridization on polytene chromosomes (2).

^b Relative to the Mayotte level.

^c ND, not detected.

collection of natural populations, which provides examples of different *tirant* dynamics, is an ideal tool for deciphering such mechanisms.

Furthermore, it is tempting to propose that these different natural strains illustrate different time periods of the history of the *tirant* endogenous retrovirus in the *D. simulans* genome (26). When an active copy of *tirant* is inserted into a favorable genomic site, it can be transcribed and translated and can retrotranspose to other sites, thereby increasing its copy number. This was likely the situation for the Mayotte strain. In the context of the “arms race” hypothesis, the *D. simulans* genome likely develops strategies to combat this retroviral invasion, such as posttranscriptional locking, which appears to be the case in the Makindu strain. Such locking may also occur in *D. melanogaster* populations, where the number of euchromatic insertions is relatively high compared with *D. simulans*, although no significant transcript levels can be detected in these populations. However, because both species display very different dynamics related to transposable elements (12), we may assume that *tirant* regulation is under the control of many distinct mechanisms.

ACKNOWLEDGMENTS

This work was supported by the ANR grant GENEMOBILE from the CNRS, FINOVI, IUF, and CIBLE 2008 (Région Rhône Alpes).

We thank Christian Biémont for his useful discussions and Geoffrey Hutinet for his technical help. The English quality was certified by American journal experts.

REFERENCES

- Belshaw R, et al. 2004. Long-term reinfection of the human genome by endogenous retroviruses. *Proc. Natl. Acad. Sci. U. S. A.* 101:4894–4899.
- Biémont C, et al. 2003. Worldwide distribution of transposable element copy number in natural populations of *Drosophila simulans*. *Evolution* 57:159–167.
- Braslet E, et al. 2006. Viral particles of the endogenous retrovirus ZAM from *Drosophila melanogaster* use a pre-existing endosome/exosome pathway for transfer to the oocyte. *Retrovirology* 3:25.
- Cañizares J, Grau M, Paricio N, Moltó MD. 2000. Tirant is a new member of the gypsy family of retrotransposons in *Drosophila melanogaster*. *Genome* 43:9–14.
- Chalvet F, et al. 1999. Proviral amplification of the gypsy endogenous retrovirus of *Drosophila melanogaster* involves env-independent invasion of the female germline. *EMBO J.* 18:2659–2669.
- Esnault C, et al. 2008. High genetic differentiation between the M and S molecular forms of *Anopheles gambiae* in Africa. *PLoS One* 3:e1968.
- Fablet M, McDonald JF, Biémont C, Vieira C. 2006. Ongoing loss of the tirant transposable element in natural populations of *Drosophila simulans*. *Gene* 375:54–62.
- Galtier N, Gouy M, Gautier C. 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comp. Appl. Biosci.* 12:543–548.
- Heidmann O, Vernochet C, Dupressoir A, Heidmann T. 2009. Identification of an endogenous retroviral envelope gene with fusogenic activity and placenta-specific expression in the rabbit: a new “syncytin” in a third order of mammals. *Retrovirology* 6:107.
- Kim A, et al. 1994. Retroviruses in invertebrates: the gypsy retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* 91:1285–1289.
- Leblanc P, et al. 2000. Life cycle of an endogenous retrovirus, ZAM, in *Drosophila melanogaster*. *J. Virol.* 74:10658–10669.
- Lerat E, Burt N, Biémont C, Vieira C. 2011. Comparative analysis of transposable elements in the melanogaster subgroup sequenced genomes. *Gene* 473:100–109.
- Malik HS, Henikoff S, Eickbush TH. 2000. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses. *Genome Res.* 10:1307–1318.
- Mallet F, et al. 2004. The endogenous retroviral locus ERVWE1 is a bona fide gene involved in hominoid placental physiology. *Proc. Natl. Acad. Sci. U. S. A.* 101:1731–1736.
- Marsano RM, et al. 2000. The complete tirant transposable element in *Drosophila melanogaster* shows a structural relationship with retrovirus-like retrotransposons. *Gene* 247:87–95.
- Misseri Y, Labesse G, Bucheton A, Terzian C. 2003. Comparative sequence analysis and predictions for the envelope glycoproteins of insect endogenous retroviruses. *Trends Microbiol.* 11:253–256.
- Moltó MD, Paricio N, López-Preciado MA, Semeshin VF, Martínez-Sebastián MJ. 1996. Tirant: a new retrotransposon-like element in *Drosophila melanogaster*. *J. Mol. Evol.* 42:369–375.
- Pélisson A, et al. 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.* 13:4401–4411.
- Pelisson A, Mejlumian L, Robert V, Terzian C, Bucheton A. 2002. *Drosophila* germline invasion by the endogenous retrovirus gypsy: involvement of the viral env gene. *Insect Biochem. Mol. Biol.* 32:1249–1256.
- Rohrman GF, Karplus PA. 2001. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. *BMC Evol. Biol.* 1:1.
- Rozas J, Rozas R. 1995. DnaSP, DNA sequence polymorphism: an interactive program for estimating population genetics parameters from DNA sequence data. *Comput. Appl. Biosci.* 11:621–625.
- Tanda S, Mullor JL, Corces VG. 1994. The *Drosophila* tom retrotransposon encodes an envelope protein. *Mol. Cell. Biol.* 14:5392–5401.
- Tcheressiz S, et al. 2002. Expression of the Idefix retrotransposon in early follicle cells in the germarium of *Drosophila melanogaster* is determined by its LTR sequences and a specific genomic context. *Mol. Genet. Genomics* 267:133–141.
- Terzian C, Pélisson A, Bucheton A. 2001. Evolution and phylogeny of insect endogenous retroviruses. *BMC Evol. Biol.* 1:3.
- Teyssset L, et al. 1998. A Moloney murine leukemia virus-based retroviral vector pseudotyped by the insect retroviral gypsy envelope can infect *Drosophila* cells. *J. Virol.* 72:853–856.
- Vieira C, Fablet M, Lerat E. 2009. Infra- and transspecific clues to understanding the dynamics of transposable elements, p 115–123. *In* Transposons and the dynamic genome, genome dynamics and stability. Springer-Verlag, Berlin, Germany.
- Viggiano L, Caggese C, Barsanti P, Caizzi R. 1997. Cloning and characterization of a copy of tirant transposable element in *Drosophila melanogaster*. *Gene* 197:29–35.