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The complete *Tirant* transposable element in *Drososphila melanogaster* shows a structural relationship with retrovirus-like retrotransposons

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Abstract

We have determined the structure and organization of *Tirant*, a retrotransposon of *Drosophila melanogaster* reported in literature to be responsible for four independent mutations. *Tirant* is a long terminal repeat (LTR) retrotransposon 8527 bp long. It possesses three open reading frames (ORF) encoding Gag, Pol and Env proteins with a strong similarity with *ZAM*, a recently identified member of the *gypsy* class of retrovirus-like mobile elements. Molecular analysis of the *Tirant* genomic copies present in four *D. melanogaster* strains revealed that most of them are defective, non-autonomous elements that differ in the position and extension of the conserved internal portion. Defective elements lacking the Gag ORF but retaining the Env ORF are abundant in heterochromatin. Four discrete *Tirant* transcripts are observed during embryogenesis in the strain *Oregon-R*, the smaller of which, 1.8 kb in size, originates from the splicing of a primary transcript and leads to a subgenomic RNA coding for the Env product.

Keywords: Envelope protein; Genomic analysis; LTR transposons; Virus-like elements

1. Introduction

Retrotransposons are a class of mobile elements that transpose via the reverse transcription of an RNA intermediate (Boeke et al., 1985; Garfinkel et al., 1985). They are found in a wide range of eukaryotes, and it is believed that they are ubiquitous components of eukaryotic genomes. The transposition process may have important consequences for the host altering genome structure or transcriptional activity of nearby genes (Finnegan, 1989). Based on their structural features, these mobile elements can be divided into poly-A-containing and long-terminal-repeat (LTR)-containing retrotransposons; the latter class shares most structural and functional features with the proviral form of vertebrate retrovirus. Both classes of retrotransposons usually contain two open reading frames (ORFs) indicated as Gag (encoding the capsid protein) and Pol (including protease-, reverse transcriptase-, RNase H- and integrase-encoding domains). Some LTR-containing retrotransposons contain a third ORF that is structurally located and partially homologous to the Env (envelope) protein of vertebrate retroviruses.

Many LTR retrotransposons have been identified in the genome of *D. melanogaster*, and the genetic and molecular characterization of *gypsy*, a three-ORF-containing retrotransposon, demonstrated that retrotransposon of the *gypsy* class can be considered as a true invertebrate retrovirus (Kim et al., 1994; Song et al., 1994). A noteworthy feature of *gypsy* is the control of its activity by a nuclear gene, *flamenco* (Pélisson et al., 1994), a finding that poses interesting questions about the relationships that can be formed between the mobile elements and the host genome during evolution.

Abbreviations: *adh*, alcohol dehydrogenase gene; CCD, cooled charge coupled; DAPI, 4′,6′-diamidine-2′-phenylindole dihydrochloride; *emc*, extramacrochaetae gene; *mgs*, mitochondrial glutamine synthetase gene; *Ser*, Serrate gene.

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Tirant is an LTR retrotransposon of *D. melanogaster* that has been shown to be associated with four independent mutations (Garrel and Modolell, 1990; Caggese et al., 1992; Thomas et al., 1995; Wu and Gibson, 1998), but only the *Tirant* copy inserted in the 5′ region of the *mgs* gene has been sequenced (Viggiano et al., 1997). However, in every case studied, the insertion affecting the gene activity has been found to be approximately 5.0 kb long. Sequencing of the *Tirant* copy associated with the *mgs* mutation revealed the presence of an ORF with similarity to the *envelope* gene of the *gypsy*-like retrotransposons, while *gag* and most of the *pol* region were not found. Thus, it was concluded that this insertion was a defective copy of an unidentified full-length retrotransposon originated by the reverse transcription.

In this paper, we present the structure of a complete *Tirant* element. Different strains of *Drosophila melanogaster* contain different defective *Tirant* copies, but all strains examined possess at least one complete element, and comparison of complete and defective copies suggests that specific repeated sequences are involved in the generation of some of the deleted elements. We have also analyzed the expression pattern of *Tirant* during *Drosophila* development, and we have investigated its phylogenetic relationships with other retrotransposons of the class characterized by the presence of three ORFs.

2. Materials and methods

2.1. DNA preparation and Southern blot analysis

Drosophila strains used in this study are as follows: *Oregon-R* stocked in Bari since 1980; *Charolles* from a collection of Dr Dimitri, University of Rome, Italy; W80 *Bakup* from the National *Drosophila* Species Center, Bowling Green State University, Bowling Green, OH; *FS(PM11-19)/Cy* carrying a *Tirant* element in the mitochondrial glutamine synthetase gene (*mgs*) (Caggese et al., 1992) balanced with the *Cy* chromosome.

Genomic DNA was prepared using repeated phenol chloroform extraction (Roberts, 1998) from adults of strains maintained at 25°C on standard cornmeal–sucrose–yeast medium. Restriction-digested DNA was blotted onto Hybond-N filters by capillary action in 20× SSC. Hybridization and washing were as described in Maniatis et al. (1989). Fragments used as probes were gel-purified and labeled with [a-^{32}P]dATP by random priming (Boehringer). The probes used were as follows: Tir-env: 0.8 kb *Ava*II–*Cla*I fragment subcloned from Tir-21B (p21/FS in Viggiano et al. 1997); Tir-gag: 1.0 kb PCR fragment amplified using primers 5′-ACACGTTCAGTTGTTGGAG and 5′-CAAACT-ATGTGAAGGTGCT designed on the basis of Tir-36D3 sequence.

2.2. Genomic libraries, databanks search and analysis

The lambda genomic library of *Oregon-R* strain in λ GEM12 (Promega) (kindly provided by L. Viggiano) was screened (Maniatis et al., 1989) with a ^{32}P-labeled Tir-env probe. Eight random clones among 40 positive plaques were purified. Restriction fragments hybridizing to the *env* probe were then subcloned into pUC19 vector plasmid. The inserts were sequenced using the Sequenase V.2 sequencing kit (Amhersham) with the forward and reverse primers of pUC19 vector or with synthetic oligonucleotides from the *env* region. Sequences database searches were performed using the BLAST tool (Altschul et al., 1990) and the EDGP (European *Drosophila* Genome Project, http://edgp.ebi.ac.uk) or BDGP (Berkeley *Drosophila* Genome Project, http://www.fruitfly.org) blast servers. Multiple protein sequence alignments were performed with Clustal W (Thompson et al., 1994). The protein distance matrix was analyzed by the Maximum Likelihood method (PROTDIST) of the PHYLIP package (Felsenstein, 1993). Phylogenetic trees were generated using the Neighbor-Joining and UPGMA methods. Protein-distance analyses were based on alignments of a 223–236 amino acid portion of the conserved RT and RNaseH domains in the Pol protein. The Accession Nos of the analyzed retrotransposon are: X01472 (*17.6*), B24872 (*297*), X04132 (*412*), U89994 (*Burdock*), M12927 (*gypsy*), AF039416 (*nomad*), M32662 (*TED*), Z24451 (*Tom*), U60529 (*yoyo*), AJ000387 (*ZAM*).

2.3. RNA extraction and Northern blot analysis

Total RNAs from aged dechorionated embryos were extracted using a guanidine-thiocyanate and phenol extraction method (Roberts, 1998). Poly(A)$^+$ RNA was isolated from oligo(dT)-cellulose and separated on formaldehyde denaturing 1% agarose gels (Maniatis et al., 1989). Hybridization conditions for Northern blots were the same used for Southern blots.

2.4. PCR amplification

PCR amplification of genomic DNA was performed with the Expand™ High Fidelity PCR System (Roche) using the conditions and procedures described in the manufacturer's protocols. RT-PCR was performed using 1 μg of 9–12 h embryo poly(A)$^+$ RNA and the Titan One-Tube RT-PCR kit (Roche). For both PCR and RT-PCR, the primers were 5Us (5′-TCCCGGGTA-CCGGTAGGATGTCCTAC-3′) corresponding to nucleotides 425–441 of *Tirant* in 36D3 region plus a *Sma*I–*Kpn*I linker in the 5′ end and E3r (5′-TCC-CGGGTACCATGTTGCCGGTAACCAC-3′) corresponding to nucleotides 6854–6872 of *Tirant* in 36D3 region plus a *Sma*I–*Kpn*I linker in the 5′ end. The

RT-PCR products were cloned into the *Kpn*I site of the pUC19 vector and sequenced as described above.

2.5. In-situ hybridization

Salivary glands chromosomes from third-instar *Oregon-R* larvae were prepared essentially as described in Pardue (1986). The plasmids pTir-Gag and pTir-Env were labeled by nick translation with the fluorescent Cy3-dCTP precursor (Amhersham). Chromosomes were stained with DAPI. Digital images were obtained using a Leica DMRXA epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Gray-scale images, obtained separately recording Cy3 and DAPI fluorescence by specific filters, were computer-colored and merged for the final image using the Adobe Photoshop software.

3. Results

3.1. Search for a complete Tirant element

To isolate a complete *Tirant* element, we first screened a genomic lambda library from the *D. melanogaster Oregon-R* strain using as a probe a O.8 kb *Ava*II–*Cla*I fragment encompassing the *env* region (Tir-env in Fig. 1) in Tir-21B, a previously cloned defective *Tirant* element (Viggiano et al., 1997). About 40 positive plaques were found out of 50 000 phages screened, showing, in accord with the Southern blot analysis of Moltò et al. (1996), that *Tirant* is present in the *D. melanogaster* genome as a moderately repeated sequence.

Eight independent clones were characterized by restriction mapping and partial sequencing. During this preliminary characterization, it was realized that four of the isolated clones contained sequences only 80% similar to Tir-21B. These *Tirant*-related elements also contain deletions and frameshift mutations, and, as they probably represent relics of ancient elements, they were not analyzed any further. Among the four phages whose inserts contained sequences more than 98% identical to Tir-21B, one possessed the same structure of Tir-21B, and among the remaining three, only one, λ Ti-A3, was informative since it contained a 1.0 kb DNA sequence not present in Tir-21B. Unfortunately, the λ Ti-A3 clone contains only part of a presumably complete *Tirant* element fragmented during the cloning procedure because its sequence contains a cloning site.

However, a BLAST search of the *Drosophila* Databanks at EDGP and BDGP using the new DNA sequence found in Tir-A3 plus the adjacent *env* region allowed the identification of three *Tirant* elements in completely sequenced cosmid and P1 clones. Table 1 lists these elements. They are all flanked by a GC-rich four-base direct repeat as previously reported regarding the *mgs Tirant* insertion (Viggiano et al., 1997), but differ in size and derive from different genomic locations. The analysis of the longest sequence, a 8527 bp sequence from the 36D3 polytenic region (Tir-36D3), revealed three ORFs, while the shorter elements, Tir-38E and Tir-2B, possess, like Tir-21B, only part of the three ORFs assumed to characterize a complete *Tirant* element. Fig. 1 shows the structure of the presumably complete element Tir-36D3 and the extent of the deletions in the three defective elements analyzed so far.

Careful inspection of the nucleotide sequences of complete and defective elements allowed us to identify the presence of short direct repeats in Tir-36D3 flanking the regions deleted in Tir-38E and Tir-21B. Thus, a

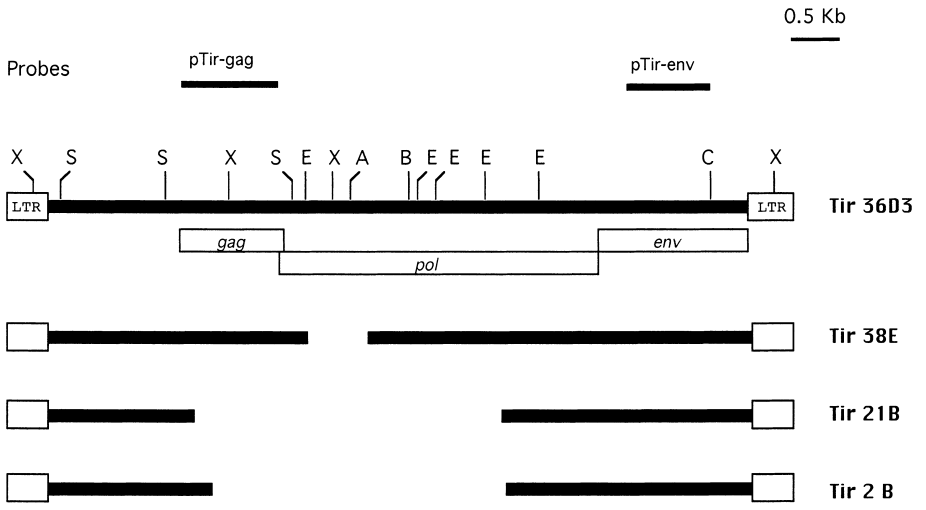

Fig. 1. Comparison of complete and deleted copies of *Tirant*. The position and extension of the deletions present in the defective Tir-38E, Tir-21B and Tir-2B elements are shown beneath the complete Tir-36D3 element. The three ORFs encoding the Gag, Pol and Env proteins are also shown. Letters in Tir-36D3 represent the restriction sites *Xho*I (X), *Sal*I (S), *Eco*RI (E), *Xba*I (A), *Bgl*II (B), *Sac*I (C). Above the map is shown the position of the gag and env fragments used as probes in hybridization experiments.

Table 1
Tirant elements present in the database

Insertion site	Strain	Length (bp)	Target site	Accession No.
2B	Oregon-R	5.481	CACG	AL031027[a]
21B	FsPM11	5.184	CGCG	X93507
36D3	y cn bw	8.527	CGCG	AC005444[b]
38E-6-9	y cn bw	7.817	CCCG	AC004759[c]

[a] Tirant is at position 35 530–41 101.
[b] Tirant is at position 50 230–59 729.
[c] Tirant is at position 71 472–79 288.

possible origin of the defective *Tirant* elements could be the recombination in these repeated sequences, as shown in Fig. 2. No repeats were found in the region flanking the deletion in Tir-2B.

3.2. *Predicted* Tirant *gene products and* Tirant *relationship with other retrovirus-like retrotransposons*

The finding of ORF1 and ORF2 in Tir-36D3 enabled us to characterize in more detail the *Tirant* element whose analysis was previously limited to the ORF3 (Viggiano et al., 1997).

Tirant ORF1 is a 377-amino-acid protein showing a basic segment in the N-terminal region. This predicted protein has 55% identity and 71% similarity with the Gag protein of the *ZAM* retrotransposon of *D. melanogaster* and also shares less similarity with the Gag protein of the lepidopteran *Trichoplusi ni TED* element, and with *yoyo* of *Ceratitis capitata*.

Tirant ORF2 spans 3549 nucleotides. In Tir-36D3, there is an extra T in a run of 5 T at position 3068 and a stop codon at position 5444 not present in the corresponding region of Tir-38E. Without the extra T and the stop codon, ORF2 would encode an 1183-amino-acid protein. Analysis of the putative Tir-36D3 ORF2 sequence using the BLAST-X program (Altschul et al., 1990) identified similarities with four domains of the *pol* gene in the following order: protease, reverse transcriptase, RNase H and integrase. Highest scores are obtained by comparison with the *ZAM* element, but extended regions of similarity with other retrovirus-like transposons are easily detected. Thus, *Tirant* ORF2 shares significant similarity with other Pol polyproteins (Xiong and Eickbush, 1990). Moreover, the conserved regions include the YxDD box of the catalytic center in the reverse transcriptase domain (Yuki et al., 1986) and the DD35E motif of the active site in the integrase domain (Polard and Chandler, 1995).

The phylogenetic relationship between *Tirant* and other retrotransposons was investigated by aligning the amino acid sequence of the conserved RT-RNaseH domains of nine retrotransposons containing three ORFs and using the two ORFs containing transposon *412* as the outgroup. The region analyzed encompasses 223–234 amino acids and corresponds to two of the seven domains of the Pol polyprotein identified by Xiong and Eickbush (1990).

Trees generated by the Neighbor-Joining (N-J) and UPGMA methods are shown in Fig. 3. The two trees differ only in the position of *nomad*, which, by the UPGMA method, is grouped with *yoyo*, while by the N-J method, it appears to be in a separate branch. *Tirant* is grouped with *ZAM* and *Ted*, and shares a more distant relationship with *Tom*, *297* and *17.6*. These elements appear to define a class distinct from the class including *gypsy*, *Burdock*, *yoyo* and *nomad*. This phylo-

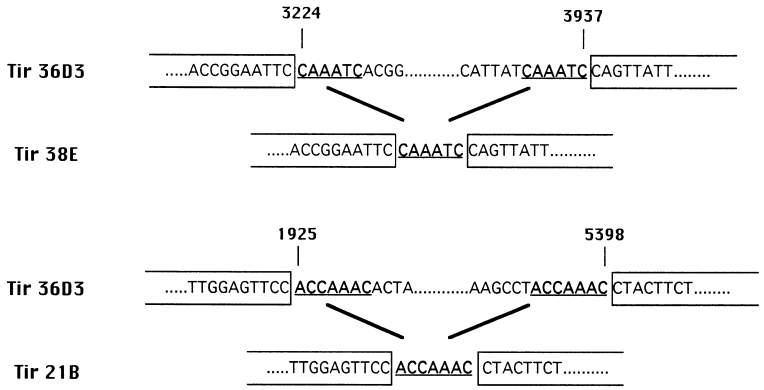

Fig. 2. Origin of deletions in Tir-38E and Tir-21B. Recombination between the repeated sequences CAAATC and ACCAAAC found in the complete Tir-36D3 element could give rise to the deleted copies.

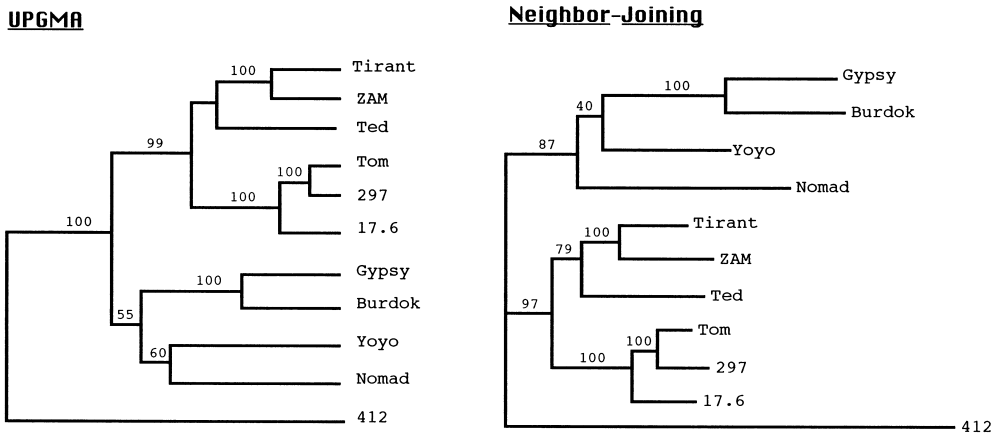

Fig. 3. Cladograms based on the alignment of the RT/RnaseH domains of the indicated retrotransposons. The length of the branches in the Neighbor-Joining tree indicates the divergence between the predicted amino acids sequences of the analyzed Rt/RnaseH regions. Bootstrap values on 100 simulations are indicated at each node.

genetic tree is supported by the finding that the primer binding site of the elements of the first class shows a strong similarity to the t-RNASer, while the homologous site of the elements of the second class shows similarity to t-RNALys. Similar phylogenetic relationships have been reported by other authors (Tanda et al., 1988; Walen and Grigliatti, 1998).

3.3. *Genome analysis of* Tirant *elements*

The analysis of λ clones and the BLAST search revealed that the genome of *D. melanogaster* contains several defective copies of *Tirant* differing in the extension and position of their deletions.

On the basis of the restriction map of Fig. 1, the DNA of the *D. melanogaster* strains, *Oregon-R*, *Charolles*, W80 *Bakup*, and *(FS)PM11-19/Cy* was digested with *Hin*dIII, an enzyme not expected to recognize any restriction site in a complete *Tirant* element. Two identical filters were then hybridized with Tir-gag and Tir-env probes (see Fig. 1) and the pattern of hybridization analyzed. As shown in Fig. 4, the four strains provide patterns different from each other with both probes, suggesting different locations of the *Tirant* elements in their genome, as typical of transposable elements. Many hybridizing bands are smaller than 8.5 kb, the size of Tir-36D, and hence they derive either from copies with internal deletions or from copies that acquired an internal *Hin*dIII site. In addition, comparison of the patterns given by the digested DNAs with each of the two probe shows that while both probes hybridize to some bands, other bands either appear specific to one probe or show a clearly different relative intensity of hybridization to the probes. These results confirm that many of the *Tirant* elements present in the genome of the analyzed strains differ in their structural organization.

Long-template PCR was also performed to detect a

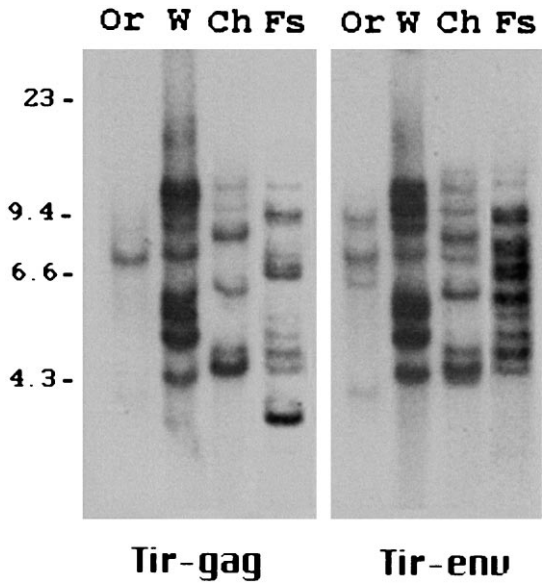

Fig. 4. Genomic analysis of *Tirant* copies. DNAs from the *D. melanogaster* strains *Oregon-R* (Or), W80 *Bakup* (W), *Charolles* (Ch) and *FS(PM11-19)/Cy* (Fs) were digested with *Hin*dIII. Identical amounts of each digested DNA were loaded in two different slots of the same agarose gel. The filter blot was then cut in halves and hybridized with the gag and env probes, respectively.

presumably complete *Tirant* element in the genome of the four *D. melanogaster* strains analyzed. Primer 5Us and E3r were designed on the basis of the Tir-36D sequence. In the presence of a complete or almost complete *Tirant* element, these two primers are expected to amplify an internal 6.4 kb fragment. The PCR results are shown in Fig. 5. It is quite evident that every strain analyzed possesses a variety of amplified fragments representing different defective copies since amplified bands still hybridize with the gag probe (Fig. 5A). However, the 6.4 kb PCR band expected from a complete element is the only one common to all strains

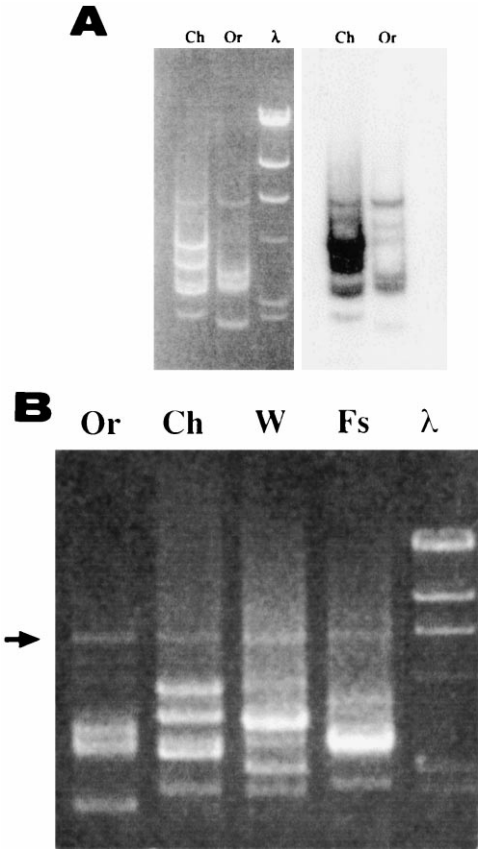

Fig. 5. PCR amplification of *Tirant* elements. (**A**) Pilot experiment showing that most of the ethidium bromide PCR bands (left panel) from *Charolles* and *Oregon-R* strains hybridize with the internal gag fragment used as probe (right panel). (**B**) More detailed pattern of the DNAs from the strains described in Fig. 4 when amplified using the primers and the conditions described in Section 2. Note the different pattern of amplified bands in the four samples analyzed and the presence of a unique 6.4 kb common band (arrow). Lambda marker bands (λ) are *Hin*dIII fragments of 23, 9.6, 6.6, 2.2 and 2.0 kb (from top to bottom).

analyzed, suggesting that at least one complete element must be present in their genome (Fig. 5B).

In-situ hybridization over polytene chromosomes of the *Oregon-R* strain allowed detection of a different distribution of structurally different *Tirant* elements within the genome. As shown in Fig. 6, only the Tir-env probe labels the chromocenter, and hence, this heterochromatic region appears enriched in *Tirant* elements with deletions extending into the *gag* ORF.

3.4. *Analysis of* Tirant *transcripts*

The pattern of *Tirant* transcription during development was tested by Northern blotting of poly(A)$^+$ RNAs extracted from different developmental stages of *D. melanogaster*. Strong signals can be detected with the env probe during embryogenesis, while in the larval, pupal and adult stages, the transcripts are barely detectable. Expression of *Tirant* gene products during embryo development has also been reported by Thomas et al. (1995).

Fig. 7A shows the pattern obtained analyzing samples defining 3 h intervals of embryogenesis. Maximal expression is observed in 9–12 h old embryos. Four transcripts are detected, the larger being more than 7.0 kb long and the smaller about 1.8 kb. These two transcripts presumably represent the full length and a spliced form of the primary transcript. Two other more abundant transcripts in the range of 4–6 kb, are also clearly visible. Interestingly, the transcription pattern of *Tirant* in *Oregon-R* appears to differ markedly from the transcription pattern of the elements *gypsy*, *ZAM* and *nomad* of *D. melanogaster* and *Tom* of *D. ananassae*, as these four well-characterized retrovirus-like retrotransposons usually produce only two transcripts (Kim et al., 1994; Tanda et al., 1994; Leblanc et al., 1997; Walen and Grigliatti, 1998).

In order to further characterize the *Tirant* transcripts, we isolated cDNA fragments amplified by RT-PCR using the 5Us and E3r primers and poly(A)$^+$ RNA from 9–12 h embryos. This PCR amplification produced two bands of 0.4 and 1.3 kb (data not shown). The cloning and sequencing of the 0.4 kb fragment allowed us to identify the splice junction shown in Fig. 7B. The splicing uses typical GT and AG splicing sites and puts an upstream ATG translation start in frame with the *env*-encoding region (Fig. 7C). A similar type of processing has been demonstrated for the *gypsy*, *ZAM*, *nomad* and *Tom* retrotransposons and for a number of retroviruses.

4. Discussion

4.1. *Structure, organization and expression of* Tirant *elements*

Combining the results of sequencing λ clones containing partial *Tirant* sequences with a BLAST search of the public databases, we have been able to determine the length and structure of a complete *Tirant* element. The *Tirant* copy inserted at 36D3 in the *D. melanogaster*, *y cn bw* strain, exhibits all the characteristic features of a retrotransposon and strong similarities to other retrovirus-like elements. Its most interesting structural feature is the presence of three ORFs in the typical location and corresponding to the *gag*, *pol* and *env* genes. However, the presence of a stop codon and of frameshift mutations in the *pol* gene indicates that Tir-36D3 is not autonomous. The other *Tirant* copies identified and sequenced, Tir-2B, Tir-21B and Tir-38E, also cannot be autonomous due the presence of large deletions affecting different part of the retrotransposon genes. On the basis of the estimated length of the as-yet unsequenced *Tirant*

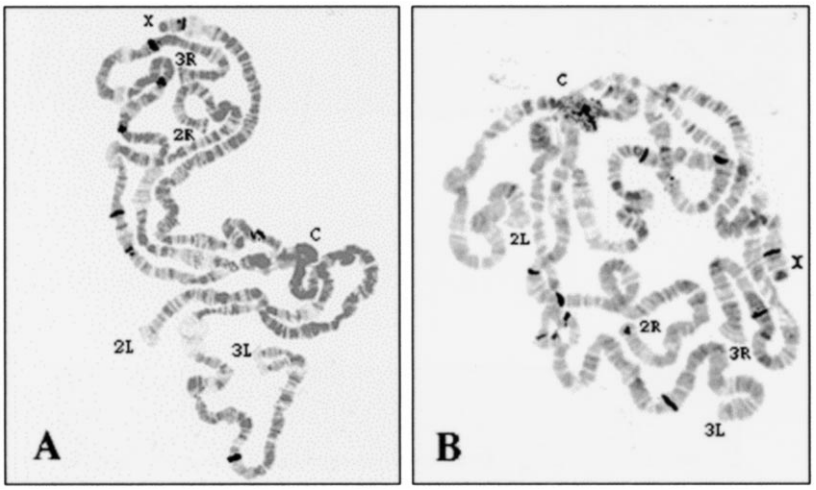

Fig. 6. In-situ hybridization. Polytene chromosomes from *Oregon-R* salivary glands were hybridized with gag (**A**) and env (**B**) probes. The gag probe detected nine signals, the env probe 11. Note also that only the env probe labels the chromocenter.

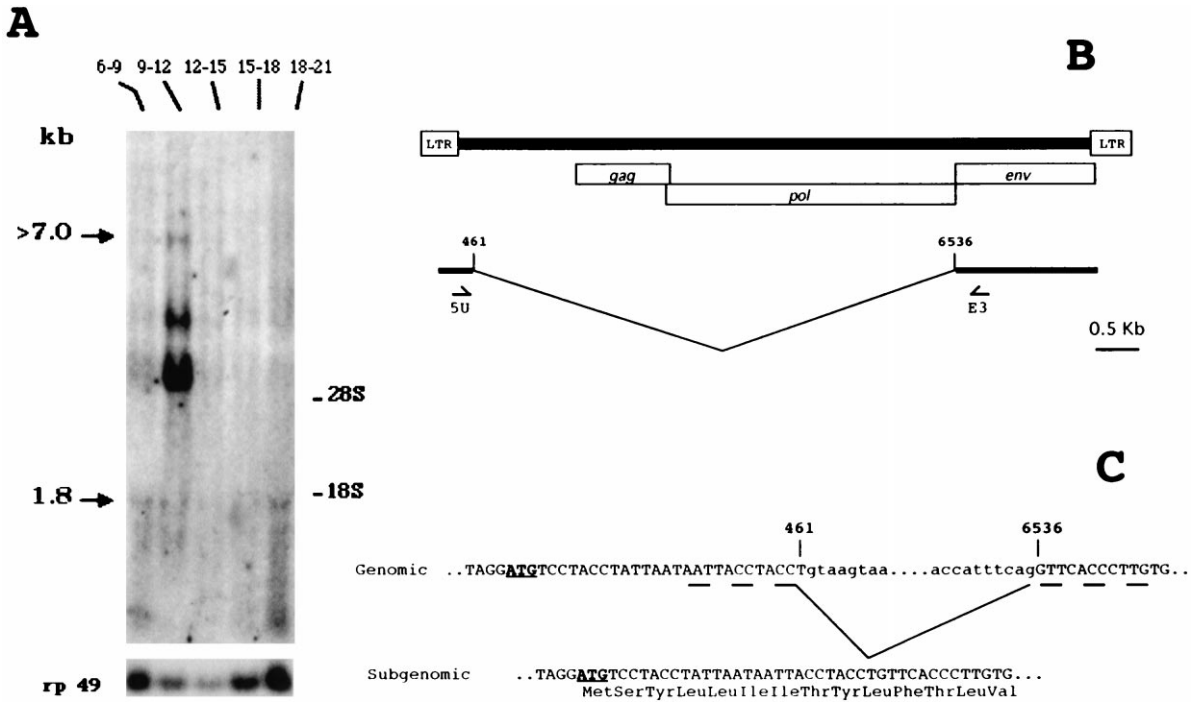

Fig. 7. Expression and structure of *Tirant* transcript. (**A**) Northern blot of poly(A)$^+$ RNAs from different embryos developmental stages probed with the env fragment of *Tirant*. The position of eukaryotic ribosomal RNA is shown on the right. Below is the hybridization signal of the ribosomal protein rp49 (O'Connell and Rosbash, 1984) probe used on the same filter to check for the amount of RNA loaded. (**B**) Structure of a complete *Tirant* element, showing the splicing event giving rise to the env transcript. (**C**) Nucleotide sequence of genomic and spliced subgenomic product as revealed by RT-PCR experiments. The in-frame ATG translation start is shown in bold and underlined.

elements inserted in the *emc*, *adh* and *Ser* genes (Garrel and Modolell, 1990; Wu and Gibson, 1998; Thomas et al., 1995), it is clear that these copies of the element are defective as well. Taken together, these data indicate that defective *Tirant* elements are abundant in the genome of *D. melanogaster*. This suggestion is supported by the long-template PCR results (Fig. 5) showing that the DNAs of all the strains analyzed produce, besides the band expected from the complete element, many amplified bands derived from presumably defective elements.

Defective retrotransposons with internal deletions have been reported (see, for example, Bayev et al., 1984; Inouye et al., 1986), and they appear preferentially located in the pericentromeric heterochromatin (Vaury et al., 1989). In accord with these results, we show that

Tirant elements deleted in the *gag* region are abundant in the chromocenter; however, similar deleted elements (for example, Tir-2B and Tir-21B) are also found in the euchromatin. Since Tir-2B and Tir-21B were detected in two different strains, each containing only one of the two elements, they must have been generated by transposition events mediated in trans by products of an autonomous element.

Transcription analysis has revealed developmental regulated expression of *Tirant*. In addition to the primary and *env* spliced form of transcripts, two abundant RNAs in the range of 4–6 kb are present in the 9–12 h embryos. The origin of these unusual RNAs could be either the transcription of the many defective copies present in the genome or the transcription of copies driven by an endogenous promoter. These two possibilities are not mutually exclusive. The Northern blots analysis of independent strains and the cloning and sequencing of these RNAs will elucidate their exact formation.

4.2. Tirant *is strongly related to* ZAM *elements*

Phylogenetic analysis suggests that *Tirant* and *ZAM* share a common ancestor (Fig. 3). Their Gag, Pol and Env proteins show, respectively, 47.6, 61.8 and 48.2% global identity. Like *Tirant*, *ZAM* transposes into a GC-rich target site (GCGCGC) (Leblanc et al., 1999), and both elements have the same tRNASer primer binding site. Another structural similarity between the two retrotransposons is the presence of a repeated region downstream from the 5′LTR. *Tirant* possesses six repeats of 106 nucleotides, while *ZAM* has two repeats approximately 300 bp long in the same position. At present, the function of these repeats is unknown, but in *gypsy*, a similar array of two 109 bp repeats, each containing four copies of a repeated sequence homologous to the octamer motif found in transcriptional enhancers and promoters of eukaryotic genes (Rosales et al., 1987), followed by another imperfect repeat of about 100 bp containing an additional four copies of the octamer-like motif, has been shown to induce a mutant phenotype due to an effect on a distally located enhancer (Geyer et al., 1988; Peifer and Bender, 1988). The putative functional significance of similar repeated regions in other LTR retrotransposons has been discussed in McDonald et al. (1997). However, the LTRs of *ZAM* and *Tirant*, except for the 5′-AGTTA…TAACT-3′ terminal sequence, show only 50% similarity. It appears, therefore, that the two transposons have shown several different specific properties while maintaining their original transposition machinery somewhat unchanged during their long evolutionary history (Moltò et al., 1996; Baldrich et al., 1997).

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