Available online at www.sciencedirect.com
SCIENCE DIRECT
Gene 357 (2005) 122 – 128
GENE
SECTION
EVOLUTIONARY GENOMICS
www.elsevier.com/locate/gene

Evidence for a functional interaction between the *Bari1* transposable element and the cytochrome *P*450 *cyp12a4* gene in *Drosophila melanogaster*

Renè Massimiliano Marsano [a], Ruggiero Caizzi [b], Roberta Moschetti [b], Nikolaj Junakovic [c,*]

[a] *Dipartimento di Genetica, Università di Parma, 43100 Parma, Italy*
[b] *Dipartimento di Genetica e Microbiologia, Università di Bari, 70126 Bari, Italy*
[c] *Istituto di Biologia e Patologia Molecolari C.N.R, c/o Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza", 00185 Roma, Italy*

Received 18 March 2005; received in revised form 22 June 2005; accepted 23 June 2005
Available online 1 August 2005
Received by W. Makalowski

Abstract

Previous studies of the genomic distribution of the transposon *Bari1* in *Drosophila melanogaster* have revealed an element which is fixed at division 91F in over 90 lab and natural populations. Here we report about the structural and transcriptional features of the insertion site which was studied in sublines isolated from an exceptional Drosophila line polymorphic for the presence/absence of *Bari1* at 91F. The insert is located at the 3′ end of the *cyp12a4* gene that belongs to the cytochrome *P*450 family. In flies with the insert the transcript of this gene encompasses 18 nucleotides of the transposon, it is shorter and is about tenfold more abundant compared to flies devoid of it. Although the hypothetical selective agent remains unknown, these data are suggestive of a selective advantage brought about by the *Bari1* insert and are reminiscent of recent evidence for functional mutagenesis of *cyp6g1*, another *P*450 gene, brought about by *Accord* and *Doc* transposable elements in *D. melanogaster* and *Drosophila simulans*.

Keywords: *Bari1* transposon; Fixed insertions; *cyp12a4* gene; 3′ end mRNA1

1. Introduction

The contribution of transposable elements (TEs) to the evolution of their hosts has been debated for over two decades. A testable question in this matter is whether occasionally elements undergo fixation, possibly due to mutations beneficial to the host. The initial inability to find such elements has contributed to the selfish DNA hypothesis which posits that TEs are essentially parasitic (Charlesworth et al., 1994). Other authors, however, viewed hypothetical functional TE–host interactions as a dynamic process where detection of fixed elements depends on the timing of the interaction and the intensity of selective pressure. Ancient insertions may lose their identity due to point mutations and deletions thus turning undetectable with probes homologous to active transposons (Britten, 1997; Brosius, 1999; Fedoroff, 1999; Makalowski, 2000; Kidwell and Lisch, 2001; Petrov et al., 2003). For example, the analysis of genomic sequences has shown that a substantial fraction of human genes contains remnants of TE sequences in both regulatory and coding regions (Nekrutenko and Li, 2001; Jordan et al., 2003; van de Lagemaat et al., 2003). Recent beneficial insertions may fail to meet the fixation criterion as they are polymorphic for the presence/absence and may take a long time to reach fixation, depending on the selective advantage they confer. In the absence of these criteria, the only beneficial inserts detectable by in situ hybridization (the most popular approach in Drosophila) are

Abbreviations: DDT, 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane); EDTA, ethylenediamine tetraacetic acid; kb, kilobases; RACE, rapid amplification cDNA ends; SDS, sodium dodecyl sulphate.

* Corresponding author. Tel.: +39 6 4991 2786; fax: +39 6 4991 2500.
E-mail address: nikolaj.junakovic@uniroma1.it (N. Junakovic).

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.06.005

the ones that are recent enough to be structurally intact and old enough to reach fixation in the particular population studied. Although this may be a rather narrow window of opportunity, recent reports suggest that beneficial TE insertions may not be all that rare as previously estimated. The *S* element associated with the *Hsp70* genes (Maside et al., 2002), the *Accord* element upstream of the *cyp6g1* gene (Daborn et al., 2002), the *Doc* element inserted in the phosphotransferase gene (Petrov et al., 2003) and the *Doc* element upstream of the *cyp6g1* gene in *Drosophila simulans* (Schlenke and Begun, 2004) are all structurally conserved and fixed or high frequency in unrelated populations.

Here we report about an element of *Bari1*, a *Tc1*-like transposon family, which has been previously found fixed at 91 F in about 60 *Drosophila melanogaster* lab stocks and 30 natural populations (Caggese et al., 1995; Junakovic, unpublished). Sublines with and without the insert have been isolated from an exceptional stock polymorphic for this trait. The insertion has been mapped to the 3′ end of *cyp12a4* gene, a member of the cytochrome *P*450 family involved in the metabolism of a myriad small molecules including xenobiotics responsible of insecticide resistance (Stoilov, 2001). The only structural difference between the two allelic forms of the gene is an intact, transposition competent, *Bari1* element, which is present in the "filled" and absent in the "empty" flies. If present, the element contributes with its first 18 nucleotides to the *cyp12a4* transcript; in addition, the transcript is shorter and it is about tenfold more abundant than in the absence of the element.

This is suggestive of a functional interaction between a transposon and a host gene, similarly to insertions of *Accord* and *Doc* elements located upstream of *cyp6g1* in *D. melanogaster* and *D. simulans* (Daborn et al., 2002; Schlenke and Begun, 2004). In addition, this is the first empyrical evidence for transcriptional regulation brought about by a transposable element inserted at the 3′ end of the affected gene.

2. Materials and methods

2.1. Drosophila stocks

The stock studied in this report was established from a single female inseminated in the wild, collected in central Italy in 1997. The initial purpose was to follow the instability of *Bari1* elements in search of novel insertion events. It was at generation 25 that we noticed for the first time that the 2.5 kb *Hin*dIII band diagnostic of the fixed element (see Section 3.1) was polymorphic among the individuals in one of the four lines studied. Thus, we are unable to tell whether one of founder parents was heterozygous from the start for the presence/absence of *Bari1* at 91 F or whether excision occurred in the lab. *y, cn bw sp* is from Bloomington Drosophila Stock Center, Indiana University. *Canton-S* and *Oregon-R* are from the Department of Genetics and Molecular Biology, Rome University.

2.2. Southern blotting

DNA from individual flies was extracted and digested with *Hin*dIII, as previously described (Di Franco et al., 1995; Junakovic, 2004). Digests were loaded on vertical ($38 \times 18 \times 0.3$ cm), 0.8% agarose gels and run for 16 h at 80 V. Transfer from gel to membrane (Hybond N+, Amersham) was carried out in the vacuum blotting unit (VacuuGene XL, Pharmacia) in 0.4 N NaOH. DNA was labelled by nick translation. Hybridization was carried out in the Church hybridization medium (0.5 M phosphate buffer, 7% SDS, 10 mM EDTA, pH 7.2) at 65 °C. Washing was carried out in 2× SSC, 0.1% SDS with several changes, initially at room temperature and then at 65 °C. Autoradiographs were exposed for 24–48 h at −70 °C (for additional details see Junakovic, 2004). The probes used in this study derive from the subcloning of previously characterized fragments harbouring *Bari1* transposon (Caizzi et al., 1993). *Bari1* probe is the *Hin*dIII–*Sma*I internal fragment of pB1-1; *cyp12a4* probe is a *Hin*dIII–*Eco*R1 fragment of pB/91F (see also Fig. 2A).

2.3. Sequencing of the cyp12a4 gene and flanking region

The "empty" and "filled" sites (from sublines 5 and 8, respectively) were characterized by sequencing the PCR products amplified with the following primers: 91F_cF (5′ CCAGCGTGTCTACCCTCTTGT position 39743–39763 in AE003725) and 91F_1R (5′ GGCAGTTTTGTTTTCGTTCGT position 46010–45990 in AE003725) (see also Fig. 2). Sequences have been deposited with the EMBL/GenBank Data Libraries under accession nos. AJ748833 and AJ748834.

The tools used to assess the integrity of the *cyp12a4* promoter are: McPromoter (Ohler et al., 2002, available at http://genes.mit.edu/McPromoter.html) and the Neural Network Promoter Prediction (Reese, 2001, available at http://www.fruitfly.org/seq_tools/promoter.html) both designed to determine the exact location of *D. melanogaster* RNA polymerase II transcription start sites.

2.4. Northern blot and 3′-RACE analysis

PolyA+ mRNA was prepared from 5 days old adults with the Quickprep mRNA Purification kit (Amersham). The RNA was quantified by OD absorbance, sized electrophoretically in a 1.2% agarose-formaldehyde gel, blotted to Nylon membrane and hybridized at 65° with a mixture of ^{32}P-labelled *cyp12a4* and ribosomal protein rp49 probes. Autoradiographs were exposed for 2 and 36 h. The shorter exposure was aimed at the prominent signal of rp49 which was used as internal control for quantifying the mRNA per lane. The 3′ end of the mRNAs was analysed with the 5′/3′

RACE kit from Roche. Two nested primers of the *cyp12a4* sequence were used: 91F_fF (5′-TTTGAGGCGATGAGA-CAGGAC-3′) and cypa4_1U (5′-CCCAATCGTCCAGG-CAACTAT-3′) at positions 41247–41268 and 41373–41393, respectively, in the scaffold AE003725. The RACE products were cloned into the pGEM-T vector (Promega) and three clones per RACE reaction of the two strains were sequenced (accession number AJ890254).

2.5. DDT resistance assay

Flies were tested by the contact assay as previously described (Daborn et al., 2001). Briefly, glass scintillation vials were pretreated by rolling them with 200 μl of acetone solution containing 5 or 2 μg of DDT (Aldrich) until the liquid evaporated. 20 females, 2–5 days old, were placed in the vials plugged with cotton soaked in 5% sucrose. Percent of mortality was measured after 16 h of treatment. Seven replicates were done for each strain and the average mortality was calculated.

3. Results

3.1. Origin and preliminary characterization of the sublines with and without Bari1 at 91F

The genomic distribution of *Bari1* elements has been previously studied in 21 Drosophila lab stocks, four populations and 80 individuals freshly collected in distant geographical areas (Soriano et al., 2002 and our unpublished results). This work was carried out by the Southern blotting technique, the genomic *Hin*dIII digests being tested with the *Bari1* probe outlined in Fig. 2A. All samples exhibited a band of 2.5 kb which was interpreted as due to the element fixed at 91 F previously described by Caggese et al. (1995). This interpretation was confirmed by the finding that in the sequenced Drosophila genome, the virtual *Hin*dIII fragment of the *Bari1* element located at 91 F is 2.5 kb long. We were then surprised to see that this band was polymorphic for the presence/absence among individuals of an isofemale line started from the wild (see Materials and methods). To establish homozygous lines of the two types, 10 crosses single male × virgin female were set up and, after the females had laid eggs, the parental patterns were analyzed. As shown in Fig. 1A, this allowed to distinguish between the parents homozygous for the absence of the 2.5 kb band (pairs 1, 5 and 7) and parents where the 2.5 kb band is present in at least one parent (pairs 2, 3, 4, 6, 8, 9 and 10). An overview of the insertion site was gained from the analysis of the sequenced *D. melanogaster* genome which does bear a *Bari1* insert at 91 F, as expected (AE003725). Inspection of the region flanking the insert revealed that the *Hin*dIII fragment bearing homology to *Bari1* should have homology to the gene *cyp12a4* as well (see Fig. 2A). To check this, the filter in Fig. 1A was stripped and

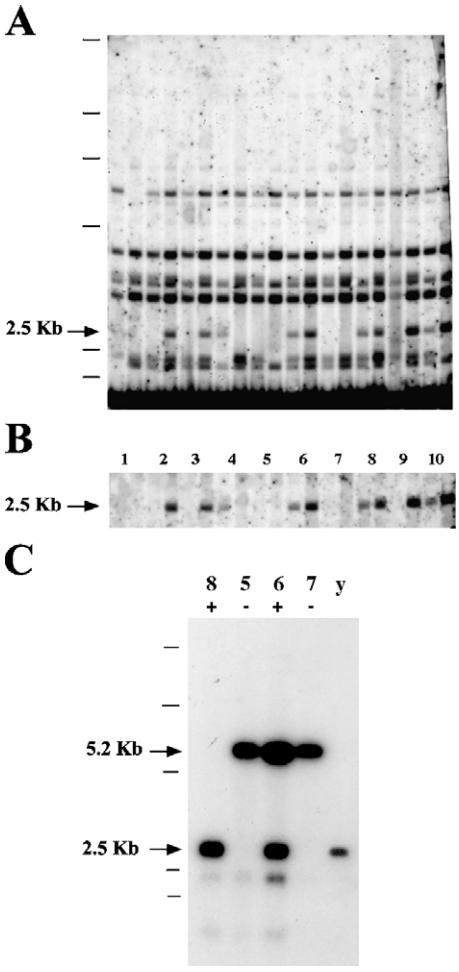

Fig. 1. Isolation and characterization of the sublines with and without *Bari1* at 91F. (A) Southern picture of 10 pairs of single fly parents of the stock in which the 2.5 kb band, most likely corresponding to the *Bari1* element inserted at 91F (see Section 3.1), is polymorphic. Genomic DNA was digested with *Hin*dIII, the probe is *Bari1* as shown in Fig. 2A. In the parents 1, 5 and 7 the 2.5 kb band is absent, whereas it shows up in at least one parent of the other pairs. The marker is λ DNA Š digested with *Hin*dIII which yields fragments of 2.0, 2.2, 4.6, 6.5, 9.4 and 23.1 kb. (B) The filter shown in previous figure has been stripped and rehybridized with the probe a4 in Fig. 2A, homologous to the portion of the *cyp12a4* internal to the fragment bearing the *Bari1* insert. (C) DNA from pools of flies of the "filled" (+) and "empty" (−) sublines established from the pairs 8, 6, 5 and 7 was tested with the same *cyp12a4* probe as above. As expected from homozygous "empty" parents, the sublines 5 and 7 lack the 2.5 kb band and are homozygous for the 5.2 kb band; of the two "filled" sublines, 8 is homozygous for the 2.5 kb band and 6 is heterozygous for both. The bands below 2.5 kb are due to a partial homology with the adjacent *cyp12a5* gene. The reference stock *y, cn bw sp* (*y*) was also analyzed as an additional example of "filled" site.

rehybridized with the *cyp12a4* probe. As shown in Fig. 1B, a 2.5 kb band that reproduces the polymorphism in presence/absence (and intensity) of the *Bari1* counterpart is revealed. To isolate the two allelic forms, sublines from the "empty" parents 5, 7 and the "filled" ones 6, 8 were established and analysed by the same approach. As shown in Fig. 1C, DNA samples from pools of flies probed with *cyp12a4* reveal that sublines 5 and 7 are homozygous for a 5.2 kb band, as expected from the *Hin*dIII map in

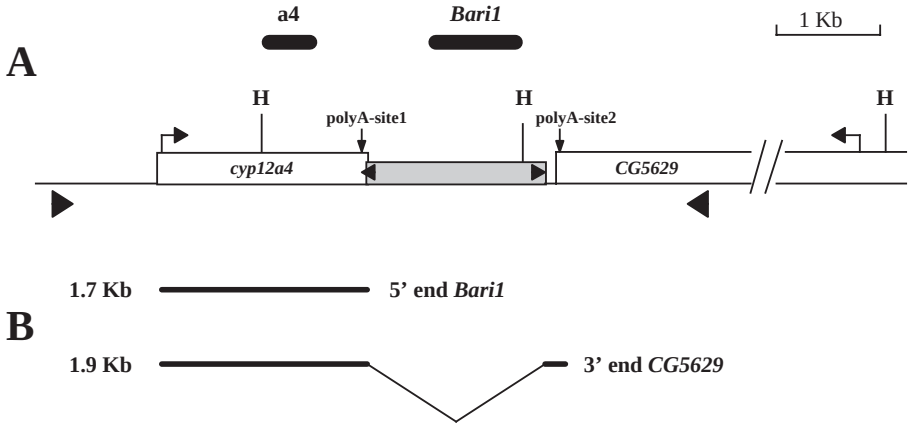

Fig. 2. (A) Outline of the 91F region in the "filled" strain. The *cyp12a4* and *CG5629* genes (white boxes) and respective transcriptional orientation (converging arrows) is shown. The *Bari1* transposon is depicted as a grey box with its terminal inverted repeats (arrowheads). The black boxes represent the probes (a4 and *Bari1*) used in the Southern blot and FISH experiments. The location of the restriction sites *Hin*dIII (H) and of the two polyadenylation sites used in the "filled" (polyA-site1) and "empty" (polyA-site2) strains is shown. The arrowheads below the diagram represent the primers used to amplify and sequence the region from the "empty" and "filled" sublines. (B) The two transcripts of *cyp12a4* from the "empty" and "filled" strains differ at their 3′ end: the 5′ end of *Bari1* element contributes with 18 nucleotides to the variant of 1.7 kb; in the absence of the element, the transcript of 1.9 kb encompasses 75 bases of the downstream *CG5629* gene transcribed in opposite direction.

AE003725; of the two "filled" sublines, 8 is homozygous for the 2.5 kb band and sublines 6 is heterozygous for both.

Finally, FISH analysis confirmed that the sublines 5 and 7 bear genuine "empty" sites for *Bari1* insertion at division 91 F as opposed to a polymorphism in the *Hin*dIII restriction sites (not shown).

3.2. Is the Bari1 element at 91 F transposition competent?

To assess the structural integrity of the local element, we have carried out a sequence comparison between *Bari1* elements located at known divisions of polytene chromosomes in various Drosophila stocks. The elements compared were from 47D (accession number X67681; Caizzi et al., 1993), 41AB, 55F and 91F of the *Oregon-R* stock (Moschetti et al., 1998) and from 95AB, 21DE, 102EF, 55F and 91 F of the *y, cn bw sp* stock, the sequenced one. We define the elements from 47D, 41AB, 95AB, 21DE, 102EF as active because they are polymorphic for the presence/absence (Caggese et al., 1995; our unpublished results); the elements from 55F and 91F are high frequency and fixed, respectively. Two main points emerge from this comparison: i) the inverted repeats of the elements at 91F are well conserved and ii) one transition G>A at position 1002 in the transposase domain, resulting into the substitution glu>lys, is present in both the element from 91F and the one from 41AB, known to be active. In other words, the element at 91F does not bear unique rearrangements potentially affecting its activity.

3.3. Structural and transcriptional analysis of the insertion region

As previously mentioned, the sequenced *D. melanogaster* genome does bear, at division 91F, a *Bari1* insert located downstream to the *cyp12a4* gene (AE003725; Fig. 2A). The cDNA sequence of the *cyp12a4* gene (AY070663), reveals that the 15 nucleotides upstream of the polyA tail belong to the 5′ end of *Bari1*. We then resolved to compare by Northern analysis the *cyp12a4* transcripts from "empty" and "filled" alleles. Preliminary data showed that in adult flies the levels of expression were low, especially in flies from the "empty" subline. Thus, more polyA+ RNA from this subline were loaded. As shown in Fig. 3A, in the presence of *Bari1*

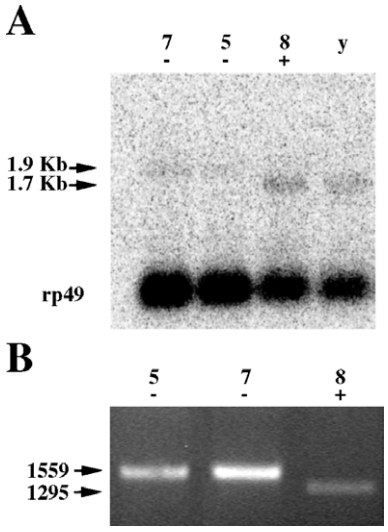

Fig. 3. Expression of *cyp12a4* gene in "empty" and "filled" sublines. (A) polyA mRNAs was extracted from adults of the "empty" sublines 7 and 5, from the "filled" subline 8 and from the the *y, cn bw sp* strain, which is also of the "filled" type. About twice as much mRNA was loaded from the "empty" sublines. The filter was co-hybridized with *cyp12a4* and rp49 probes (Fig. 2A). The latter gives rise to the prominent band (exposure time 2 h) and is used as internal control of the amount of RNA loaded. The mobility of *cyp12a4* mRNA bands (exposure time 36 h) is indicated by arrows. (B) PCR products from 3′-RACE of sublines 5, 7 and 8. The size of the fragments, as determined by sequencing, is indicated on the left.

the *cyp12a4* transcript is 200 pb shorter and the amount is about tenfold higher.

The 3′ end of the *cyp12a4* transcripts from "empty" and "filled" sublines was analysed by 3′-RACE procedure and the PCR products cloned and sequenced (Fig. 3B). In the "filled" subline 8, three additional nucleotides were found (3′ end position 42789 in AE003725, also see AY070663) extending to 18 nucleotides the overlap between the 3′ end of *cyp12a4* and the 5′ end of *Bari1*. In the longer mRNA from "empty" sublines 5 and 7, the 264 additional nucleotides are due to the readthrough transcription of downstream sequence (3′ end position 44787 in AE003725) including the 75 nucleotides at the 3′ end of the adjacent *CG5629* gene that is transcribed in opposite orientation. These results are outlined in Fig. 2A where the location of two putative polyA-sites (ATTAAA at positions 42757 and AATATA at 44761) is shown. Both can be used in insects (Beaudoing et al., 2000), the former is used in the "filled" line, whereas the latter is used in the "empty" one, although both are present in the longer transcript. This suggests that *Bari1* plays a role in the choice of the poly(A) signal upstream of its insertion site, as reported for other sequences downstream of genes (Beaudoing and Gautheret, 2001).

Finally, to find out whether a feature other than the insert could account for the above difference in size and amount of the transcript, the regions around the "empty" and "filled" *cyp12a4* gene (from the sublines 5 and 8, respectively) have been sequenced (see Fig. 2A and Material and Methods for the position of primers used in PCR experiment). Upstream the *cyp12a4* transcription starting point, five nucleotide substitutions were found in the "empty" subline 5 that do not appear to affect the *cyp12a4* promoter as assessed with two promoter prediction tools (see Materials and methods).

At the "empty" insertion site the di-nucleotides TG, from the 3′ end of *Bari1* and TA, from the target site duplication, are joined (Fig. 4). This gives rise to a 4 bp transposon "footprint" which is believed to result from DNA repair of the gap left behind following excision (Plasterk et al., 1999). In turn, this suggests that the element at 91F may undergo excision, consistent with the above evidence for transposition competence.

3.4. Is DDT the selective agent of Bari1 at 91F?

Overtranscription correlated with the insertion of a transposable element has been linked to DDT resistance in the case of the *cyp6g1* gene (Daborn et al., 2002) but we had

... **TA**....*Bari1*....*TG***TA**... "filled" 91F

...**TA***TG***TA**... "empty" 91F

Fig. 4. The insertion site in the "filled" subline 8 (and *y, cn bw sp*) and in the "empty" subline 5. The *Bari1* element is in italic; the di-nucleotides TA, in boldface, are the target site duplication. The transposon "footprint" diagnostic of excision is underlined.

Table 1
Test of DDT resistance

Stock	% mortality (DDT 5 μg/vial)	% mortality (DDT 2 μg/vial)
y, cn bw sp	1.00	1.00
Canton-S	1.00	0.95
Oregon-R	0.9	0.78
Subline 8 ("filled")	0.71	0.24
Subline 5 ("empty")	0.68	0.33

two reasons for believing that the story would be different in the case of *cyp12a4*: one is that the 2.5 kb *Bari1* band, corresponding to the element at 91F, has been observed in Southern pictures of two *Canton-S* and two *Oregon-R* stocks (Soriano et al., 2002). These stocks were collected in the 1930s, prior to the usage of DDT and have not been contaminated by more recent flies because they appear devoid of *P* elements (our unpublished results) which invaded *D. melanogaster* about half a century ago; the other is that only *cyp6g1* is necessary and sufficient for resistance to DDT of all 90 *P*450 genes tested, thus including *cyp12a4* (Daborn et al., 2002).

However, in another DDT resistant line overtranscription of two cytochrome *P*450 genes (*cyp6g1* and *cyp12d1*) has been reported (Brandt et al., 2002), thus raising the formal possibility that *cyp12a4* as well may contribute to DDT resistance. To check this, the "empty" and "filled" sublines were tested for DDT resistance along with three additional stocks (*y, cn bw sp*, *Canton-S*, and *Oregon-R*), all bearing the *Bari1* insert at 91F (see above). As shown in Table 1, no significant difference is detected between the two sublines, although they appear to be somewhat less susceptible than the other stocks tested.

4. Discussion

This report is the direct follow up of previous studies of the genomic distribution of the elements of the transposon family *Bari1*. The initial work, carried out by in situ hybridization, reported an element present at 91F in all populations tested (Caggese et al., 1995; our unpublished results). Subsequently, in a survey carried out by the Southern blotting technique, a 2.5 kb band was found in all samples tested and assigned to the fixed element (Soriano et al., 2002). In addition to extending the number of the samples analyzed, the latter work showed that the *Bari1* element was inserted at the same site within the 91F band. We report now about the isolation and characterization of "filled" and "empty" alleles of this insertion site. The insert is right next to the 3′ end of the *cyp12a4* gene. In the presence of *Bari1* the amount of *cyp12a4* transcript is about 10 times higher and its size is 264 nucleotides shorter compared to the "empty" allele at 91F.

This is comparable to the correlation, reported in *D. melanogaster* and *D. simulans*, between the insertion of

transposons upstream to another *P*450 gene, *cyp6g1*, and its overtranscription (Daborn et al., 2002; Schlenke and Begun, 2004). Such correlation appears somewhat more direct in our case because the "filled" and "empty" alleles share a common genomic background. In addition, the 18 nucleotides of *Bari1* at the 3′ end of the *cyp12a4* transcript and the apparent usage of a specific poly(A) signal in the presence of the insert are suggestive of a direct role of the transposon in the expression of this gene. In the presence of the insert, the shorter transcript is more abundant, possibly because the signal AUUUA in the 3′UTR destabilizes the longer variant (van Hoof and Parker, 2002); alternatively, the 3′ end of the longer *cyp12a4* transcript could pair with the *CG5629* transcript proceeding from opposite orientation (see Fig. 2B) and trigger RNAi mediated degradation. Another possibility is that the *Bari1* element acts as "insulator" or "gene spacer" that prevents *cyp12a4* transcript from turning unstable because of an ill defined termination caused by hindrance from the *CG5629* transcript. Either way, the primary event affecting the abundance of *cyp12a4* mRNA would be the insertion of the *Bari1* element.

It has been known for some time that TEs may affect gene expression and the number of candidate examples has risen dramatically thanks to studies carried out in silico. One of the results is the key concept that TEs occur more often in genes related to response to external stimuli than in other gene classes (van de Lagemaat et al., 2003). This provides an additional, indirect, link between *cyp12a4* and *cyp6g1* which are isoforms of the *P*450 family involved in a number of interactions with the environment (Stoilov, 2001). Another finding reported from in silico work is that gene expression may be affected by inserts at both the 5′ and the 3′ end of genes and that in the latter case they may serve as polyadenylation sites (van de Lagemaat et al., 2003). The example of the ING1 tumor suppressor gene terminating in a DNA transposon is reminiscent of *cyp12a4* terminating in *Bari1*, also a DNA transposon. Combined in silico and experimental evidence suggest that in mammals L1 may affect gene expression by slowing down RNA polymerase and by producing premature polyadenilation (Han et al., 2004). While the analogy with our results is limited to the (hypothetical) choice of alternative poly(A) sites, these data add to the broader concept of TEs from different structural classes affecting transcription of host genes in distantly related organisms, in a variety of ways.

In the absence of a detectable difference in DDT resistance between "filled" and "empty" alleles, the hypothetical selective agent remains unknown. A hint on the time of establishment of the initial insertion is provided by the observation of the 2.5 kb *Bari1* band in Southern pictures of *Canton-S* and *Oregon-R* stocks which were collected in the 1930s prior to the use of DDT. Thus, finding out what causes selection may prove difficult because it requires reconstruction of environmental parameters over 70 years old without any hint as to whether to look for a man-made selective agent or an adaptation normally encountered by wild populations.

We think that most of the evidence available for high frequency/fixation of TEs can be viewed as the unpredicted outcome of studies initially dealing with other subjects such as genomic distribution and instability of TEs (Maside et al., 2002; our results), a particular *P*450 gene (Daborn et al., 2002), ectopic recombination along the genome (Petrov et al., 2003) or the haplotype structure near *Sr-CII* gene (Schlenke and Begun, 2004). The first experimental approach explicitly aimed at detection of putative beneficial TEs has been published very recently (Franchini et al., 2004). The results suggest that TE-gene associations that meet the basic requirements for an adaptive role are more numerous than previously estimated, even though the study has been limited to LTR-retroelements and to the 5′ end of genes (Franchini et al., 2004). Our results, along with the cases of the *S* element and of the ING1 tumor suppressor gene in mouse (Maside et al., 2002; van de Lagemaat et al., 2003), show that fixation meeting the above requirements extends to DNA elements and that insertion at both 3′ and 5′ ends may be correlated with upregulation of the affected gene. Considering this variety of elements and ways they can affect genes, we think that more data, obtained by dedicated experimental approaches, will be required to assess the evolutionary impact of TEs in Drosophila.

Acknowledgments

This work was supported by Ministero dell'Istruzione e della Ricerca Scientifica, Consiglio Nazionale delle Ricerche and Fondazione Cenci Bolognetti Institut-Pasteur.

References

Beaudoing, E., Gautheret, D., 2001. Identification of alternate polyadenylation sites and analysis of their tissue distribution using EST data. Genome Res. 11, 1520–1526.

Beaudoing, E., Freier, S., Wyatt, J.R., Claverie, J., Gautheret, D., 2000. Patterns of variant polyadenylation signal usage in human genes. Genome Res. 10, 1001–1010.

Brandt, A., et al., 2002. Differential expression and induction of two Drosophila cytochrome *P*450 genes near the Rst(2)ddt locus. Insect Mol. Biol. 11, 337–341.

Brosius, J., 1999. Genomes were forget by massive bombardments with retroelements and retrosequences. Genetica 107, 209–238.

Britten, R.J., 1997. Mobile elements inserted in the distant past have taken on important functions. Gene 205, 177–182.

Caggese, C., Pimpinelli, S., Barsanti, P., Caizzi, R., 1995. The distribution of the transposable element *Bari-1* in the *Drosophila melanogaster* and *Drosophila simulans* genomes. Genetica 96, 269–283.

Caizzi, R., Caggese, C., Pimpinelli, S., 1993. *Bari-1*, a new transposon-like family in *Drosophila melanogaster* with a unique heterochromatic organization. Genetics 133, 335–345.

Charlesworth, B., Sniegowski, P., Stephan, W., 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371, 215–220.

Daborn, P.J., Boundy, S., Yen, J.L., Pittendrigh, B., ffrench-Constant, R.H., 2001. DDT resistance in Drosophila correlates with *cyp6g1* overexpression and confers cross-resistance to neonicotinoid imidacloprid. Mol. Genet. Genomics 266, 556–563.

Daborn, P.J., et al., 2002. A single *P*450 allele associated with insecticide resistant in *Drosophila*. Science 297, 2253–2256.

Di Franco, C., Terrinoni, A., Galuppi, D., Junakovic, N., 1995. DNA extraction from single *Drosophila* flies. Drosoph. Inf. Serv. 76, 172.

Fedoroff, N.V., 1999. Transposable elements as a molecular evolutionary force. (Molecular strategies in biological evolution). Ann. N.Y. Acad. Sci. 870, 251–264.

Franchini, L.F., Ganko, E.W., McDonald, J.F., 2004. Retrotransposon-gene associations are wide-spread among *D. melanogaster* populations. Mol. Biol. Evol. 21, 1323–1331.

Han, J.S., Szak, S.T., Boeke, J.D., 2004. Transcriptional disruption by the L1 retrotransposon and implications for mammalian transcriptomes. Nature 429, 268–274.

Jordan, I.K., Rogozin, I.B., Glazko, G.V., Koonin, E.V., 2003. Origin of a substantial fraction of human regulatory sequences from transposable elements. Trends Genet. 19, 68–72.

Junakovic, N., 2004. Southern blotting analysis of individual Drosophila flies. In: Miller, W.J., Capy, P. (Eds.), Mobile Genetic Elements and their Application in Genomics, Methods in Molecular Biology and Medicine, vol. 260. Humana Press Publisher, pp. 41–58.

Kidwell, M.G., Lisch, D., 2001. Perspective: transposable elements, parasitic DNA, and genome evolution. Evolution 55, 1–24.

Makalowski, W., 2000. Genomic scrap yard: how genomes utilize all that junk. Gene 259, 61–67.

Maside, X., Bartolomé, C., Charlesworth, B., 2002. *s*-element insertions are associated with the evolution of the *hsp70* genes in *Drosophila melanogaster*. Curr. Biol. 12, 1686–1691.

Moschetti, R., Caggese, C., Barsanti, P., Caizzi, R., 1998. Intra- and interspecies variation among *Bari-1* elements of the melanogaster species group. Genetics 150, 239–250.

Nekrutenko, A., Li, W.H., 2001. Transposable elements are found in a large number of human protein-coding genes. Trends Genet. 17, 619–621.

Ohler, U., Liao, G., Niemann, H., Rubin, G.M., 2002. Computational analysis of core promoters in the *Drosophila* genome. Genome Biol. 3 (research0087.1-0087.12).

Petrov, D.A., Aminetzach, Y.T., Davis, J.C., Bensasson, D., Hirsh, A.E., 2003. Size matters: non-LTR retrotransposable elements and ectopic recombination in Drosophila. Mol. Biol. Evol. 20, 880–892.

Plasterk, R.H.A., Izsvak, Z., Ivics, Z., 1999. Resident aliens: the Tc1/*mariner* superfamily of transposable elements. Trends Genet. 15, 326–332.

Reese, M.G., 2001. Application of a time-delay neural network to promoter annotation in the *Drosophila melanogaster* genome. Comput. Chem. 26, 51–56.

Schlenke, T.A., Begun, D.J., 2004. Strong selective sweep associated with a transposon insertion in *Drosophila simulans*. Proc. Natl. Acad. Sci. U. S. A. 101, 1626–1631.

Soriano, S., Fortunati, D., Junakovic, N., 2002. Evidence for the host contribution in the definition of preferential insertion sites of the elements of *Bari1* transposon family in *Drosophila melanogaster*. J. Mol. Evol. 55, 606–615.

Stoilov, I., 2001. Cytochrome *P*450s: coupling development and environment. Trends Genet. 117, 629–632.

van de Lagemaat, L.N., Landry, J.R., Mager, D.L., Medstrand, P., 2003. Transposable elements in mammals promote regulatory variation and diversification of genes with specialized functions. Trends Genet. 19, 530–536.

van Hoof, A., Parker, R., 2002. Messenger RNA degradation: beginning at the end. Curr. Biol. 12, R285–R287.