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

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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.

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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′