



Spatiotemporal transcription of the *P* element and the 412 retrotransposon during embryogenesis of *Drosophila melanogaster* and *D. willistoni*

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Abstract

Transposable elements (TEs) are mobile nucleotide sequences which, through changing position in host genomes, partake in important evolutionary processes. The expression patterns of two TEs, *P* element transposon and 412 retrotransposon, were investigated during *Drosophila melanogaster* and *D. willistoni* embryogenesis, by means of embryo hybridization using riboprobes. Spatiotemporal transcription patterns for both TEs were similar to those of developmental genes. Although the two species shared the same *P* element transcription pattern, this was not so with 412 retrotransposon. These findings suggest that the regulatory sequences involved in the initial development of *Drosophila spp* are located in the transposable element sequences, and differences, such as in this case of the 412 retrotransposon, lead to losses or changes in their transcription patterns.

Key words: *Drosophila*, *P* element, 412, transposable element, embryonic development.

Received: November 9, 2010; Accepted: July 27, 2011.

Transposable elements (TEs), already described for virtually all species, contribute as a substantial fraction in genome size (González *et al.*, 2008). They are nucleotide sequences that can move throughout the genome of host organisms (Kidwell and Lisch, 2000).

In *Drosophila* species, the *P* element, one of the best-studied TEs, was first described as that responsible for hybrid dysgenesis in *D. melanogaster* (Kidwell, 1983), a syndrome caused by TE mobilization, particularly in germ-line cells, and which leads to gonadal atrophy and consequential sterility. With the exception of *D. melanogaster*, this particular element has not been found in the *melanogaster* subgroup of already studied *Drosophila* species. In addition, there is only one nucleotide difference between the *P* element sequence found in *D. melanogaster* and the sequences in the *willistoni* subgroup (Daniels *et al.*, 1990; Clark and Kidwell, 1997), thereby inferring the horizontal transfer of the *P* element of one of the species in the *willistoni* subgroup to *D. melanogaster*.

In contrast, the wide distribution of the 412 retrotransposon in the *melanogaster* subgroup gives to under-

stand that it was already present in the subgroup's ancestor (Cizeron *et al.*, 1998). Although complete copies have been found in several species, the sequences in *D. melanogaster* and *D. simulans* are located in the euchromatic part of chromosome arms, thereby inferring their active state. In *D. willistoni*, and when using Southern blot, the size of the hybridized band infers the existence of a complete copy of the element, whereas with chromosome hybridization, most sequences are encountered in the chromocenter, a possible indication of an old invasive process and their silencing (Capy *et al.*, 1991; Periquet *et al.*, 1994; Cizeron *et al.*, 1998).

The importance of TEs lies in their being one of the many endogenous mechanisms that generate mutations capable of causing diseases or evolutionary adaptation (Kidwell and Lisch, 2000; González *et al.*, 2008). Taking into consideration how long *P* and 412 TEs have been present in the genomes of *D. melanogaster* and *D. willistoni*, the aim was to understand the behavior of TEs in different genomes, through the detection and description of their expression patterns in *D. melanogaster* and *D. willistoni* embryos.

The strains *D. melanogaster* Harwich (H) (from the USA, sampled in 1967) and *D. willistoni* 17A2 (from southern Brazil (30°05' S, 51°39' W), and sampled in the beginning of the 1990's), were raised in a cornmeal me-

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dium (Marques *et al.*, 1966), at a constant temperature and humidity (17 +/- 1°; 60% rh). 0-18 h embryos, collected from an oviposition medium (1.5% agar, 15% honey, 10% yeast, Ponceau dye and 0.3% propionic acid), were dechorionated with 5% bleach for 5 min. They were then fixed and hybridized according to the Tautz and Pfeifle (1989) method. The embryonic stages were identified according to the criteria described by Campos-Ortega and Hartenstein (1985).

P-element riboprobes were synthesized from *KpnI*-*EcoRI* restriction fragments of $\pi\pi 25.1$ plasmid (GenBank X06779; O'Hare and Rubin, 1983). This fragment was cloned into the pSPT18 plasmid *KpnI* - *EcoRI* restriction site. For obtaining strand-specific probes, the plasmids were linearized with *HindIII* restriction enzyme. The 412 riboprobes were synthesized from a p412TOPO plasmid, produced by subcloning of a 780-bp sequence obtained from the cDM2042 clone (GenBank X04132; Yuki *et al.* 1986) and produced by PCR using the following amplification primers: 412RTS (5'-GCGATTGCCATTTGGCT T-3') and 412RTA (5'-TTCTCGATGGTGAACCCCA-3'). The plasmid was linearized with *HindIII* restriction enzyme, in order to obtain a strand-specific probe. By using a DIG RNA labeling kit (Roche) according to manufacturer's instructions, 1 μ g of the templates was transcribed with T7 RNA polymerase to synthesize the antisense riboprobe. Detection was carried out with antibody anti-digoxigenin (Roche), and the colorimetric reaction with 4-nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) (Sigma). Embryos were photographed using a Stemi 2000-C (Zeiss) stereomicroscope and a CC-8703 (GKB) high-resolution digital color camera.

The spatiotemporal expression-pattern of the *P* element during the various phases of *D. melanogaster* and *D. willistoni* embryonic development can be observed in Figure 1. Apparently, the pattern is very similar in both species. The *P*-element hybridization signal is diffuse during the syncytial-blastoderm stage (Figure 1A). In the cellular blastoderm stage, the hybridization signal encircles the embryos, apparently accumulating in the cells being formed (Figure 1B). In the gastrulation stage, hybridization with the *P* element probe occurred in the region of the ventral furrow (Figure 1C, in the initial ventral furrow, cell invagination in *D. melanogaster*, and in the ventral furrow in *D. willistoni*) and the signal follows mesoderm migration that extends ventrally towards the posterior region and dorsally towards the anterior region surrounding the embryo (Figure 1C). During germ band extension, *P*-element expression was detected throughout the mesoderm and in the posterior midgut primordium (Figure 1D). During germ band retraction, *P* transcripts spread through the mesoderm and into the ventral nerve cord (Figure 1E). The ventral, posterior and anterior regions remained heavily labeled during dorsal closure, thus implying no change in the pattern described

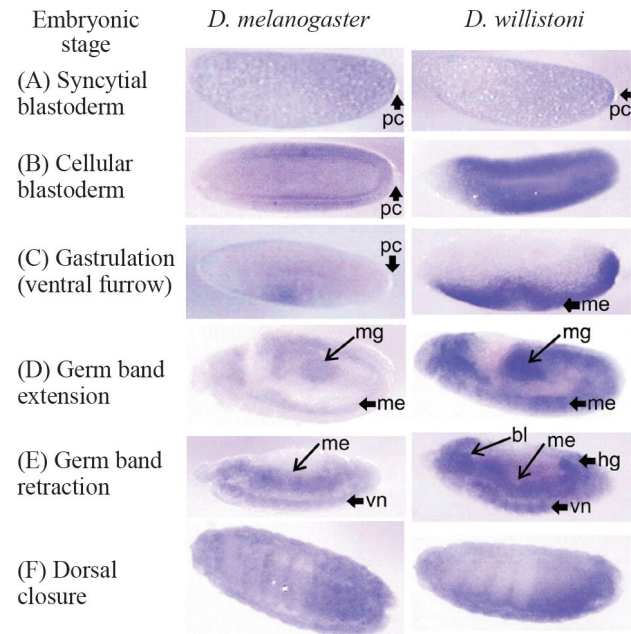


Figure 1 - *P* element transcriptional pattern during embryonic development in *D. melanogaster* - Harwich strain, and *D. willistoni* - 17A2 strain, in syncytial blastoderm (A), cellular blastoderm (B), gastrulation (C), germ band extension (D), germ band retraction (E) and dorsal closure (F). pc - polar cells, me- mesoderm, mg- midgut, vn- ventral nerve cord, bl- brain lobe, hg- hindgut. Anterior region to the left, lateral view, except in *D. melanogaster* embryo F, dorsal view.

for the germ-band retraction stage. In this stage and during head involution we observed a slight segmentation of the stain, but it is not possible to specify the number and identity of each segment.

Contrary to what was observed for the *P* element, the two species did not share the same 412 retrotransposon hybridization pattern (Figure 2). Although in the *D. melanogaster* cell line, the pattern was the same as that already described by Ding and Lipshitz (1994) (Figure 2A, 2B and 2C), in *D. willistoni*, transcripts were detected only in the central nervous system during the germ band retraction stage, as seen in Figure 2D. In addition to being more restricted, hybridization was weaker in *D. willistoni* than in *D. melanogaster*.

TEs can move throughout the genome, their expression patterns possibly being affected by genomic promoter regions depending on insertion sites. Thus, their different expression patterns during embryogenesis of *D. melanogaster* and *D. willistoni* were expected because TEs are inserted in different genome sites in the two species. In contrast, the regulation of TE transcription by *cis*-regulatory sequences has been suggested in several studies (Bröner *et al.*, 1995; Kerber *et al.*, 1996; Deprá *et al.*, 2009). Ding and Lipshitz (1994), by showing that, 14 of the 15 retrotransposons, had the same transcription pattern during embryogenesis, in four polymorphic *D. melanogaster* strains, proposed that *cis*-regulatory sequences drive TE-transcription. This was corroborated in the present study, in

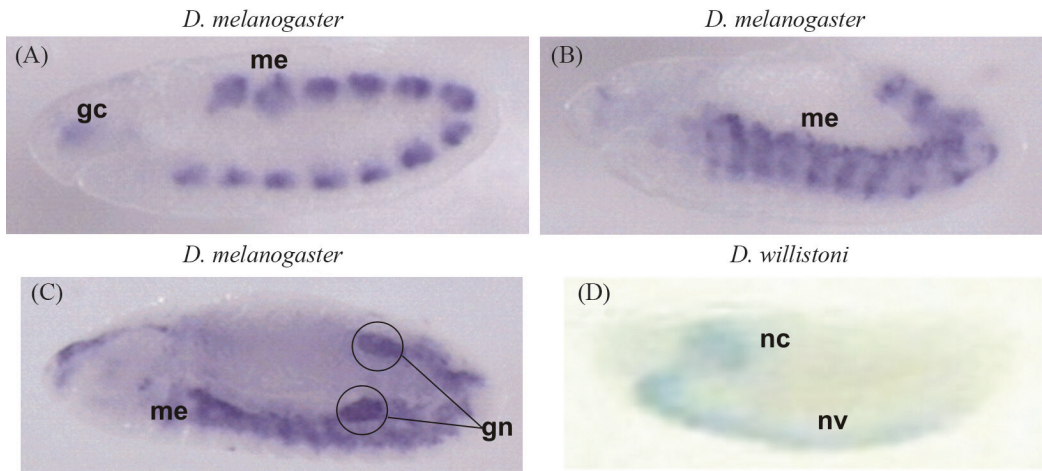


Figure 2 - 412 retrotransposon transcription pattern in *D. melanogaster* embryo, Harwich strain, in germ band extension (A and B) and germ band retraction (C), and *D. willistoni*, 17A2 strain in germ band retraction (D). cc- cellular cluster, me- mesoderm, gn- gonads, cn- central nervous system, and vn- ventral nerve cord. Anterior region to the left, lateral view in A, B and D, and dorsal view in C.

that the *P* element pattern in both species remained the same, thereby implying that *P* element spatiotemporal expression follows its own regulatory sequence, and that transcriptional dependence on host promoters is unlikely.

High preservation of the *P* element sequence between species and consequently, conservation of the regulatory region, should be considered. Simultaneously, the difference in the nucleotide sequence of the 412 retrotransposon in *D. melanogaster* and *D. willistoni* (blast-performed with the canonical 412 retrotransposon sequence – GenBank X04132 - in the *D. willistoni* genome- *taxid:7260*, thereby detecting low identity of the 5' sequence, but high identity of the sequence region used as probe) is in agreement with the different expression-patterns encountered during embryogenesis.

The loss of sequence identity may be responsible for the difference in the hybridization pattern observed in both *D. melanogaster* and *D. willistoni*. In the latter, the sequence difference not only promoted the loss of the mesoderm and gonad transcription patterns found in *D. melanogaster*, but also induced CNS function gain. Obviously, the interactions between retrotransposon sequences with genomic regulatory regions should not be overlooked.

Transcription during embryogenesis also implies post-transcriptional regulation of TE mobility, seeing that the strains under study are not hypermutable. Both transposase and 66 kDa repressor transcripts have been detected in *D. melanogaster* and *D. willistoni* embryos (Blauth *et al.*, 2009). In this case, inactive heteromultimers may form after transduction (Gloor *et al.*, 1993). Antisense transcripts, possibly capable of impeding mobility via RNA interference, have also been detected (Blauth *et al.*, 2009). To date, no transposition silencing system has been described for the regulation of 412 retrotransposon.

TEs may reorganize genomes and promote genetic variability by means of chromosome rearrangement, gene

disruption, gene duplication, exon shuffling, epigenetic effects, or gene expression reorganization, not only by interrupting regulatory sites, but also through their having accompanying *cis*-regulatory sequences, which possibly play the role of promoters for host genes close to their insertion sites (Feschotte and Pritham, 2007; Wagner and Lynch, 2010). Pereira *et al.* (2009) attributed 20% of the differences in rodent expression profiles to TE insertion, thus in agreement with findings reported by Urrutia *et al.* (2008), who also found a correlation between differential expression in humans and mice, and the number of *Alu* sequences. Recently, it was shown that upregulation of the *Cyp6g1* gene that induces resistance to a variety of insecticide classes, resulted from the LTR insertion upstream of the *Accord* gene (Chung *et al.*, 2007). The creation of new regulatory networks is widely accepted as the main promoter of macroevolution via gene heterotopy or heterochrony between species (Carroll, 2008; Wagner and Lynch, 2010). Herein, the proposal is the creation of a new regulatory network, probably by accumulating mutations in the 412 retrotransposon in *D. willistoni*, as observed in the hybridization pattern during embryogenesis.

Acknowledgments

We thank CNPq and FAPERGS for their financial support to this study.

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Associate Editor: Ricardo Guelerman P. Ramos

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