HSP70 OF *D. MELANOGASTER* ARE "HOTSPOTS" FOR P ELEMENT INSERTIONS

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Introduction

The 70 kDa family of hsps is a major system of cellular response to stress in nearly all organisms (Zatsepina et al.,2001). Recent studies have shown that promoter regions of these genes in *D. melanogaster* sometimes are disrupted by transposable elements (TE) (Lerman et al.,2003). Such insertions may play an important role in microevolution of *hsp70* genes and in organism adaptation to local thermal environment. In this study we investigated the frequency of P element-containing construction (*EPgy2*) insertions into the *hsp70* genes using *D. melanogaster* model system. Our results show that promoter regions of *hsp70 Aa* and *Ab* are "hotspots" for *EPgy2* insertions.

Material and methods

***Drosophila* stocks**: *D. melanogaster* strains, containing *EPgy2* insertions, were obtained from the Berkeley Drosophila genome project from university of California, Berkeley. All flies were cultured at 25^0C on a yeast, molasses and agar medium. **Genetic crosses:** we used genetic crosses (Fig.1) to generate strains that may have the additional copy(s) of the starting element *EPgy2*. Local transpositions from the starting element *EPgy2* were generated in the F1 males with *Δ2-3* transposase source. The new insertions were recovered in the F2 males with elevated eye pigmentation**. Southern blot analisis**: genomic DNA was prepared as described by (Zatsepina et al., 2001). Each DNA sample was digested with BamHI/HindIII endonucleases, separated by agarose gel electrophoresis, transferred to the Hybond N+ membrane. Hybridization was carried out as described by (Zatsepina et al., 2001) with radioactively labeled XhoI/BamHI-fragment complementary to 5'-end and BamHI/SalI-fragment complementary to 3'-end of *hsp70 D. melanogaster*. **PCR screens**: the polymerase chain reactions were performed for 30 cycles. We employed primers from 5'- and 3'-ends of P element as described by (Timakov et al., 2002), primers from 5'- and 3'-ends of *hsp70 genes* of

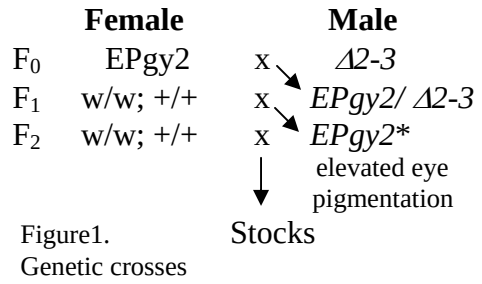

Figure1. Genetic crosses

D. melanogaster with in outward and inward orientations, from *CG18347* and from *aurora*.

Sequencing: to identify the *EPgy2* insertion sites we used the direct sequencing of the PCR products with appropriate primers. Sequencing was performed in the centre "Genome" of EIMB RAS (http://www.genome-centre.narod.ru/).

Results and discussion

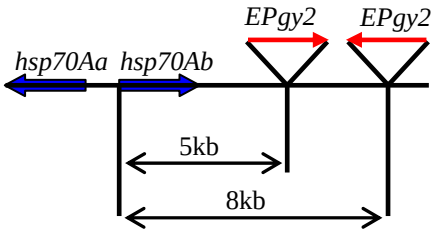

Figure 2. Location of the starting element *EPgy2* in two stock strains *D. melanogaster*

We investigated the frequency and localization of P element-base construction transpositions in two deletion strains of *D. melanogaster*, which contain the starting element *EPgy2* located at 5 and 8 kb distance from *hsp70Ab* (Fig.2). Local transpositions were produced by special genetic crosses (see above). *EPgy2* contains mini-white as a marker (Hugo J. Bellen et al., 2004) and flies carrying an additional copy of this construction showed elevated eye pigmentation. We have found that overall frequency of *EPgy2* transposition was 7,5% (562/7400 males). To detect insertions in *hsp70* genes we used the method of Southern blot hybridization. DNA digestion with BamHI/HindIII endonucleases and subsequent hybridization with 5'- and 3'- end of *hsp70* allowed to identify insertions in different copies (six total) of *hsp70* genes (Fig. 3A).

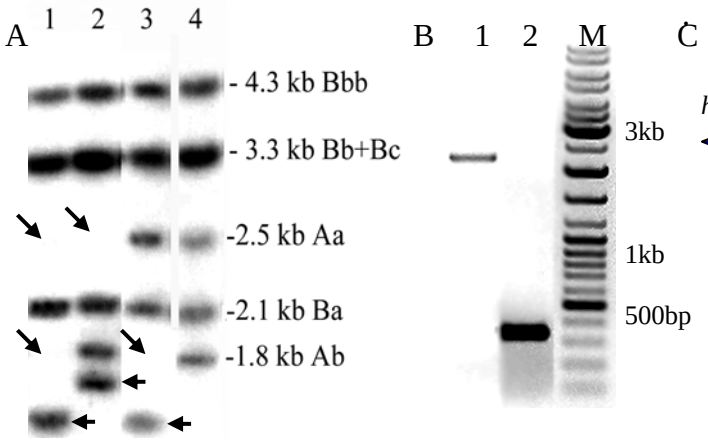

Figure 3A. Hybridization with 5'-end of *hsp70*. Changing of banding pattern in Southern hybridization due to insertion of EPgy2 is marked with arrows.*EPgy2* insertions in *hsp70Aa, Ab* (lane 1), *hsp70 Aa* (lane 2), *hsp70Ab* (lane 3);

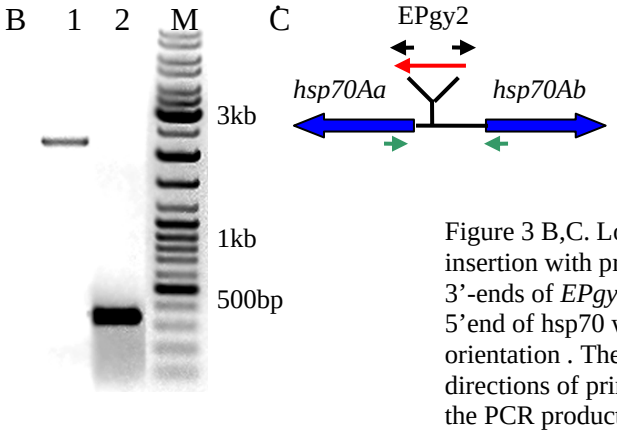

Figure 3 B,C. Localization of *EPgy2* insertion with primers from 5'- and 3'-ends of *EPgy2* and primers from 5'end of hsp70 with an outward orientation . The arrows indicate the directions of primers (5' to 3') (C); the PCR products that were produced from these primers (B – lane 1, 2). Molecular size standards are shown on the right.

The frequency of *EPgy2* transposition into *hsp70* genes is 8,2% (31/375). The genomic location and orientation of insertion *EPgy2* were confirmed by PCR. We scanned the genomic region near starting element by using several PCR reactions with primers to 3'- and 5'-ends of *EPgy2*,

and primers to promoter, structural and 3'-flanking regions of *hsp70* genes. Results of one of these reactions are depicted in the Figure 3B, C. Our analysis of first construction (starting element located at 8 kb distance from *hsp70Ab*) has shown that: *EPgy2* inserts in opposite; *EPgy2* inserts in promoter region of *hsp70* (within 44 - 256bp interval) preferentially at positions -112 and -113 bp upstream from transcriptional start; the majority of transpositions were detected in *hsp70Aa* (87%) and the rest in *hsp70Ab* (13%); no local insertions were found in the coding region or in 3'-flanking region; no local insertions were found in *hsp70Ba, Bb, Bc, Bbb* (87C locus). Herein we present localization (upstream from transcriptional start, bp) and frequency (%) of *EPgy2* insertions in *hsp70Aa, Ab* genes: -112, -113bp (58%), -151 (12,9%), -256 (6,4%), -58 (6,4%), -44 (3,2%), -56 (3,2%), -160 (3,2%), -190 (3,2%), -153 (3,2%). Analysis of second construction (starting element located at 5 kb distance from *hsp70Ab*) confirmed our results on highly non-random distribution in the process of transposition.

Conclusions

High frequency of P element-base construction insertions into *hsp70* promoters indicate that these regions are hot spots for P element transpositions. The target preference of such insertions could be explained if the *EPgy2* predominantly inserts into the "open" chromatin containing an active promoter of *hsp70* genes. Thermotolerance experiments revealed significant differences in the survival of strains with insertions in *hsp70* loci in comparison with the original strain. Basing on thermotolerance experiments exploring the transgenic strains we speculate that such insertions may play an important role in the microevolution of *hsp70* genes and in organism adaptation to thermal environment.

References

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