Cloning and characterization of β-esterase (Est B) gene in *Drosophila virilis*.

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β-esterases of *Drosophila* display marked variation in their patterns and sites of the expression. Est 5 of *D. pseudoobscura* is expressed in the eyes and haemolymph (Lunday and Farmer, 1983). Est 6 of *D. melanogaster* is highly expressed in the sperm ejaculatory duct of the adult male (Sheehan *et al*., 1979). The variation of gene expression in the *Est 5B* of *D. pseudoobscura* and the *Est 6* of *D. melanogaster* has been examined (Tamarina *et al*., 1997). In the β-esterase of *D. virilis*, two types were detected, one of which is specific for the genitalia (Est S) (Sergeev *et al*., 1993) and the other for the adult head and haemolymph (Est B) (Sasaki, 1974). In order to compare *Est 5B* and *Est 6* with *Est B*, I examined in the first place, the DNA sequence and transcripts of the *Est B* gene in *D. virilis*.

The cDNA and genomic libraries of adult flies of *D. virilis* were constructed in lambda gt10 and lambda dash, respectively. The cDNA library was screened with partial *Est 6* gene of *D. melanogaster* as a probe. The genomic library was screened with the *Est B* cDNA of *D. virilis*. The region of the *Est B* gene was subcloned in pBluescript and sequenced on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

mRNA in adult flies of *D. virilis* was prepared by use of a QuickPrep mRNA Purification Kit (GE Healthcare). The primers designed for 5' RACE were as follows: Pesb, 5'- P-ACAGCTCTGACTAG -3'; A1esb, 5'- ATGCGAATCGACTTATC -3'; A2esb, 5'-GGATCAGCAAAGGTAGC -3'; S1esb, 5'- CCGGGCAACTTTGGATTA -3'; S2esb, 5'-TAGACGTGCGTTTGAGCT -3'. For 5' RACE, 1 μg of mRNA was reverse-transcribed into cDNA by using a 5'-Full RACE Core Set (Takara) in the presence of Pesb primer according to the instructions of the manufacturer. The cDNA was amplified by use of an LA PCR Kit (Takara) with A1esb and S1esb primers for the first step and with A2esb and S2esb primers for the second step. PCR amplifications were carried out for 25 cycles under the following conditions: 94ºC, 60ºC and 72ºC for 30 sec for the respective steps.

One positive cDNA clone was isolated by screening of about 8×10^3 plaques of the cDNA library. The sequence analysis indicated that the clone was 1.3 Kbp in length, though it was not a full-length one. Two positive genomic clones were isolated by screening of about 7.2×10^4 plaques by use of the cDNA as a probe. I determined the nucleotide sequences of 3-Kbp stretches of the genomic regions.

Sequence analyses of cDNA and genomic DNA revealed that the *Est B* gene comprised two exons, one is 1381bp and the other 236bp, separated by a short intron of 62bp. Poly A signal was identified 252bp downstream of the stop codon. The exon–intron organization of *Est B* was the same as those of *D. pseudoobscure* and *D. melanogaster*, *Eat 5B* and *Est 6*. Nucleotide sequences in the coding region of *Est B* showed 67.3% and 62.9% similarities to those of *Est 5B* and *Est 6*, respectively. These values are low in comparison with other genes encoding constitutive proteins, suggesting high variability of the *esterase* genes in *Drosophila*. The deduced amino acid sequence of Est B consisted of 538 residues, shorter 7 residues than Est 5B and 6 residues than Est 6. Alignment of amino acid sequences of Est B, Est 5B, and Est 6 (Figure 1) indicated that the N-terminal signal

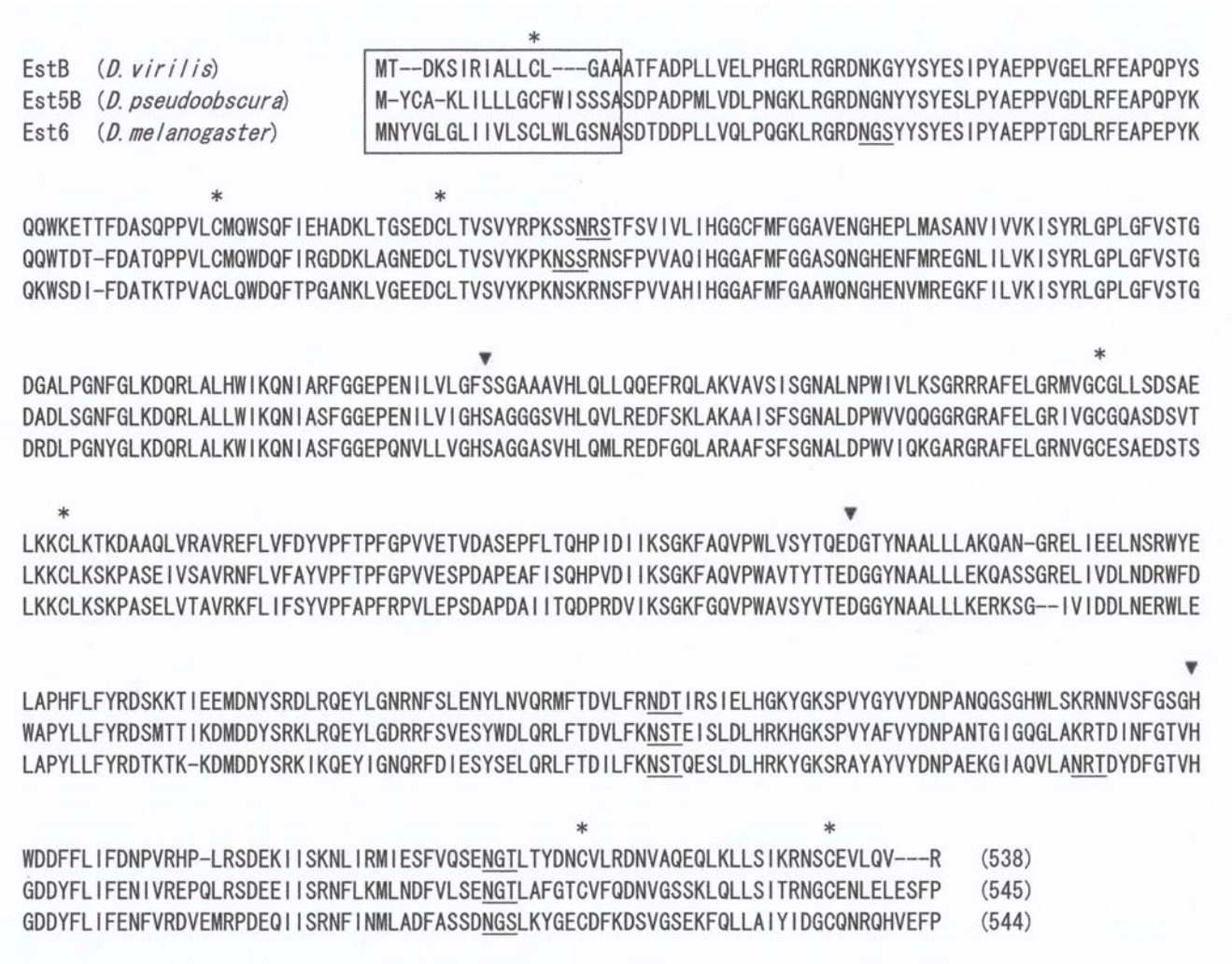

Figure 1. Sequence alignment of the β-esterase of *D. virilis*, *D. pseudoobscura* and *D. melanogaster*. The 3 noncontiguous residues for the catalytic activity are indicated by triangle. The seven cysteine residues conserved among *Drosophila* species are indicated by an asterisk. The putative N-glycosylation sites are underlined. The N-terminal signal sequences are boxed.

peptides, seven cysteine residues, three noncontiguous catalytic residues, and two N-glycosylation sites were found in the same positions. Comparison of hydropathy profile of these proteins among three species showed that the C-terminal region of Est 6 enzyme composed of a monomer was more hydrophilic than those of Est 5B and Est B such as a dimer (data not shown).

A 5'RACE analysis identified two transcriptional start sites (Figure 2). The one transcript started at -32 and the other transcript started at -387, in which the 71 bps (from -321 to -251) in the 5' leader sequence were spliced. The *Est 6* of *D. melanogaster* has multiple transcriptional start sites between nucleotides -38 and -32 (Collet *et al*.,1990). These results showed that the transcripts of these esterases examined are similar to each other, except for the transcriptional pattern in the 5' region. Therefore, it is likely that different transcripts at the 5' region of the gene are useful for specific tissue expression.

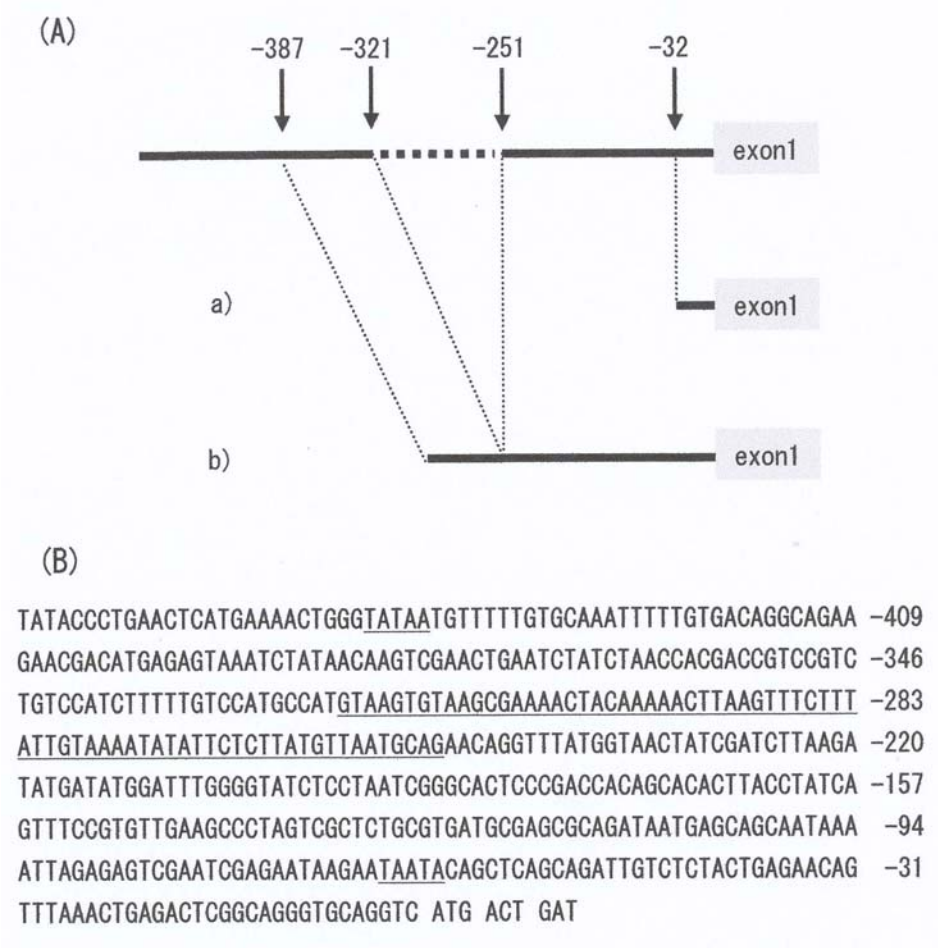

Figure 2. (A) Two transcriptional patterns a) and b) of the 5' region of *EstB* gene. Intron is indicated by dotted line. (B) Nucleotide sequences of the 5' region of *EstB* gene. Transcriptional start sites are boxed. Intron is double-lined. Putative TATA boxes are underlined.

References: Collet, C., K.M. Nielsen, R.J. Russell, M. Karl, J.G. Oakeshott, and R.C. Richmond 1990, Mol. Biol. Evol. 7: 9–28; Lunday, A.J., and J.L. Farmer 1983, Biochem. Genet. 21: 453-463; Sasaki, F., 1974, Jan. J. Genetics 49: 223-232; Sergeev, P.V., G.N. Yenikolopov, N.I. Peunova, B.A. Kuzin, R.A. Khechumian, L.I. Korochkin, and G.P. Georgiev 1993, Nucleic Acids Res. 21: 3545–3551; Sheehan, K., R.C. Richmond, and B.J. Cochrane 1979, Insect Biochem. 9: 443-450; Tamarina, N.A., M.Z. Ludwig, and R.C. Richmond 1997, Proc. Natl. Acad. Sci. USA 94: 7735-7741.

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