The expression of esterase S gene of *Drosophila virilis* in *Drosophila melanogaster*

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Abstract ***Drosophila melanogaster*** **was transformed with the esterase S gene from** ***Drosophila virilis*****. This gene is strongly activated in ejaculatory bulbs of mature males of** ***Drosophila virilis*****. The closely related gene from** ***Drosophila melanogaster*** **is activated in ejaculatory ducts. The tissue- and stage-specific expression of incomplete genomic copy of the esterase S gene integrated into the** ***Drosophila melanogaster*** **genome is the same as in** ***Drosophila virilis*****. These data show that tissue and stage specificity is determined by relatively small 5′ regulatory region of the esterase S gene. The comparison between deduced amino-acid sequences of the esterase S of** ***Drosophila virilis*** **and esterase 6 of** ***Drosophila melanogaster*** **was performed. These sequences revealed 50% homology.**

Key words: Esterase; Regulation of expression; *Drosophila virilis*

1. Introduction

The central problem in eukaryotic molecular biology is to understand the mechanisms by which specific genes are expressed in a temporal or tissue-specific manner.

The *estS* gene of *Drosophila virilis* encoding esterase S is an attractive model for studies of gene expression control as this gene is switched on at a well defined period of development (the 3rd day after emergence), and in one tissue (the epithelium of ejaculatory bulbs in males) [1,2]. The enzyme is carboxylic-ester hydrolase; its enzymatic activity is determined by hydrolyzing β-naphtylacetate [3,4]. The enzyme is responsible for processes of females fertilization. Esterase S is accumulated in ejaculatory bulbs, excreted and transferred into the genitals of females upon copulation [1–5]. Earlier we described the cloning and some aspects of regulation of *estS* gene of *Drosophila virilis* [6–8]. The *estS* gene has very specific mechanisms of gene regulation, since it has two alternative promoters, distal P1 and proximal P2. The level of transcription of mRNA from the proximal promoter is 100 times higher than the level of transcription of mRNA from distal promoter. Both promoters have different tissue- and stage specificity.

Among two promoters described, one, the P2 promoter, possesses very high tissue and time specificity. It is active only in ejaculatory bulbs epithelium starting from the third day after emergence. The role of P2 activity in females is not clear. In *Drosophila melanogaster*, esterase 6 is recovered not only in ejaculatory ducts of males, but also in fat bodies of females [9,10]. Fat bodies may be the place of low esterase S expression in *Drosophila virilis* females as well. An important goal is to detect the sequences determining such a high specificity of the P2 promoter. It should be pointed out that many sequences similar to other enhancer elements are located in this region.

The distal P1 promoter possesses a broader tissue specificity. Though active in ejaculatory bulbs, it is also used for transcription in several other tissues (imaginal disk in the third instar larvae). However this mRNA seems to be not utilized due to translation control (third factor). For example, gene expression is inhibited at the translational level due to the presence of at least two active ORFs [11,12,13]. The combination of such factors may completely eliminate the *estS* gene expression from the male tissues other than epithelium of ejaculatory bulbs.

It seemed interesting to understand what kind of regulatory elements (*cis* or *trans*) are involved in control of tissue-specific gene expression. For resolving this problem we used p-element transformation of *estS* gene in *D. melanogaster*. The expression of the most closely related gene *est6* of *D. melanogaster* occurred in adult males in ejaculatory ducts, while the expression of *estS* gene occurred in the ejaculatory bulb of mature males.

We have determined that relatively small region of the *estS* bagene is responsible for tissue specificity and that the tissue specificity of *estS* gene expression in transformed *Drosophila melanogaster* is the same as in *Drosophila virilis*. The esterase S enzyme is excreted and transferred to the female genitals upon copulation.

2. Materials and methods

2.1. P-element transformation

A 1.2-kb *Eco*RI *D. virilis* genomic fragment containing the part of the *estS* gene was subcloned into CaSpeR vector [14] for a p-element-mediated transformation. This genomic fragment contains a relatively small regulatory region of the *estS* gene (about 400 bp) and the coding part contained a putative active center of the esterase S enzyme. The sequence of this region (EMBL Accession No. X70351) [7] and schematic map of construction is shown in Fig. 1a,b. Df(1) line of *D. melanogaster* was used for transfection. The injection of the construction was carried out in embryos of *Drosophila melanogaster*. Second generation flies were taken for immunochemical analysis.

2.2. Analysis of the estS gene expression in D. melanogaster

Enzyme synthesis was controlled with monoclonal antibodies against the esterase S enzyme. Obtaining of the monoclonal antibodies was previously described in [15,16]. Tissues were dissected by hand, rinsed in homogenization buffer and then ground and loaded onto gels. Analysis of enzyme products of the injected construction was carried out

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Abbreviations: SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; *estS*, esterase S; *est6*, esterase 6.

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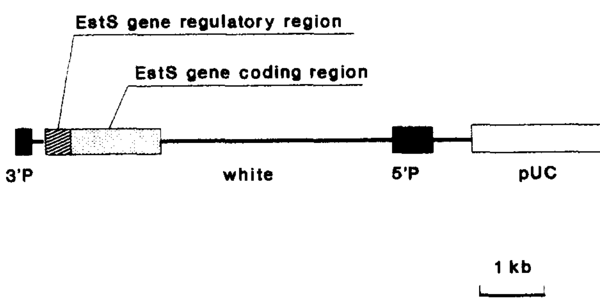
electrophoretically (15% polyacrylamide gel in the presence of 1% SDS [17]). Thus separated enzymes were transferred onto nitrocellulose filters and then the filters were treated with 3% BSA (Sigma) in PBS during 3 h. Treatment with antibodies against esterase S was carried out in 3% BSA, PBS, pH 7.4, 0.1% Tween-20 during 3 h. Washing was performed in 0.1% Tween PBS. Further procedures were as in [16].

3. Results and discussion

3.1. *The esterase S from* Drosophila virilis *and esterase 6 from* Drosophila melanogaster *are conserved*

We have previously described the cloning and sequencing of the *estS* locus from *Drosophila virilis*. We have performed the analysis of a nucleotide sequence of a cDNA copy of the *estS* gene and have deduced putative amino acid sequence of *estS*. In this paper we compare the putative amino acid sequences of *estS* and of the most closely related enzyme in *D. melanogaster est6*. The comparison of these sequences shows 50% homology (Fig. 2) The exon-intron structures of both genes are similar. The *est6* of *D. melanogaster* has only one promoter and a relatively short 5′ untranslated mRNA region [18]. Both enzymes contain a signal peptide. The most similar region is the one near the putative active center of the esterases. The C-termini of the enzymes possess a lesser homology.

In in vitro experiments, we have also determined whether some upstream sequences might be involved in the *estS* transcription control. It was found that the removal of the region from −830 to −475 bp did not influence the *in vitro* transcription, while the removal of the segment from −475 to −115 bp reduced transcription from the promoter P2 four-fold [19]. Thus, the 5′-upstream sequences could activate the P2 promoter even in the in vitro system. We suggest that this area contains *cis*-regulatory control elements. In order to detect this region and to study whether it can regulate tissue and stage specific expression of the *estS* gene we made constructions based on CaSpeR vector [14].



a

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aattctgact tcaaagtaat tttcaaatat ataaattgag aagaggagtt ctaagtgata   60
cggggagatt tgagctcagc tgatatttct tttataagct catttgcgtt cacttttacg  120
aaagtcagcg cgtgattcat aattttacag ttgctaagag cggaacaact tagaaatggg  180
gaatcatcta ttaaagaccc cagaaactaa gcgctgaacc caacttccaa actctgtggc  240
ctctataaaa caaatggcag agtcttaaat tttgaaactc agtttaactg ccaatgccaa  300
ggggccagcc atcggaacta atcgaatagc tgtcttcttt tctcacgtga acaaaactta  360
gactcaagtg accataaaaa gggagttggc tgtctccgtt tcgaacaatt caagtttgag  420
ctccgtcctg tgcacaccat gactcaaata ctgttgccga tcgcgttgct ctgtctgttt  480
gcagcatcaa ccctcagcaa tcccctgctc gtggagttgc caaatggaga attgcgggga  540
cgcgacaatg gattctatta cagctacgag tcaatacctt atgccgaacc cccaatcgat  600
gatctgtgct tggaagaacc tcgtccctat accgaaagat gggaaaatac ctttgacgcg  660
actcggcccc cagttgactg cctgcagTGG AGTCAACTCA TTTCACAGCC TAATAAGCTG  720
ACAGGGAGCG AGGACTGTCT AACCGTCAGC ATCTACAAGC CAAAGAATCT GACTCGCATC  780
TCTTTTCCGG TGGTGGCGCA TATATTTGGC GGCGGCTGGT CATTTGGTGC TGCGATCGAT  840
GACGGAGTGA GGCCCTTCAG CAGCAGCGGC AATGTGATAG TGGTGAAGAC AACCACAGAG  900
TGGGAGCGCT TGGGTTTTAT GAGCACTGGT GATTCTGTGA TTCCGGGCAA CTTCGGACTA  960
AAGGATCAGC GTCTGGCAAT CAAATGGATT AGGAACAACA TTGCACGCTT TGGCGGAGAT 1020
CCACATAATA TAATTCTTCT CGGTTTCAGT ACAGGCGGCT CCTCGGTGCA CTTGCAGCTT 1080
ATGCACAAGG AATATGGACA GCTGGTGAAG GGGGCCATAT CCATTAGTGG AACTGCAACG 1140
GTTCCCTGGG CTGTACAGGC CAATGCACGT GATCTCGCAT TCCGATATGG CAAACTCTTG 1200
GGTTGTAATA ACCCTAAAAA TTCACGCGAG CTGAAAGATT GCCTGAAAAA AACGGATGCG 1260
GAAGAATT
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b

Fig. 1. (a) A schematic map of the construction used for transfection of *Drosophila melanogaster*. (b) A nucleotide sequence of the *estS* gene of *Drosophila virilis* inserted in the CaSpeR vector. The initiating translation codon underlined, the sequence encoding putative active center of esterase is designated in bold cases, the sequence of putative signal peptides is designated in italic cases, the region to which the antibodies have been obtained is designated in upper cases.

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      Length = 542     Matches/length =  50.0 percent

1   M-TQIL-LPIALLCL---FAASTLSNPLLVELPNGELRGRDNGFYYSYESIPYAEPPIDD  EstS
1   MNYVGLGLIIVLSCLWLGSNASDTDDPLLVQLPQGKLRGRDNGSYYSYESIPYAEPPTGD  Est6

56  LCLEEPRPYTERWENTFDATRPPVDCLQWSQLISQPNKLTGSEDCLTVSIYKPKNLTRIS  EstS
61  LRFEAPEPYKQKWSDIFDATKTPVACLQWDQFTPGANKLVGEEDCLTVSVYKPKNSKRNS  Est6

116 FPVVAHIFGGGWSFGAAIDDGVRPFSSSGNVIVVKTTTEWERLGFMSTGDSVIPGNFGLK  EstS
121 FPVVAHIHGGAFMFGAAWQNGHENVMREGKFILVKISYRLGPLGFVSTGDRDLPGNYGLK  Est6

176 DQRLAIKWIRNNIARFGGDPHNIILLGFSTGGSSVHLQLMHKE-YGQLVKGAISISGTAT  EstS
181 DQRLALKWIKQNIASFGGEPQNVLLVGHSAGGASVHLQ-MLREDFGQLARAAFSFRGNAL  Est6

235 -PWAVQANARDLAFRYGKLLGCNNPKNSRELKDCLKKTDAEEFVSTLRHLQVFDYVPFGP  EstS
240 DPWVIQKGARGRAFELGRNVGCESAEDSTSLKKCLKSKPASELVTAVRKFLIFSYVPFAP  Est6

295 FGPVVESPEVESPFLTELPLDTIRSGNFAQVPWLASYTPEEELNTRWNELAPYFLAYPYT  EstS
300 FSPVLEPSDAPDAIITQDPRDVIKSGKFGQVPWAVSYVTEDDLNERWLELAPYLLFYRDT  Est6

375 LKRSEMNAHSQKLKYQYLGYKNFSVVNYFDVQRLFTNELYKKGIELSLDSHRKHGASPVY  EstS
379 KTKKDMDDYSRKIKQEYIGNQRFDIESYSELQRLFTDILFKNSTQESLDLHRKYGKSPAY  Est6

435 AYVYDNPADKSLAQFLAKRSDISLGTGMGDDYYLLMNNPLR-EPLRADEKIVSWKLVKMV  EstS
439 AYVYDNPAEKGIAQVLANRTDYDFGTVHGDDYFLIFENFVRDVEMRPDEQIISRNFINML  Est6

494 EDFA--AHETLVYDDCVFPNNLGKKKFQLVVIGRNYC-KQLEVESFARHGVQ          EstS
499 ADFASSDNGSLKYGECDFKDSVGSEKFQLLAIYIDGCQNRQHVE-FP               Est6
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Fig. 2. The comparison between amino acids sequences of the esterase 6 of *Drosophila melanogaster* and the esterase S of *Drosophila virilis*.

3.2. *P-element-mediated transformation*

A 1.2 kb *EcoRI D. virilis* genomic fragment containing the region coding for the active centers of the enzyme was inserted in the CaSpeR vector (Fig. 1). This vector contains a mini gene *white*. It makes possible to screen easily the transformed flies. Earlier we have obtained the antibodies to the esterase S [15,16]. These antibodies have high affinity level to the esterase S of *Drosophila virilis* but they have also a low affinity level to the esterase 6 of *Drosophila melanogaster*.

At the next stage the transformed flies of *D. melanogaster* were selected and the enzymes from various tissues of adult males and females were prepared. The immunochemical analysis demonstrated that tissue-specific expression of the esterase S of *Drosophila virilis* occurs in bulbs of mature males. Cross-hybridization occurs also with endogenous esterase 6 of *Drosophila melanogaster*. It is worth noticing that the increase of the esterase S expression depends on the stage of development of adult males and coincides with that in *Drosophila virilis*. The expression of the esterase 6 of *Drosophila melanogaster* occurs in ejaculatory ducts. A weak signal was obtained from genitals of males, but this signal was not detected when only ejaculatory bulbs were taken for immunochemical reactions. We also have detected a low level of expression of esterase S gene though in more degraded form in genitals of females after copulation (Fig. 3). These data suggest that a part of esterase S with signal

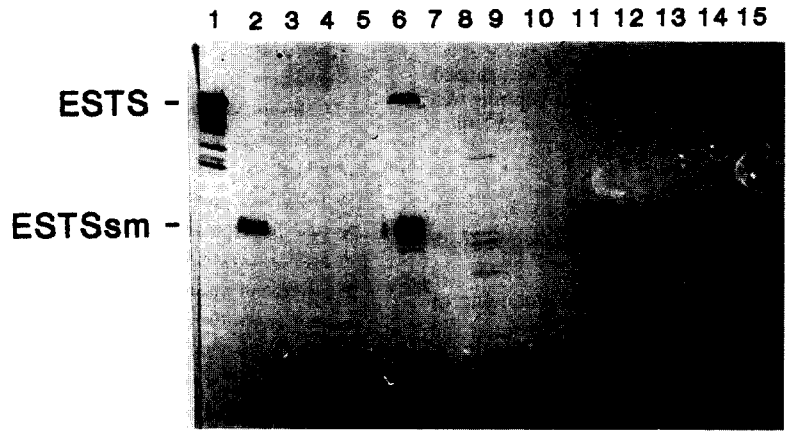

Fig. 3. This figure shows the results of immunochemical analysis of transformed flies of *Drosophila virilis*. Lanes 1,15, enzymes were isolated from ejaculatory bulbs of *Drosophila virilis*, (the lower signals are from products of degradation of esterase S); lanes 2–14, enzymes were isolated from of transformed *Drosophila melanogaster*: 2, from ejaculatory bulbs of 3 day males; 3, ejaculatory ducts; 4, paragonii; 5, testicles; 6, genitals of 4 day male; 7, the same male without genitals; 8, genitals of females upon copulation; 9, genitals of virgin females; 10, female without genitals; 11,13, genitals of different stage males; 12,14, the same males without genitals. ESTS, full length esterase S; ESTSsm, incomplete esterase S.

peptide is excreted and transferred into female genitals upon copulation. The processes of excretion and transferring of enzymes are very conservative in both species of *Drosophila*.

The most interesting and important result of our experiments is that the tissue and stage specificity of the *estS* gene of *Drosophila virilis* in transformed*Drosophila melanogaster* flies is the same as in *D. virilis*. A relatively small regulatory region of esterase S gene is responsible for tissue and stage specific activation of *estS* gene. A 396 bp fragment of the regulatory region of the *estS* gene upstream to the major transcription initiation site can switch on the *estS* gene at precisely determined time and place. These data agrees with data obtained in the in vitro transcription system. Removal of different parts in 5′ and 3′ regions of the *estS* gene shows that deletions in 5′ regulatory region of the *estS* gene up to 115 bp to the major transcription initiation site can reduce the transcription in vitro four-fold. The length of the 5′ regulatory region of the *estS* gene was very important for experiments on in vitro transcription. The DNA fragments with a 5′ region containing 830, 750, 450, 390 bp to the major transcription initiation site were also used in such experiments. The efficiency of in vitro transcription of these deletion constructions was practically the same [7,19].

Computer analysis of the region from −390 to −60 to the major transcription initiation site shows that this region contains many sequences similar to the most common enhancer elements of eukaryotic genes. Probably they determine the high efficiency of *estS* gene in vitro transcription. The region of DNA containing the *estS* gene downstream to the major transcription initiation site practically does not influence the in vitro transcription.

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