muscles (Orchard, 1982) into hemolymph (Davenport and Evans, 1984: Hirashima and Eto, 1993). Bearing in mind these data and present results (the increase of Tyr. OA, NA, and DOPA contents after 15 min of stressor action) the most likely explanation of the observed changes in the levels of biogenic amines and their precursors in *D. virilis* under stress is as follows. A sharp increase in the contents of DA and OA results from their release from depot. The increase of product content may decrease the activity of the synthesizing enzymes, tyrosine hydroxylase (TH) and tyrosine decarboxylase (TDC), via a feedback mechanism, and we have demonstrated precisely this (Rauschenbach *et al.*, 1995: Sukhanova *et al.*, 1997). A sharp decrease in TH and TDC activities may, in turn, lead to the increase of Tyr pool, and this, again, we have precisely shown (Figure 1A). An increase in Tyr content would decrease the activity of alkaline phosphatase (APh), the Tyr-controlling enzyme. Indeed, it has been demonstrated that the activity of APh decreases under stress (Sukhanova *et al.*, 1996).

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Regulation of the expression of tissue-specific esterase in *Drosophila*.

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The esterase genes family was described in *Drosophila virilis* and *D. melanogaster* (Korochkin, 1980). The deduced amino acid sequences of tissue specific esterase S *D. virilis* and esterase 6 *D. melanogaster* reveals 50% homology. The exon-intron structure of both genes is similar (Sergeev *et al.*, 1995). The *est6* gene of *D. melanogaster* has only one promoter while gene *estS* of *D. virilis* has two.

Drosophila melanogaster was transformed with the esteraseS gene from *D. virilis*. This gene is strongly activated in ejaculatory bulbs in mature males of *D. virilis*. The closely related gene from *D. melanogaster* is activated in ejaculatory ducts. A genomic copy of this gene including 400 bp of 5' regulatory region was integrated into the genome of *Drosophila melanogaster* (Figure 1). The tissue- and stage-specific expression of genomic copy of the esterase S gene integrated into the *D. melanogaster* genome is the same as in *D. virilis*. The products of the transferred genes were detected in ejaculatory bulbs of transgenic flies (Korochkin *et al.*, 1995). The results suggest that this specificity is evidently determined by the regulatory region of the esterase S gene and controlled by cis mechanism and at the transcriptional level: *estS* gene of *D. virilis* is transcriptionally active only in ejaculatory bulbs of transgenic flies (Figure 2). "Transgenic product" could be transferred from male ejaculatory bulbs of transgenic flies into female genitals upon copulation, with subsequent degradation there. The using of lacZ reporter gene shows that tissue and stage specificity depends upon a relatively small 5' regulatory region of the esterase S 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 in many places of the developing animal, but DNA upstream from this fragment inhibits this gene activity in all organs excluding the ejaculatory bulb. 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 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. Computer analysis of the region from -390 to -60 to the major transcription initiation site shows that this region contains many sequences similar to

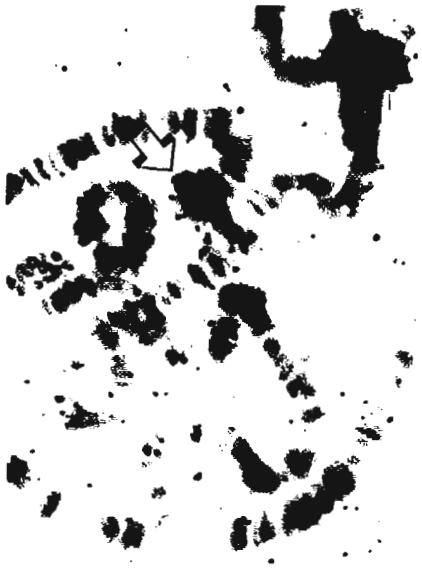

Figure 1. Cytological hybridization with polytene chromosomes from transformed flies using ^{3}H-labeled DNA probe.

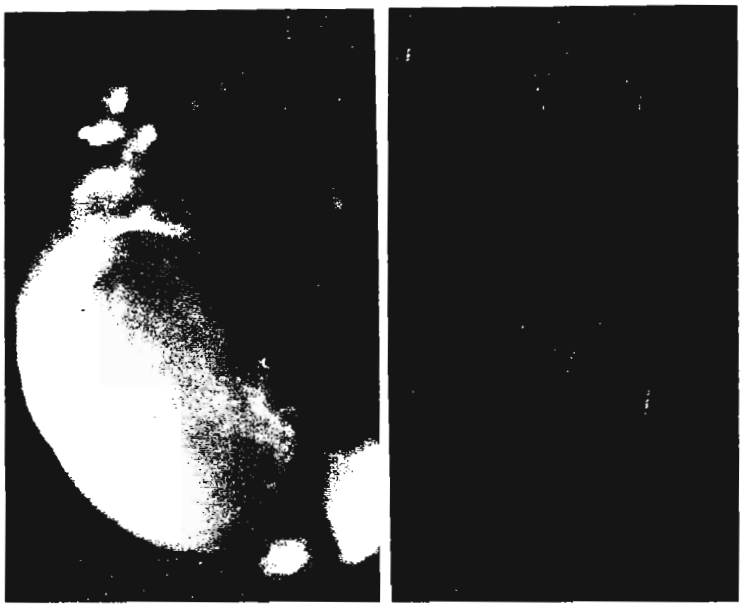

Figure 2. Results of *in situ* hybridization on total preparations of *D. melanogaster* reproductive system with DNA probe containing the *estS* gene of *D. virilis*. In transgenic animals (left) fluorescence is observed only in ejaculatory bulbs. In control (right) fluorescence is absent. Fluorescent method with digoxygenin was used.

the most common enhancer elements of eukaryotic genes. They are important for the high efficiency of *estS* gene *in vitro* transcription. We were interested in characterizing the regulatory elements of the *estS* gene of *D. virilis* which are responsible for its tissue specific expression. To localize these regulatory regions, several deletion constructs of upstream fragment of genomic copy of *estS* were made. One fragment, which includes DNA sequence from -3430 bp to +570 bp relative to the start of transcription, was subcloned first from plasmid pBR322 with previously cloned genomic sequence of *estS* into pBluescript II SK+ for making deletions. Then the obtained DNA fragments of *estS* were subcloned into pCaSper -augbgal for fly

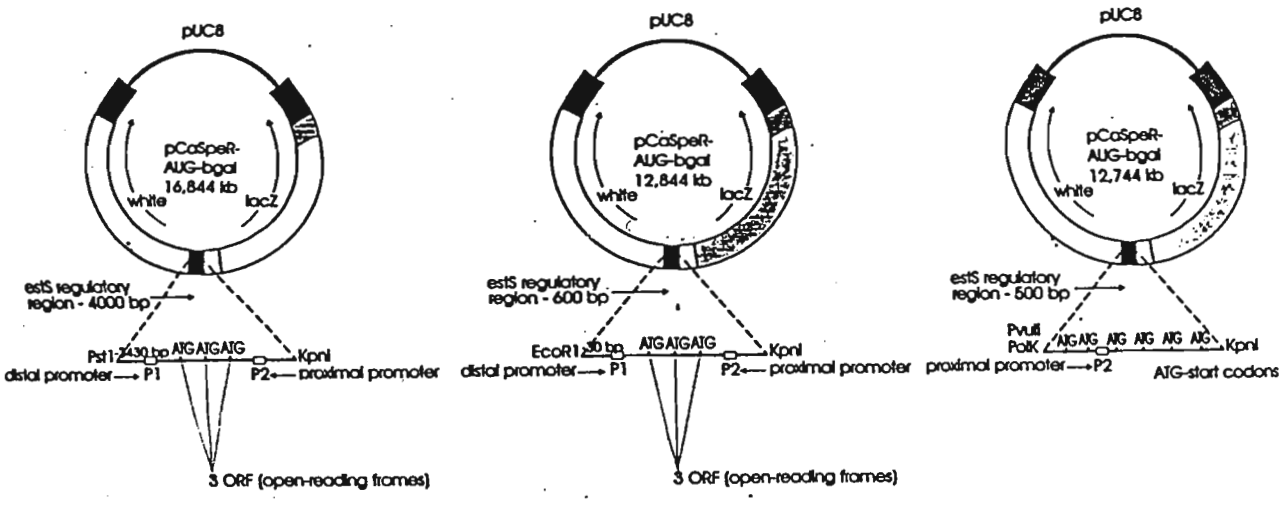

Figure 3. The constructs which were used for the analysis of the function of *estS* regulatory region.

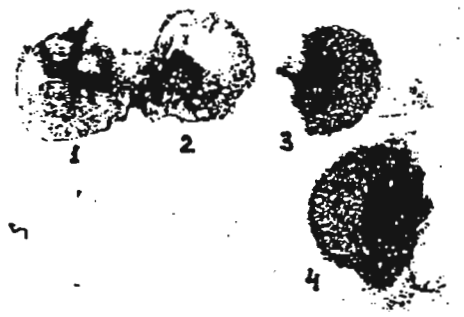

Figure 4. Ejaculatory bulbs of transformed flies. 1,2 – organs were isolated from males containing a small fragment of regulatory region; 3,4 – organs were isolated from males containing a long fragment of regulatory region.

transformation. The first construct contained 4 kb *Hind*III-*Cla*I fragment (from -3430 to +570 bp), the second one – 600 bp sequence (from -30 to +570), and the third one – 500 bp (+500 bp). The first two constructs included both presumed promoters – P1 (distal) and P2 (proximal) – with three small ORFs in between. The third construct included just P2 promoter (Figure 3). Downstream region between the P2 promoter and the *Cla*I site contained 5' universal ATG codons. All plasmid constructs were grown in *E. coli*, isolated by the usual alkaline lysis procedure, and purified by ultracentrifugation in CsCl. Five transgenic fly lines were produced with these constructs. To follow the insertion we used genetic markers *Cy*, *L*, *D*, and *Sb*. The integration of construct into the genome was confirmed first by Southern blotting, and then localized on polytene chromosomes by *in situ* hybridization with [^{3}H]-labeled DNA probe. The construct insertion was mapped to the 3rd chromosome in four transgenic fly lines, and to the X chromosome in one line. Efficiency of transformation was ~ 5% from surviving flies. To characterize specificity and activity of regulatory sequences of interest, the β-gal activity was analyzed in 20-30 flies of both sexes in three independent experiments. The whole flies were punctured with a dissecting needle in regions of the abdomen and thorax and then stained in buffer containing X-gal. The staining of flies transformed with the third construct containing only one promoter revealed β-gal expression in a wide range of organs: in esophagus, intestines, in some parts of muscles, legs, proboscis, and Malpighian tubules. However, the expression in ejaculatory bulbs was law (Figure 4). The second construct with two promoters and short upstream region has also revealed non-specific expression in many organs, though the level of expression was significantly lower than that of the third construct, and weak staining of ejaculatory bulbs was detected.

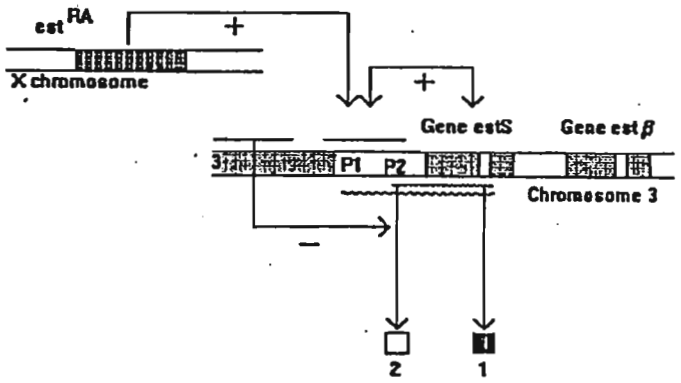

Figure 5. Scheme of the regulation of the tissue-specific expression of *estS* gene in ejaculatory bulbs of *Drosophila virilis*.

The expression level of the first construct with both promoters and big upstream region (3.4 kb) was higher in ejaculatory bulbs than the expression of the second construct (however, not very significantly). The staining of other organs in this case was substantially reduced and varied from weak to non-detectable depending on the animal (Figure 4). Transcription of gene *estS* regulating esterase enzymatic activity is initiated with P1 promoter in different organs including ejaculatory bulbs. There is a regulatory region located upstream of P1 which suppresses *estS* expression everywhere but ejaculatory bulbs. Thus, both positively and negatively regulating elements participate in determining the organ-specific pattern of *estS* gene expression (Figure 5).

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