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A relatively small 5' regulatory region of esterase S gene of *Drosophila virilis* determines the specific expression as revealed in transgenic experiments

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Expression of the esterase S gene of *Drosophila virilis* was studied in transgenic experiments. Truncated genomic copy of this gene including 400 bp of 5' regulatory region was integrated into the genome of *Drosophila melanogaster*. The products of the transferred gene were detected. It was found that strict temporal and tissue specificity of the esterase S gene expression is conserved in transformed flies. The results suggest that this specificity is evidently determined by the regulatory region of the esterase S gene and controlled by *cis* mechanism.

The gene of esterase S *(estS)* of *D. virilis* encodes the enzyme (carboxylic-ester hydrolase) involved in the processes of female fertilization [1-3]. Gene has previously been genetically isolated, cloned and sequenced in our laboratory. We have also described some features of the *estS* expression in *Drosophila virilis* [3-5]. This gene possesses strict stage and tissue specific expression [1, 2]. *EstS* has

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Abbreviation: *estS*, esterase S gene.

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two promoters which evidently determine the complex mechanism of gene regulation [4]. Thus an investigation of the mechanisms underlying *estS* expression can shed light upon an important problem of molecular genetics - how eukaryotic genes are regulated.

In the present work we conducted transgenic experiments to investigate specificity of the *estS* expression to find out whether it is determined by its own regulatory elements or solely depends on *trans*-activation. An example of the latter way of gene regulation is found in *trans*-activation of glutathione transferase P gene during chemical hepatocarcinogenesis of rat revealed by transgenic experiments [6]. The transformation approach was previously used for investigation of regulatory elements of esterase 6 gene of *D. melanogaster* [7]. The gene, a homologue of *estS* of *D. virilis*, was found to have about 1 kb 5' upstream region containing putative regulatory sequences which determine specific expression of the gene. But the mechanism of this regulation is still vague. It is worth noting that *estS* and esterase 6 gene have different spatial, temporal, and sex-specific patterns of expression [3, 8].

In this communication, we describe an experiment with transgenic flies which allowed us to identify the regulatory region responsible for specificity of *estS* expression and propose a *cis*-mechanism the gene regulation.

MATERIALS AND METHODS

Transgenic construct

For P-element transformation we used CaSpeR vector [9] containing a 4.1 kb fragment of gene *white* as a genetic marker. Part of the genomic *estS* copy (~1.3 kb) was inserted into the vector using Eco RI - Xbal restriction sites. This *estS* fragment includes the part of coding sequence (~900 bp) with the region corresponding to the enzyme active center (197-213 position of amino acid sequence of esterase S). The plasmid construct (designated as TG7) also contains about 400 bp of an upstream regulatory region of *estS* with distal and proximal promoters. The directions of *estS* and *white* gene transcription coincide (Fig. 1).

Microinjections

An isolated plasmid with genetic construct was purified by equilibrium centrifugation in CsCl-etidium bromide gradients (45,000

rpm for 48 hours in a Beckman Ti65 rotor [10]). Microinjections of the purified plasmid into embryos of Df(1) line of *Drosophila melanogaster* were carried out by standard method [11]. Second generation flies were taken for immunochemical analysis.

Genetic procedures

Flies surviving after microinjections were individually crossed with the $y^{+}w$;Cy/L;D/Sb flies. With fertile flies, the efficiency of transformation was 44.6%. Transformed flies were identified by phenotypic manifestation of marker gene *white* in the F1 generation [9].

DNA hybridization

To localize the positions of transgenic construct insertion, cytological hybridization with polytene chromosomes was performed. For this purpose a part of TG7 construct consisting of *estS-white* fragment was used as a probe. The probe labeled with tritium or biotin-dUTP was synthesized by nick translation procedure [10, 12]. Identification of the insertion positions was carried out after hybridization by autoradiography and light microscopy.

The *estS* fragment of TG7 was taken for the synthesis of a digoxigenin-labeled probe by random-primed reaction [10, 12]. Digoxigenin-fluorescein hybridization and detection assay [12] was used for *in situ* hybridization with total preparations of the genital system. We also used at this stage a luminescent microscope ML-2 (LOMO, Russia).

Analysis of gene expression

Enzyme synthesis was assayed with antibodies to the esterase S enzyme. The production of the antibodies was previously described in [13]. Tissues were hand dissected, rinsed in homogenization buffer, then ground and loaded onto gels. Analysis of enzyme products of the injected construct was carried out by electrophoresis in 15% polyacrylamide gel in the presence of 1% SDS [14]. Thus separated enzymes were transferred onto nitrocellulose filters, then the filters were treated with 3% BSA (Sigma) in PBS for 3 hours. Exposure to antibodies to esterase S was carried out in 3% BSA, 0,1% Tween 20, PBS, pH 7.4, for 3 hours. Washing was performed in 0.1% Tween PBS. Further procedures were as in [14].

RESULTS AND DISCUSSION

Previously we found that homologous genes coding for tissue-specific esterase have distinct expression patterns in the genital systems of male flies of different species within the *Drosophila* genus [3]: the gene of esterase S is expressed in ejaculatory bulbs in some

species of *Drosophila virilis;* gene esterase 6 of *Drosophila melanogaster* (species *D. melanogaster, D. similans, D. mauritiana, D. sechelia*) is expressed in ejaculatory ducts; this gene is practically not expressed in the genital systems of male flies of *Drosophila yakuba* (species *D. yakuba, D. teissieri, D. orena, D. erecta*), nor in the genital systems of *D. obscura* and *D. pseudoobscura.* Gene *estS* is expressed in an almost fully non-specific manner in *Drosophila hydei* (*D. hydei, D. eohydei, D. neohydei, D. nigrohydei*); the product of this gene is found in different organs of male and female flies, as well as in larvae. These findings give rise to the question whether the peculiarities of esterase S gene expressions result only from the structure of a regulatory gene sequence and are governed by *cis*-mechanism, or whether they are fully determined by a *trans*-acting factor and, in this case, the gene has the expression pattern dictated by its genotypic environment.

To answer this question, plasmid TG7 containing *estS* from *D. virilis* (Fig.1) was microinjected into *D. melanogaster* embryos. The transgenic flies of *D. melanogaster* with this genetic construct inserted into their genome were obtained.

The insertions were revealed by *in situ* hybridization with polytene chromosomes using a biotin or tritium labeled DNA probe (Fig. 2). The sites of injected DNA integration were found to be typically localized on the second chromosome, and the number of insertions varied between 1 and 5 (Table 1). There was no correlation detected

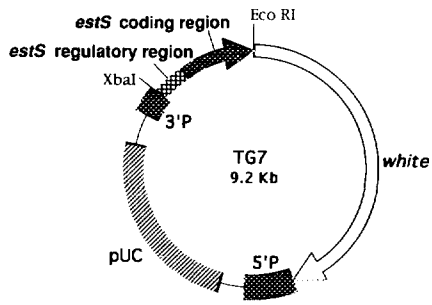

Fig. 1. Schematic map of construct TG7 used for transformation of *Drosophila melanogaster.* Arrows indicate the direction of gene transcription.

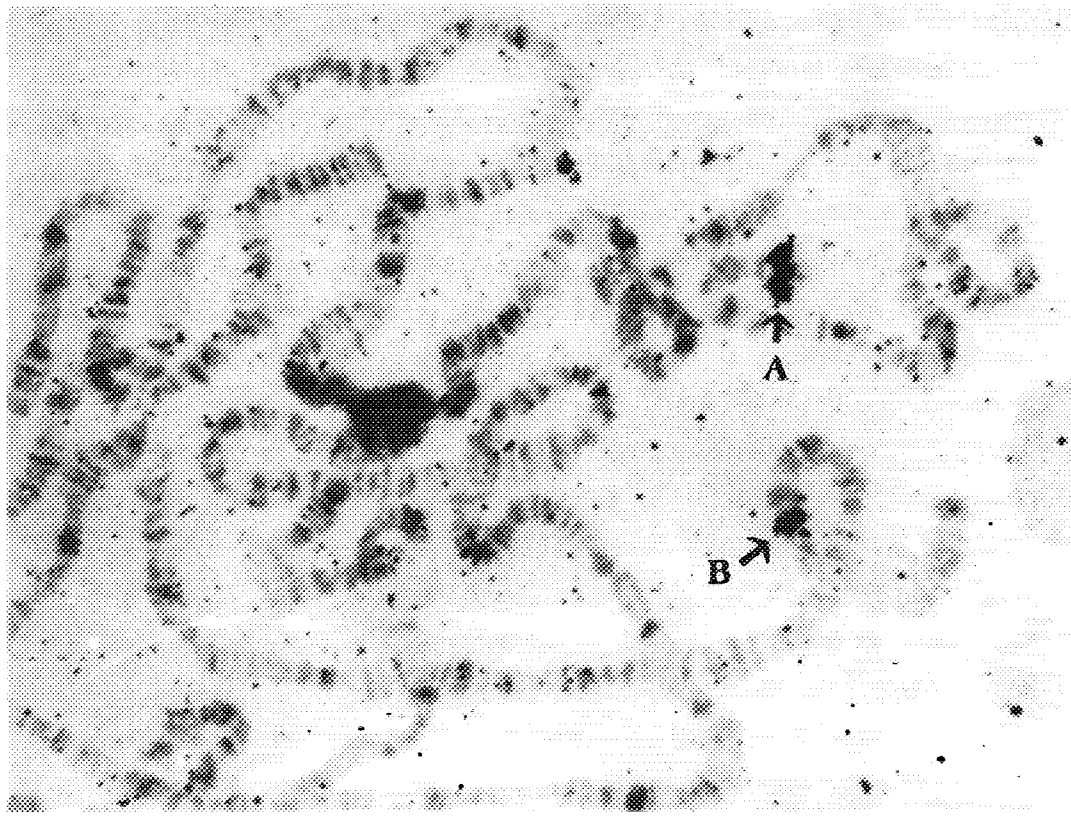

Fig. 2. Cytological hybridization with polytene chromosomes from transformed flies using ^{3}H -labeled DNA probe. Arrows indicate the chromosomal localization of: **A** - *estS* construct insertion (2R-41B); **B** - gene *white* (X-3C).

between the number of construct insertions and eye color (which depends on the level of gene *white* expression) or the level of *estS* expression (see below). Hence the functional activity of integrated

Table 1. Chromosomal localization of *estS* construction insertions in the genome of transformed lines of *D. melanogaster* revealed by cytological hybridization using ^{3}H-labeled (★) or biotin-labeled (☆) probe

Lines	Chromosomal localization										
	2L	2R						3L		3R	
	36D	41B	42D	45F	49F	55C	60A	62E	77E	88F	91C
t1		★ ☆								☆	☆
t24	★					☆		☆	★		
t12			☆	☆							
t54			★								
t56					★ ☆						
t60							☆	☆	☆		

genes is likely to be determined by the specific position of DNA insertions, rather than by the number of these insertions.

The most important observation was the fact that *estS* is transcriptionally active only in the ejaculatory bulbs of transformed male flies (Fig. 3, 4). Thus the *estS*, once integrated into genome of *D. melanogaster*, conserves the expression specificity. A number of polypeptide products were revealed by immunoblotting using specific antibodies (Fig. 4). Three polypeptide forms (about 30 kDa) were detected in two lines of transgenic flies (*t12* and *t24*).

A polypeptide product of *estS* was found in ejaculatory bulbs of transgenic male flies. No products were observed for control flies (line Df(1)). However, preparation from genitals of females after copulation showed some weak bands revealed by immunochemistry. We suppose that these bands resulted from the degradation of *estS* product transferred from male ejaculatory bulbs into female genitals upon copulation. The *estS* product was not detected in head or abdomen of flies, nor in male flies with ejaculatory bulbs removed.

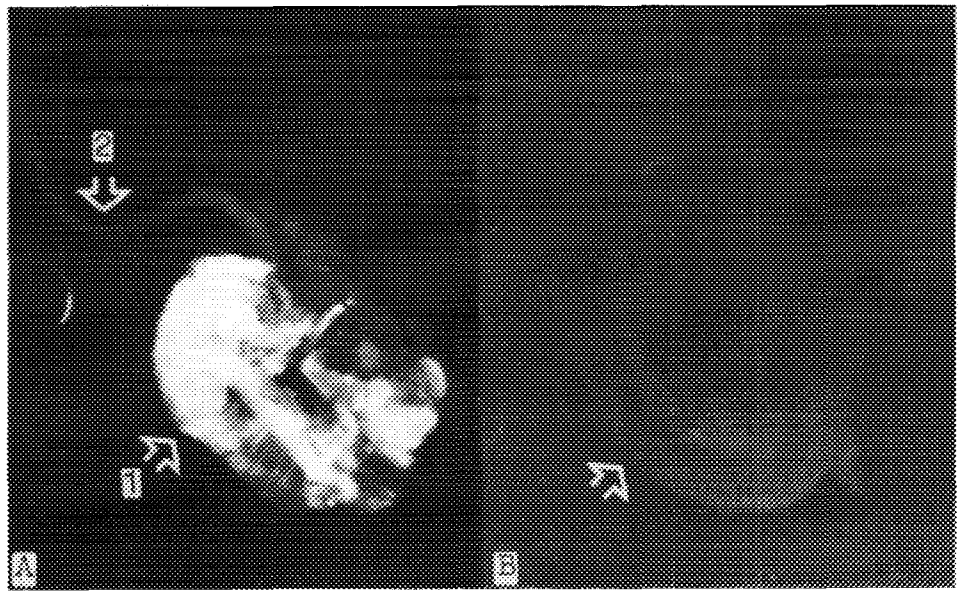

Fig. 3. Hybridization *in situ* with total preparations of the genital system of males of *D. melanogaster*. **A** - a fly from transformed stock: (1) - ejaculatory bulb, (2) - ejaculatory duct. **B** - a fly from control stock.

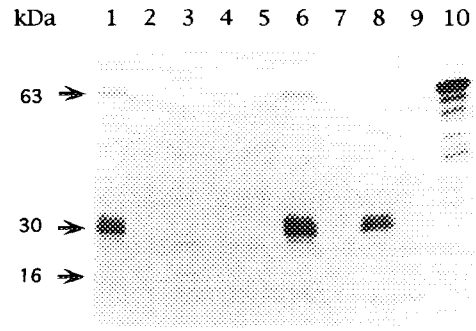

Fig. 4. Immunochemical analysis of polypeptide products isolated from *Drosophila* flies. Lines 1-9 correspond to the transformed *D. melanogaster* ; the enzymes were isolated from: (1) genitals of 4-day males; (2) 4-day males without genitals; (3) genitals of females upon copulation; (4) genitals of virgin females; (5) females without genitals; (6,8) genitals of males of different ages; (7,9) the same males without genitals. Line 10 - enzymes were isolated from the ejaculatory bulbs of *D. virilis* (the lower signals are from products of esterase S degradation). Arrows indicate the approximate molecular weights of polypeptides.

Weak immunochemical signals (corresponding to polypeptides of 15-17 kDa and about 63 kDa) were observed for male genital preparations made both from transgenic and control flies. Since such signals were detected only for adult flies (7 days after emergence), they were most probably the result of a cross immunochemical reaction with the product of esterase 6 gene of *D. melanogaster,* which reveals about a 50% homologous amino acid sequence [15]. This conclusion is supported by the fact that the intensity of these signals correlates with the activity of esterase 6. Cross reaction between the antibodies to esterase S of *D. virilis* and to esterase 6 of *D. melanogaster* has already been demonstrated [16, 17].

No correlation was observed between eye color and the intensity of the esterase product immunochemical signal for different lines of transgenic fly. Hence the transcription regulation of genes *white* and *estS* both present in the transgenic construct integrated into *D. melanogaster* genome appears to occur independently.

In summary, the results described above lead us to the following main conclusions: 1. Esterase S gene of *D. virilis* integrated along with its own regulatory region into genome of *D. melanogaster* is

transcriptionally active in a strict tissue-specific manner (only in ejaculatory bulbs starting from the third day after fly emergence); 2. The *estS* does synthesize polypeptide product in *D. melanogaster*, which could be transferred from male ejaculatory bulbs into female genitals upon copulation, with subsequent degradation there; 3. The specificity of *estS* expression is evidently determined by the upstream regulatory region of about 400 bp with *cis*-mechanism of regulation.

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