GENE STRUCTURE OF *DROSOPHILA* DIAPHORASE-1
THE DIVERSITY OF THE TRANSCRIPTS IN FEMALE AND MALE ADULT FLIES

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**ABSTRACT**
The gene DMC22E5 (ac. # CG4199) from Barkely Drosophila Genome Project (BDGP) (http://www.fruitfly.org) was found, using the partial amino acid sequences of three tryptic peptides, obtained from purified *Drosophila virilis* Diaphorase–1. This gene is located on the X chromosome at position 2C9–2C10. The structure of the gene reveals three exons and two long introns. Using Barkely Drosophila Genome Project (BDGP) we found six transcripts from this gene (ac.# CG4199RF, CG4199RC, CG4199RD, CG4199RB, CG4199RE, CG4199RA). The difference between these transcripts is in their 5’ untranslated regions (UTR) and their 3’ ends are identical. Thirty-four ESTs (expressed sequence tags) from different cDNA libraries were found in the collection of the ESTs. Most of them are from Schnaidr L2 cell culture (SH) cDNA library. The transcripts are represented at vary low level in the cells of different organs and at different stages of Drosophila development. Using RT-PCR we obtained the five of these transcripts in cDNA samples from female adult flies. We couldn’t find any of them in cDNA samples from male adult flies.

**Introduction**
It is known that the eukaryotic genes display great diversity in their structure and mechanisms of regulation. Investigation of model systems can therefore make a significant contribution toward the understanding of the genome as a whole. Study of the diaphorases [NAD(P)H: acceptor oxidoreductases; EC 1.6] in *Drosophila* as a gene-enzyme system may contribute to widening our knowledge in this sphere. Previous investigations in our laboratory have shown that these enzymes in *Drosophila* are encoded for by at least four structural genes and differ in their specificity for nicotinamide cofactors. Diaphorase-1 and diaphorase-3 can oxidize NADH as well as NADPH, while diaphorase-2' and diaphorase-2" only oxidize NADH. Genes coding didphorse-1 and diaphorase-3 were localized on the X chromosome of *Drosophila virilis* (2). Diaphorase-2’ has been identified as a dihydrolipoamide dehydrogenase with diaphorase activity (3). Diaphorase-2’’ has been identified as a xantine dehydrogenase, also showing diaphorase activity (4) and is coded by the gene *rosy*.

In the present paper we report on the gene structure in *Drosophila melanogaster* and the diversity of the transcripts in female and male adult flies.

**Materials and Methods**

**Computer analyses**
Diaphorase-1 was purified from *Drosophila virilis* (5) and partial amino acid sequences of three tryptic peptides were obtained (Petkov, P. et al. unpublished data).

The sequences of these peptides (DIA-1 pep1 - AAFGEEIGGR; DIA-1 pep2 - VNIGHYQLAQYHGR; DIA-1 pep3 -
LNTDFLANSGVR) showed similarity with the gene DMC22E5 (ac. # CG4199), that was obtained from the Barkely *Drosophila* Genome Project (BDGP) (http://www.fruitfly.org). This gene is located on the X chromosome at position 2C9-2C10, which confirms our previous results (3).

The structure of the gene reveals three exons and two long introns. Using Barkely *Drosophila* Genome Project (BDGP) we found six transcripts from this gene (ac.# CG4199RF, CG4199RC, CG4199RD, CG4199RB, CG4199RE, CG4199RA). The difference between these transcripts is in their 5’ untranslated regions (UTR). The 3’ ends of the six transcripts are identical (Fig. 1).

Four of these transcripts (ac.# CG4199RF, CG4199RC, CG4199RD, CG4199RB) have identical open reading frame (ORF) and encode one and the same protein (ac.# CG4199-PF, CG4199-PC, CG4199-PD, CG4199-PB).

The other two transcripts (ac. #CG4199RE, CG4199RA) encode two different proteins (ac. #CG4199-PE), (ac.#CG4199-PA).

cDNAs encoding these transcripts were identified in the expressed sequence tag (EST) database Barkely *Drosophila* Genome Project (BDGP). Twenty three of the EST's are from Schnaider L2 cell culture (SH) cDNA library, five – from embryo (RE and LD) cDNA libraries, four – from adult head (RH and GH) cDNA libraries, one – from testes (AT) cDNA library and one – from ovary (GM) cDNA library (Table).

**Drosopfila culture:** The adult flies of

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**Fig. 1.** Diversity in the exon-intron organization of diaphorase-1 gene. The six transcripts with their differences. Exons are shown as boxes; the numbers of nucleotides in the exons are shown above the boxes. The protein coding regions are marked with the numbers of the encoded amino acids indicated below them. The stop codons are indicated above the boxes. Introns are designated by thin lines with the numbers of nucleotides indicated above.
TABLE

D. melanogaster DIA-1 cDNAs. The six transcripts (CG4199RE, CG4199RF, CG4199RC, CG4199RD, CG4199RB, CG4199RA) and the number of ESTs for them, identified from different cDNA libraries

<table>
<thead>
<tr>
<th></th>
<th>BDGP Schneider L2 cell culture (SH)</th>
<th>BDGP embryo (LD)</th>
<th>BDGP embryo (RE)</th>
<th>BDGP head (RH)</th>
<th>BDGP head (GH)</th>
<th>BDGP ovary (GM)</th>
<th>BDGP testes (AT)</th>
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<tr>
<td>CG4199RE</td>
<td>53167, 20109</td>
<td></td>
<td></td>
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<td>22520, 05956, 22368, 15644</td>
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<td>CG4199RC</td>
<td>24769, 05213, 18368, 17018, 20904, 0219, 02121, 17830, 20386, 19004, 13127</td>
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<td>14669</td>
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<td>CG4199RA</td>
<td>23281, 37636, 05890, 26317</td>
<td>36801, 37636</td>
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<td>01986</td>
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</tr>
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</table>

*Drosophila melanogaster* were maintained on a standard corn-meal/agar medium at 25 °C (6) and the male and female adult flies were separated.

**RT-PCR.** The cDNA samples from female and male adult flies were prepared, using Oligotex mRNA Purification kit (Qiagen) and SuperScript™ II RT kit (Invitrogen).

We prepared and purified mRNA from male and female adult flies using Oligotex Direct mRNA protocol for isolation of Poly A+ mRNA from animal tissues. 150 mg material was simultaneously disrupted and homogenized using 1.0 ml OL (lysis) buffer and a rotor-stator homogenizer. 2.0 ml Buffer ODB was added to the lysate and the samples were centrifuged for 3 min at maximum speed (14 000-18 000 x g). The supernatant was transferred to a new RNase-free tube. 130 µl Oligotex Suspension was added to the samples. They were mixed thoroughly by pipetting, and were placed at 30 °C for 10 min. The Oligotex:RNA complex was pelleted by centrifugation for 5 min at maximum speed (14 000-18 000 x g). The supernatant was transferred to a new RNase-free tube. 100 µl Buffer OEB (hot 70 °C) was pipetted onto the column. The samples were centrifuged for 1 min at maximum speed. The spin column was transferred to a new RNase-free 2 ml microcentrifuge tube. 600 µl Buffer OW2 was pipetted onto the column. The samples were centrifuged for 1 min at maximum speed. The spin column was again transferred to a new RNase-free 2 ml microcentrifuge tube. 600 µl Buffer OW2 was pipetted onto the column. The samples were centrifuged for 1 min at maximum speed. The spin column was transferred to a new RNase-free 2 ml microcentrifuge tube. 100 µl Buffer OEB was added to samples and they were incubated at 70 °C for 3 min and then were placed at room temperature for 10 min. The Oligotex:mRNA complex was pelleted by centrifugation for 5 min at maximum speed (14 000-18 000 x g), and the supernatant was removed by pipetting. The pellet was resuspended in 600 µl Buffer OW1 by vortexing. The sample was pipetted onto a large spin column, which is placed in a 2 ml microcentrifuge tube. The samples were centrifuged for 1 min at maximum speed. The spin column was transferred to a new RNase-free 2 ml microcentrifuge tube. 600 µl Buffer OW2 was pipetted onto the column. The samples were centrifuged for 1 min at maximum speed. The spin column was again transferred to a new RNase-free 2 ml microcentrifuge tube. 100 µl Buffer OW2 was pipetted onto the column. The samples were centrifuged for 1 min at maximum speed. The spin column was transferred to a new RNase-free 2 ml microcentrifuge tube and 100 µl Buffer OEB (hot 70 °C) was pipetted onto the column. Pipetting 3 or 4 times the resin was resuspended. The samples were centrifuged for 1 min at maximum speed. To ensure
maximal yield we repeated this step and another 100 μl Buffer OEB was added onto the column. Pipetting 3 or 4 times the resin was resuspended. The samples were centrifuged for 1 min at maximum speed.

Using SuperScriptTM II RT (Invitrogen), we synthesized the First –Strand cDNA from purified mRNA. We used this cDNA to perform the PCR amplification using appropriate primers for the six transcripts. The reverse primer DIA.R (5’-CGGAGCTCCGTAATGGGTGC-3’) was the same for the six transcripts. We used the following forward primers: DIA.F1-2 (5’-GGCAGCTACTGGCCAGACG-3’) - for the first and the second transcript; DIA.F3 (5’-GCCTCGCATTGCATTGGC-3’) – for the third transcript; DIA.F4-5 (5’-GCATCTCGGACACGTTTGGG-3’) - for the fourth and fifth transcript; DIA.F6 (5’-GGTCTCCAGGTTCATTCCGTT-3’) - for the sixth transcript.

RT-PCR with primers (FER.3F 1 and FER.3R) for ferritin small subunit was used as quantitative control for the mRNA preparation from female and male adult flies.

The PCR amplification was performed at 94 ºC for 5 min; 30 cycles at 94 ºC for 1 min, 60 ºC for 1 min, 72 ºC for 1 min; 72 ºC for 10 min in 50 μl mixtures containing 5 μl of template cDNA. After the amplification, PCR products were separated on a 1% agarose gel. The corresponding bands were excised and purified using the Sephaglass BandPrep kit (Pharmacia Biotech Inc.). Purified PCR fragments were cloned in the pCR II vector, using the TA cloning kit (Invitrogen), and both strands were sequenced using vector- and insert-specific primers. DNA sequencing was performed by the automated sequencing facility of the Arizona Research Laboratories Division of Biotechnology, University of Arizona.

**Results and Discussion**

The information from Fig. 2 shows that the transcripts can be found in Schnaider L2 cells and are presented at vary low level in the cells of different organs and at different stages of Drosophila development.

We supposed that these six transcripts are a result of alternative splicing. Unfortunately we couldn’t find them using Northern blot hybridization (7), most probably because they are poorly represented in the cells. To resolve this problem we used RT-PCR method to find them, using samples of mRNA from female and male adult flies. The samples were prepared using Oligotex mRNA Purification kit (Qiagen) and SuperScriptTM II RT kit (Invitrogen).

We expected to receive the first and the second transcripts using forward primer

![Fig. 2. Amplified, using PCR, DNA fragments were separated on a 1%agarose gel. 1. lane-1kb. DNA ladder; 2. lane-control sample (cDNA, prepared from female adult flies with FER3.F1 and FER3.R primers); 3. lane-cDNA, prepared from female adult flies with DIA.F1-2 and DIA.R primers; 4. lane-cDNA, prepared from male adult flies with DIA.F1-2 and DIA.R primers; 5. lane-cDNA, prepared from female adult flies with DIA.F3 and DIA.R primers; 6. lane-cDNA, prepared from male adult flies with DIA.F3 and DIA.R primers; 7. lane-cDNA, prepared from female adult flies with DIA.F4-5 and DIA.R primers; 8. lane-cDNA, prepared from male adult flies with DIA.F4-5 and DIA.R primers.](image)
Results in the expected fourth and fifth transcripts. The size of the fifth and the fourth transcripts is the expected one - 652bp and 594bp respectively. We obtained them only in the cDNA sample from female adult flies (Fig. 2).

Using forward primer DIA.F5 (5'-GGTCTCCAGTTCCATTCG-3') and reverse primer DIA.R (5'-CGGAGCTCCGTAAATGGGTGC-3'), we received the expected sixth transcript with size 349bp. Again it was found only in the cDNA sample from female adult flies (Fig. 3).

The results reveal that five from the expected six transcripts are represented in female mRNA samples, but none of them in male mRNA samples. The possibility that quantitative differences in the mRNA samples result in the above mentioned differences between female and male can be excluded because the intensity of the bands obtained using forward and reverse primers (FER.3.F1 and FER.3.R) for the ferritin small subunit is one and the same in both samples (Fig. 2, Fig. 3).

These results suggest that most probably there are differences in the control of gene expression between male and female adult flies.

REFERENCES