УДК 612.014.42:612.31

GENES EXPRESSION OF CALCIUM SIGNALING MOLECULES IN SALIVARY GLANDS OF *Drosophila melanogaster* LARVAE

T. I. CHORNA[1,2], G. HASAN[1], V. V. MAN'KO[2], M. Yu. KLEVETS[2]

[1]National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India;
[2]Ivan Franko L'viv National University, Ukraine
e-mail: tanya0104@yandex.ru

Genes expression of the Itp-r-83A, Ca-P60A, olf186-F which encode inositol-1,4,5-trisphosphate receptor, endoplasmic reticulum Ca^{2+}-ATPase and Orai protein – component of the store-operated Ca^{2+}-entry respectively, was determined in salivary glands of Drosophila melanogaster larvae. For this purpose, the method of reverse transcription-polymerase chain reaction (RT-PCR) was used. Our results suggest that mentioned Ca^{2+}-transport systems play an important role in maintaining of Ca^{2+}-homeostasis in larval salivary glands of Drosophila melanogaster.

K e y w o r d s: gene expression, calcium, inositol-1,4,5-trisphosphate receptor, endoplasmic reticulum Ca^{2+}-ATPase, Orai protein, Drosophila melanogaster.

Calcium is a central player in cell physiology and is used by cells as a second messenger to control many cellular processes including muscle contraction, secretion, metabolism, neuronal excitability, cell proliferation and cell death [1]. Besides Ca^{2+} plays a key role in nuclear functions, including gene transcription and DNA replication [2]. The Ca^{2+} concentration inside cells is regulated by the simultaneous interplay of multiple counteracting processes, which can be divided into Ca^{2+} 'on' and 'off' mechanisms depending on whether they serve to increase or decrease cytosolic Ca^{2+} [3]. Different cell types express various combinations of channels, ATPases and Ca^{2+} sensors to suit their physiology. The Ca^{2+} 'on' mechanisms include channels located at the plasma membrane which regulate the supply of Ca^{2+} from the extracellular space, and channels on the endoplasmic reticulum and sarcoplasmic reticulum. An equally diverse set of 'off' mechanisms is employed by cells to remove Ca^{2+} from the cytoplasm [3]. These include Ca^{2+}-ATPases on the plasma membrane and endo(sarco)plasmic reticulum membrane, in addition to exchangers that utilize gradients of other ions to provide the energy to transport Ca^{2+} out of the cell e.g. Na^+/Ca^{2+} exchange. Mitochondria also play an important part in regulating cytosolic Ca^{2+} levels [4].

Different Ca^{2+}-transport systems (inositol 1,4,5-trisphosphate and ryanodine receptors, endoplasmic reticulum Ca^{2+}-ATPase, voltage-gated Ca^{2+}-channels, Na^+-Ca^{2+}-exchanger) have been identified in *Drosophila* adult and embryos tissues [5–9]. But the role of *Drosophila* larval salivary glands in maintaining calcium homeostasis has yet to be elucidated. As is known, salivary glands of insect larvae are easily preparable secretory organs with basic functions comparable to its mammalian counterpart. Furthermore, the availability of *Drosophila* genome sequence can be a very useful model for functional investigations. Thus, the main aim of these investigations was to use the fully available sequence information of the *Drosophila* genome project to determine which known proteins involved in calcium signalling are expressed in salivary glands by means of reverse-transcription polymerase chain reaction (RT-PCR).

Materials and Methods

The Canton-S strain was used as the wild type in this study. Flies were reared on standard cornmeal-dextrose-yeast medium at 22–25 °C.

Salivary glands were dissected under the phosphate-buffered saline (PBS) from the third instar larvae of *Drosophila melanogaster*.

Total RNA from whole larval tissues (approximately 15 larvae) and 100 salivary glands of wild type third instar larvae was extracted by standard procedures and treated with RNAse-free DNAaseI [10]. Approximately 1 μg of purified total RNA was used for reverse transcription (RT) reactions. The mixture containing RNA (1 μg/μl), 20 μM of the 3′-primer and 0.1% diethyl pyrocarbonate (DEPC) water in a total volume of 11.5 μl was heated at 70 °C for 5 minutes and added to a reverse transcription reaction containing 5×first strand buffer, 20 mM dNTPmix, 100 mM DTT, 40 unites of RNAsin and 200 unites of Moloney murine leuke-

mia virus (MMLV) reverse transcriptase (Invitrogen, USA). The RT reaction was incubated at 42 °C for 60 minutes and at 95 °C for 5 minutes. cDNA was generated using following gene specific primers:

1) *Rp49*: forward 5′-ATGACCATCCGCCCAGCATAC-3′;

reverse 5′-TTACCTCGTTCTTCTTGAGAC-3′;

2) *Itp-r-83A* (CG1063): forward 5′-GCGTGTCCGATACTTCATCAGG-3′;

reverse: 5′-GTCCATCACCAAGGGATACTCC-3′ ;

3) *olf186-F* (CG11430): forward: 5′-FWD GAGATAGCCATCCTGTGCTGG-3′;

reverse: 5′-GATGCGCCATAGTTCATGC-3’

4) *Ca-P60A* (CG3725): forward: 5′-TGGAAGACGGTCACTCGAA-3′;

reverse: 5′- GTTCCTCAAACAGGGCGAG-3′.

Gene IDs and names were obtained from Flybase (http://www.flybase.org).

Ribosomal protein 49 (*Rp49*) primers were used for internal normalization of every batch of RNA. It is known that Rp49 belongs to a family of house-keeping genes whose expression is assumed to be stable in different types of cells (Fig. 1). Polymerase chain reactions (PCRs) were performed using cDNA as a template. 25 μl reaction mixture for PCR contained: 10× assay buffer for Taq DNA polymerase, 15 mM MgCl2, 20 μM each of the 3′- and 5′-gene specific primers, 20 mM dNTPmix and 1 unit of Taq polymerase (Invitrogen, USA). Negative controls were generated by omitting reverse transcriptase of the reverse transcription and were included before setting up RT-PCRs. RT-PCRs were performed on the 96 Well Thermal Cycler, Veriti 9902 (Applied Biosystems, USA). After an initial 5 minutes at 94 °C, amplification was allowed to proceed for 30 cycles with the following regime: 94 °C for 30 s, 56 °C (for *Rp49*), 60 °C (for *Itp-r-83A* and *olf186-F*), 68 °C (for *Ca-P60A*) for 30 s, 72 °C for 30 s, then 1 cycle of 72 °C for 5 min and hold at 25 °C for 1 min. The RT-PCRs products were separated on a 1.2% agarose gel.

Results and Discussion

Recently, genome-wide RNAi screens performed in *Drosophila* cells [11] have identified a Ca^{2+}-release activated Ca^{2+}-channel (CRAC) component, *olf186-F*. It was renamed as *Drosophila* Orai (*dOrai*) [12]. Orai is involved in the positive regulation of Ca^{2+} ion transpost via store-operated Ca^{2+}-channel [11].

We carried out our experiments using both whole larval tissues and salivary glands total RNA. In this case, genes amplified DNA fragments from whole larval tissues mRNA have been shown as controls. We obtained *olf186-F* gene transcription profile of larval salivary glands mRNA (Fig. 2, lane *2*) of the same expected size (270 bp) as an *olf186-F* amplified DNA fragment from whole larval tissues mRNA (Fig. 2, lane *3*). Besides the negative control which could result from the genomic DNA contamination did not show any product (Fig. 2, lane *1*). The expression pattern of *olf186-F* suggests that Orai protein is involved in the regulation of Ca^{2+}-transport via store-operated (CRAC) channel in *Drosophila* larval salivary glands. Since the role of this protein in the regulation of exocrine secretory events has yet to be resolved, further investigations are required to understand its function in *Drosophila* larval salivary glands.

Drosophila melanogaster inositol-1,4,5-triphosphate receptor (InsP3R) gene [6] (*Itp-r-83A*) is involved in the range of biological processes such as chemosensory transduction, brain and muscle function [13], olfactory adaptation [14], normal flight behaviour [15]. As is shown in Fig. 2 (lane *4*), RT-PCR of salivary glands specific mRNA amplified fragment corresponding to the *Itp-r-83A*. Although this transcript size (180 bp) occurs to be different from whole larval tissues mRNA *Itp-r-83A* transcript size (233 bp) (Fig. 2,

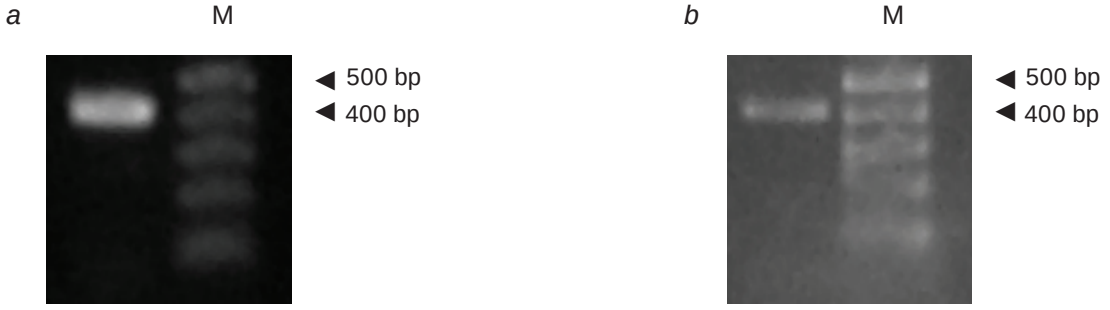

Fig. 1. Rp49 transcripts obtained by RT-PCR of RNA isolated from salivary glands (a) and whole larval tissues (b): M − marker (1 Kb + DNA ladder)

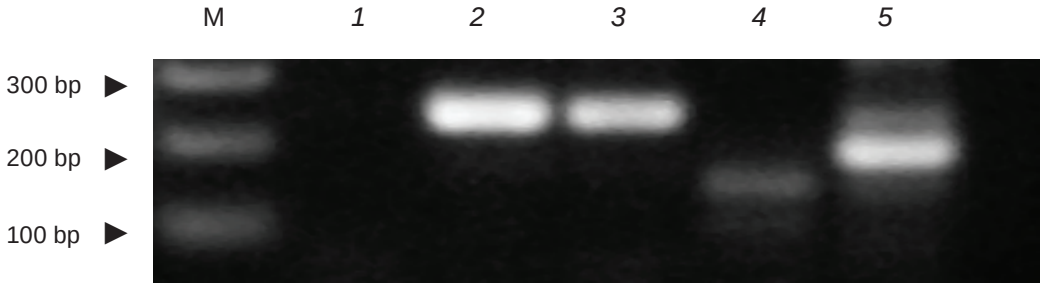

Fig. 2. olf186-F and Itp-r-83A transcripts obtained by RT-PCR of RNA isolated from salivary glands (lane 2 and lane 4 respectively) and whole larval tissues (lane 3 and lane 5 respectively): M – marker (1 Kb + DNA ladder)

lane *5*). We assume it could be due to intron regions which span the cDNA sequence. Thus, there is a salivary gland specific splice variant of *Itp-r-83A* with slightly different size from the major *Itp-r-83A* transcript in whole larval tissues.

Ca-P60A is a protein coding gene from the fruit fly *Drosophila melanogaster* and its molecular function is described as Ca^{2+}-transporting ATPase activity [16]. *Drosophila* Ca^{2+}-ATPase is highly expressed in intracellular membranes widely distributed through the cytoplasm of both neurons and muscles [17] and its expression is developmentally regulated [16]. We obtained *Ca-P60A* transcript of larval salivary glands mRNA (Fig. 3, lane *1*) which occurs to be of the same size (241 bp) as the whole larval tissues analogue (Fig. 3, lane *2*). This amplification product is unlikely to result from contamination by genomic DNA since control lacking reverse transcriptase did not show any product (Fig. 3, lane *3*). Initially, the same size *Ca-P60A* transcript was shown in adult thoraces [18].

Expression both of the *Drosophila Ca-P60A* and *Itp-r-83A* genes in larval salivary glands, observed by the RT-PCR method, suggests that, as in vertebrates [19, 20], these Ca^{2+}-transport systems also serves exocrine secretory functions. It could be possible to elucidate these functions in *Drosophila* by analyzing mutants in these genes, as well as by localizing the proteins within the larval salivary glands.

Thus we established *olf186-F*, *Itp-r-83A* and *Ca-P60A* genes expression in salivary glands of *Drosophila melanogaster* larvae. These results will give a rise to further investigations on the role of Ca^{2+}-transport systems encoded by these genes in *Drosophila* larval salivary glands functioning.

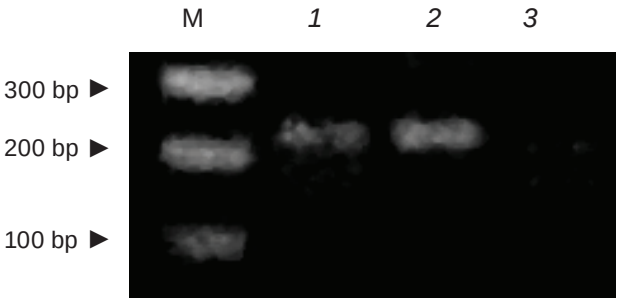

Fig. 3. Ca-P60A transcripts obtained by RT-PCR of RNA isolated from salivary glands (lane 1) and whole larval tissues (lane 2): M – marker (1 Kb + DNA ladder)

ЕКСПРЕСІЯ ГЕНІВ КАЛЬЦІЙ-СИГНАЛЬНИХ МОЛЕКУЛ У СЛИННИХ ЗАЛОЗАХ ЛИЧИНКИ *Drosophila melanogaster*

Т. І. Чорна[1,2], Г. Хасан[1], В. В. Манько[2], М. Ю. Клевець[2]

[1]Національний центр біологічних наук, Тата Інститут Фундаментальних досліджень, Бангалор, Індія;
[2]Львівський національний університет імені Івана Франка, Україна;
e-mail: tanya0104@yandex.ru

У слинних залозах личинки *Drosophila melanogaster* встановлено експресію генів *Itp-r-83A*, *Ca-P60A*, *olf186-F*, які кодують відповідно інозитол-1,4,5-трифосфатний рецептор, Ca^{2+}-АТР-азу ендоплазматичного ретикулуму та протеїн Orai – компонент депокерованого входу Ca^{2+}. Для цього використали метод зворотної транскрипції та полімеразної ланцюгової реакції. Результати свідчать про те, що вищезгадані Ca^{2+}-транспортувальні системи відіграють важливу роль у підтриманні Ca^{2+}-гомеостазу у слинних залозах личинки *Drosophila melanogaster*.

Ключові слова: експресія гена, кальцій, інозитол-1,4,5-трифосфатний рецептор, Ca^{2+}-АТР-аза ендоплазматичного ретикулуму, протеїн Orai, *Drosophila melanogaster*.

ЭКСПРЕССИЯ ГЕНОВ КАЛЬЦИЙ-СИГНАЛЬНЫХ МОЛЕКУЛ В СЛЮННЫХ ЖЕЛЕЗАХ ЛИЧИНКИ *Drosophila melanogaster*

Т. И. Чорна[1,2], Г. Хасан[1], В. В. Манько[2], М. Ю. Клевец[2]

[1]Национальный центр биологических наук, Тата Институт Фундаментальных Исследований, Бангалор, Индия;
[2]Львовский национальный университет имени Ивана Франко, Украина;
e-mail: tanya0104@yandex.ru

В слюнных железах личинки *Drosophila melanogaster* установлена экспрессия генов *Itp-r-83A*, *Ca-P60A*, *olf186-F*, которые кодируют соответственно инозитол-1,4,5-трифосфатный рецептор, Ca^{2+}-АТР-азу эндоплазматического ретикулума и протеин Orai – компонент депо-управляемого входа Ca^{2+}. Для этого использовали метод обратной транскрипции и полимеразной цепной реакции. Результаты свидетельствуют о том, что вышеупомянутые Ca^{2+}-транспортировочные системы играют важную роль в поддержании Ca^{2+}-гомеостаза в слюнных железах личинки *Drosophila melanogaster*.

Ключевые слова: экспрессия гена, кальций, инозитол-1,4,5-трифосфатный рецептор, Ca^{2+}-АТР-аза эндоплазматического ретикулума, протеин Orai, *Drosophila melanogaster*.

1. *Berridge M. J., Bootman M. D., Roderick H. L.* // Nat. Rev. Mol. Cell Biol. – 2003. – **4**, N 7. – P. 517–529.
2. *Hardingham G. E., Cruzalegui F. H., Chawla S., Bading H.* // Cell Calcium. – 1998. – **23**, N 2–3. – P. 131–134.
3. *Berridge M. J., Lipp P., Bootman M. D.* // Nat. Rev. Mol. Cell Biol. – 2000. – **1**, N 1. – P. 11–21.
4. *Bootman M. D., Collins T. J., Peppiatt C. M. et al.* // Cell & Develop. Biol. – 2001. – **12**, N 1. – P. 3–10.
5. *Sinha M., Hasan G.* // Gene. – 1999. – **233**, N 1–2. – P. 271–276.
6. *Hasan G., Rosbash M.* // Develop. – 1992. – **116**, N 4. – P. 967–975.
7. *Vazquez-Martinez O., Canedo-Merino R., Diaz-Munoz M., Riesgo-Escovar J. R.* // J. Cell Sci. – 2003. – **116**, N 12. – P. 2483–2494.
8. *Smith L. A., Wang X., Peixoto A. A. et al.* // J. Neurosci. – 1996. – **16**, N 24. – P. 7868–7879.
9. *Schwarz E. M., Benzer S.* // Proc. Natl. Acad. Sci. USA. – 1997. – **94**. – P. 10249–10254.
10. *Molecula*r Cloning / Ed. J. Sambrook., D.W. Russell. – Cold Spring Harbor Laboratory Press, 2001. – **1**. – 7.1. –8.1
11. *Feske S., Gwack Y., Prakriya M. et al.* // Nature. – 2006. – **441**. – P. 179–185.
12. *Gwack Y., Feske S., Srikanth S. et al.* // Cell Calcium. – 2007. – **42**, N 2. – P. 16232–16243.
13. *Raghu P., Hasan G.* // Develop. Biol. – 1995. – **171**, N 2. – P. 564–577.
14. *Deshpande M., Venkatesh K., Rodrigues V., Hasan G.* // J. Neurobiol. –2000. – **43**, N 3. – P. 282–288.
15. *Banerjee S., Lee J., Venkatesh K., Wu C.-F., Hasan G.* // J. Neurosci. –2004. – **24**, N 36. – P. 7869–7878.
16. *Magyar A., Bakos E., Varadi A.* // Biochem. J. – 1995. – **310**, N 3. – P. 757–763.
17. *Sanyal S., Consoulas C., Kuromi H. et al.* // Gen. – 2005. – **169**. – P. 737–750.
18. *Nair S., Agrawal N., Hasan G.* // Invert. Neurosci. – 2007. – N 7. – P. 137–147.
19. *Ashby M.C., Tepikin A. V.* // Physiol. Rev. – 2002. – **82**. – P. 701–734.
20. *Choi J.-H., Jo H., Hong J. H et al.* // Yonsei Med. J. – 2008. – **49**, N 2. – P. 311–321.

Received 10.05.2008