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Dopamine receptor type 1 of *Caenorhabditis elegans* expressing in mechanosensory neurons

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Until now the results on profiling dopamine receptors in C. elegans have been incomplete and fragmentary. The aim of this study was to investigate the expression profile of dop-1 gene in C. elegans using 3 kb promoter with 3'-end locating before ATG of dop-1 gene. Methods. The strain of C. elegans with mutant unc-119 gene was used. To check a pattern of the dop-1 expression, the promoter of this gene was amplified using PCR. The animals were co-bombarded with plasmid pPD95.77 dop-1::GFP and reporter construct containing unc-119 gene. Results. Using GFP as a reporter protein, we built a whole picture of expression of dopamine receptor type 1 in C. elegans and found that this protein could be detected only in mechanosensory neurons such as PLM, PVQR, PVQL, ALNR, ALNL, DVAR, DVC.

Keywords: dopamine receptors, mechanosensory neurons, Caenorhabditis elegans, expression.

Introduction. Dopamine is one of the most essential neurotransmitters for many animals – from nematodes to mammals. In humans it participates in the regulation of locomotor activity, thinking, emotional state, etc [1]. The disorder in metabolism of this compound may result in a series of wide-spread human diseases, including schizophrenia, Parkinson disease, and Tourette syndrome [2].

Plant parasitic nematode *C. elegans* was used as a model organism for over 20 years [3]. Recently its application has been extended to the simulation of neurodegenerative pathologies and design of medical preparations [4]. Therefore, a detailed investigation of the nervous system, including the metabolism of neuromediators and their receptors in *C. elegans*, is rather urgent.

There are four known dopamine receptors which (by analogy to human receptors) are divided into two

families: D1, including receptor type 1 (dop-1), and D2, including types 2–4 receptors (dop-2-4) [2].

Previous research on the expression profile of dopamine receptor type 1 revealed rather ambiguous results as the works demonstrated different patterns of neurons, where a reporter construction was expressed. This can be explained by uneven length and structure of promoters, used in these works [5–7]. Therefore, it was decided to conduct a more detailed analysis of expression profile of dop-1 in *C. elegans*, using 3 kb promoter with 3'-end locating before ATG of the dop-1 gene. The promoter length was selected based on the task of obtaining the most specific expression pattern, independent from other DNA regulating elements, which may be located in longer promoters and cause the expression of the reporter protein in non-target neurons.

Materials and Methods. Strains. The strain *C. elegans* with a mutant *unc-119* gene, leading to almost complete paralysis of animals, was used. The strain was obtained from the Genetic Center of *Caenorhabditis*

(USA). The cultivation was conducted on Petri dishes with NGM-agar. The nutrient medium was *Escherichia coli* bacteria of OP50 strain (OP-50).

Genetic constructions. To check the expression pattern of *dop-1*, 3 kb promoter of this gene was amplified using a protocol of single worm polymerase chain reaction (PCR) [8]. Then the promoter of *dop-1* gene was built into *pPD95.77* (*Add-gene*, USA) at restriction sites *HindIII* and *Cfr9I* to obtain the construction *pPD95.77dop-1::GFP* (Fig.1).

Gene bombardment of *C. elegans*. Animal cells were co-bombarded with plasmid *pPD95.77dop-1::GFP* and reporter construction *pRH21Unc119* (Prof. A. Fire's lab, USA) with *unc-119* gene, promoting restoration of the wild phenotype. The bombardment was

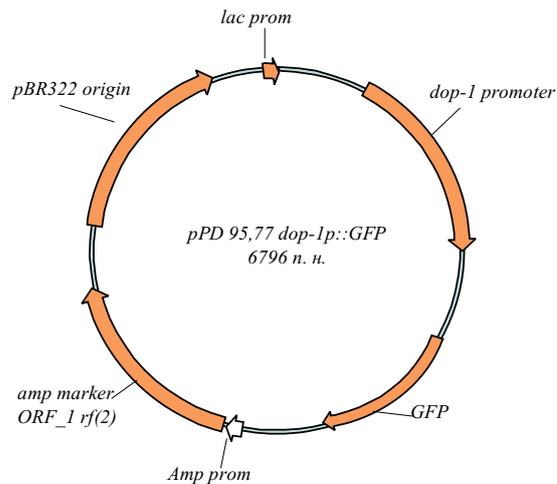


Fig. 1. Plasmid *pPD95.77dop-1p::GFP*

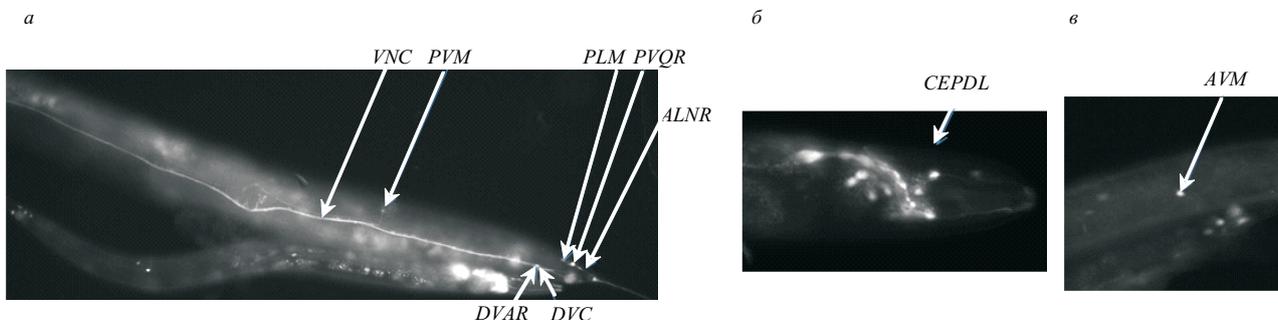


Fig. 2. *dop-1::GFP* expression in neurons *C. elegans* (a – tail section; б – head section; в – midsection). *VNC* – ventricle nerve trunk

performed using PDS-1000/He system (*Biorad*, USA) with the pressure of 9.3 MPa and 711 mm Hg in the chamber.

The delivery of DNA was performed using gold nanoparticles, covered with linearized plasmid DNA in 3:1 ratio by a standard method [9]. Two days prior to bombardment the *C. elegans* culture was synchronized, and 1 hour prior to the experiment it was transferred to dishes without bacteria. After bombardment the culture was incubated for 30 min at room temperature and sown to dishes with NGM agar and OP50.

The screening was started 3 days after bombardment and continued for 10 days using light and confocal microscopes.

Results and Discussion. The visualization of GFP expression under the control of the promoter of *dop-1* gene revealed that the expression of the target protein is mainly registered in the neurons of the head and tail

(Fig.2, a, b). In the tail of the experimental animals GFP was revealed in neurons PLM, PVQR, PVQL, ALNR, ALNL, DVAR, DVC (Fig.2, a). In the head of animals the fluorescence of GFP was detected in neurons CEPDL and CEPDR as well as in a numerous group of cells, surrounding the nervous ring (Fig.2, b), belonging to the mechanosensory neurons, accountable for the response to soft touches. Besides the cranial and caudal compartments, GFP was expressed in mechanosensory neurons PVM and AVM, located in the medium part of *C. elegans* body (Fig.2, a, c).

As GFP expression is registered only in the mechanosensory neurons it can be concluded that the dopamine receptor type 1 should determine the response to mechanic stimuli. This is indicated by the previously obtained results of changes in the reactions of animals with the *dop-1* gene knockout to mechanic stimuli. However, it is surprising that all the above-

mentioned neurons are not post-synaptic regarding dopaminergic cells of *C. elegans* which may serve as another confirmation of the hypothesis on the neuroendocrine effect of dopamine in the nervous system.

Conclusions. The expression profile of the 3 kb promoter region of dopamine receptor type 1 of *C. elegans* nematode was characterized using the expression of reporter *gfp* gene, which allows employing these results in future elaboration of new approaches to search for the means of treating diseases, related to impairment of the dopaminergic neurotransmission system.

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Ген дофамінового рецептора першого типу експресується в механосенсорних нейронах *Caenorhabditis elegans*

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Резюме

Результати досліджень експресії дофамінових рецепторів в *C. elegans* наразі залишаються неповними і фрагментарними. Мета роботи полягала у вивченні профілю експресії дофамінового рецептора типу 1 (*dor-1*) в *C. elegans* з використанням промотору розміром 3 тис. п. н., 3'-кінцева послідовність якого розташована перед кодоном АТГ гена *dor-1*. **Методи.** Культивування штаму *C. elegans*, мутантного за геном *unc-119*, з агаром NGM. Для перевірки паттерну експресії *dor-1* ампліфіковано промотор гена методом ПЛР. Клітини тварин кобомбардували плазмідною рPD95.77*dor-1::GFP* та репортерною конструкцією з геном *unc-119*. **Результати.** Із застосуванням GFP як репортерного білка вдалося повністю охарактеризувати профіль експресії дофамінового рецептора першого типу в *C. elegans*. **Висновки.** Продемонстровано, що даний білок експресується в механосенсорних нейронах, таких як PLM, PVQR, PVQL, ALNR, ALNL, DVAR і DVC.

Ключові слова: дофамінові рецептори, механосенсорні нейрони, *Caenorhabditis elegans*, експресія.

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Резюме

До настоящего времени результаты исследований экспрессии дофаминовых рецепторов в *C. elegans* остаются неполными и фрагментарными. Цель работы состояла в изучении профиля экспрессии дофаминового рецептора первого типа (*dor-1*) в *C. elegans* с использованием промотора размером 3 тыс. п. н., 3'-концевая последовательность которого расположена перед кодоном АТГ гена *dor-1*. **Методы.** Культивирование штамма *C. elegans*, мутантного по гену *unc-119*, с агаром NGM. Для проверки паттерна экспрессии *dor-1* амплифицировали промотор гена методом ПЛР. Клетки животных кобомбардировали плазмидой рPD95.77*dor-1::GFP* и репортерной конструкцией с геном *unc-119*. **Результаты.** С использованием GFP в качестве репортерного белка удалось полностью охарактеризовать профиль экспрессии дофаминового рецептора первого типа в *C. elegans*. **Выводы.** Продемонстрировано, что данный белок детектируется в механосенсорных нейронах, таких как PLM, PVQR, PVQL, ALNR, ALNL, DVAR и DVC.

Ключевые слова: дофаминовые рецепторы, механосенсорные нейроны, *Caenorhabditis elegans*, экспрессия.

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