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MUTAGENESIS TESTING USING THE *LacZ* REPORTER ACTIVITY OF THE REPARATION GENE *mus209* IN *DROSOPHILA MELANOGASTER*

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We studied a set of Drosophila melanogaster strains that could be potentially suitable for testing a variety of mutagenic factors. Their genomes contained insertions of the enhancer trap P{lacW} in which the activity of the LacZ reporter is under the control of the reparation genes' regulatory region. We demonstrated that the beta-galactosidase reporter, which is encoded by insertion of P{lacW} element in the gene mus209, is induced by irradiation in the cells of the salivary glands and wing imaginal discs. Despite the fact that the reporting coloration is not associated with the dose of radiation treatment, we found that the induction threshold of the reporter is different for these tissues. Thus, coloration in salivary glands is detectable after the dose of 200 rad and above, whereas the imaginal discs get colored with 500 rad and above. Thereby, multiple thresholds for induction of the reporter in the various tissues allow approximating the received dose.

Key words: *mutagenic factors, D. melanogaster, LacZ reporter, radiation.*

Introduction. Testing of various substances for possible mutagenic effect was performed within the U.S. National Toxicology Program (http://ntp.niehs.nih.gov).

Under this program, the following tests were conducted: Ames test [1], for mouse lymphoma cells L5178Y which detects the amount of mutations arising from the action of the mutagen in the locus of resistance to trifluorothymidine (direct result of mutations in the thymidine kinase locus) [2], cytogenetic methods of recording sister chromatid exchanges and chromosomal aberrations in Chinese hamster cells [3]. Genetic tests *in vivo* (when the whole animal is subject to action of the mutagen) commonly use: counting the micronuclei in the bone marrow of mice and rats [4], the test for sister chromatid exchanges and chromosomal aberrations in bone marrow of mice [5]. This program involves Meller-5 test of sex-linked lethals [6] and test for reciprocal translocations in *Drosophila melanogaster* [7]. Although the test for the induction frequency of mitotic crossing-over events, that are recorded as somatic *mwh* clones, is not recommended for use particularly in this program [8], it is used by researchers quite often. The drawback of these test systems is that organization of a well-equipped laboratory could be a serious challenge in order to properly set these tests, and most importantly – the following analysis would take many days.

In order to test the presence as well as the environmental toxicity of a substance a luciferase test is proposed, where a luminescence reduction of the enzyme luciferase is associated with the response to environmental pollution [9]. This approach is based on the fact of physical interaction of the enzyme with a contaminating agent, that's being said the isolation of this enzyme is required for this assay, which may overall limit the reproduction of the method. The method, however, is fast (takes a few hours), but is not suitable for mutagenic assessment, thus the idea of using a fluorescent reporter *in situ* looks interesting. Recent studies report an established human cell line that expresses a GFP reporter under the control of the promoter of the DNA reparation gene in response to irradiation [10]. Our results show that the development of high-quality *in situ* biosensors for the detection of mutagenic presence is perspective and needs further investigations [9].

In this paper we studied a set of DNA repairing genes in different tissues of *D. melanogaster*, that could be potentially suitable for testing of various mutagenic factors.

Materials and Methods. The following stocks of *Drosophila melanogaster* were used in our experiment:

10574, «y[1] w[67c23]; P{w[+mC]=lacW}mei-W68[k05603] par-1[k05603]/CyO»
11192, «cn[1] P{ry[+t7.2]=PZ}PCNA[02448]/CyO; ry[506]»

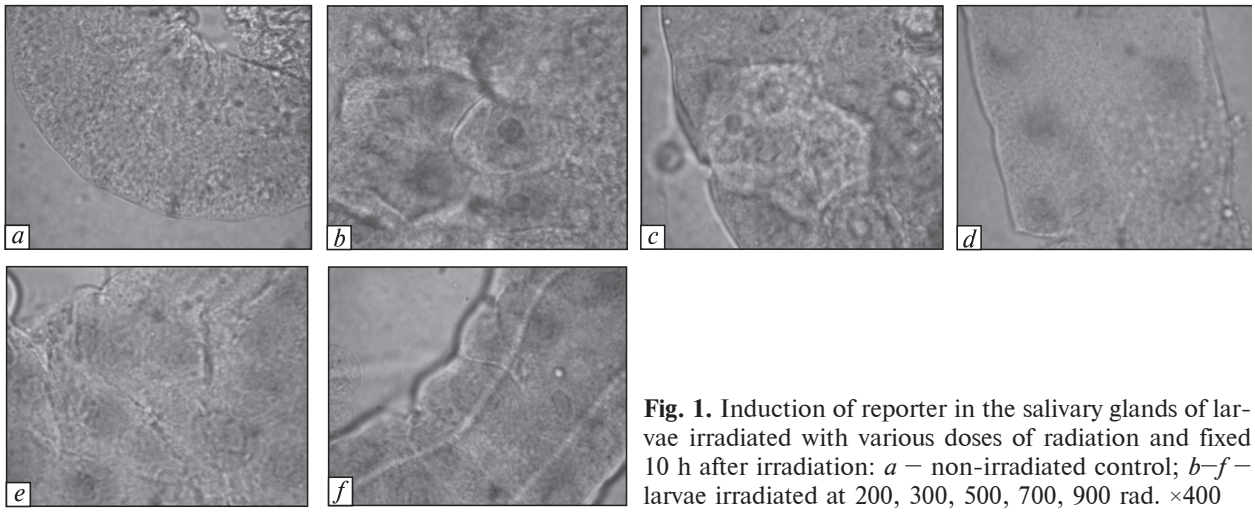

Fig. 1. Induction of reporter in the salivary glands of larvae irradiated with various doses of radiation and fixed 10 h after irradiation: *a* – non-irradiated control; *b–f* – larvae irradiated at 200, 300, 500, 700, 900 rad. ×400

13285, «y[1] P{y[+t7.7]=Mae-UAS.6.11}mus81 [GG01201] w[67c23]»
10361, «y[1] w[67c23]; P{w[+mC]=lacW}PCNA [k00704]/CyO»

The flies had been obtained in advance from the stock center in Bloomington. Irradiation of larvae was performed using the «RUM-17» emitting X-rays with the energy of 180 keV for 5–35 min. We treated the third instar of the fruit fly larvae. Histochemical stain for LacZ reporter activity was carried out as follows: the organs of larvae were isolated in Hank's solution and incubated in the 0.75 % glutaraldehyde latch, which was prepared using 0.1 M sodium cacodylate buffer. The organs had been washed in PBS (130 mM NaCl, 7 mM $Na_2HPO_4 \cdot H_2O$, 3 mM $NaH_2PO_4 \cdot 2H_2O$, pH 7,0). After that they were placed in staining solution (10 mM NaCl, 1 mM $MgCl_2 \cdot 6H_2O$, 0.3 % Triton X-100, 3.1 mM K4 [FeII (CN6)]) 3.1 mM K3 [FeIII (CN6)], 2 % X-gal).

The photography was performed on a microscope Leica DM 2000 with ×200 and ×400 magnification using the camera Canon PowerShot G5 with the software RemoteCapture 2.7.4R2.

Results and Discussion. The studied strains particularly contained the inserted enhancer trap P {lacW}. This insertion allowed us to control the regulatory region of the lac gene the activity of the histochemical reporter LacZ is, where the above insertion has occurred. The trial experiments were performed: the larvae were irradiated at a dose of 100 rad, following that a histochemical staining of the bodies was administered after 2 h of incubation. The strains #13285 and #11192 did not contain the LacZ reporter and was used as a control – to monitor the coloring level of the endogenous beta-galactosidase before and after irradiation in various organs of Drosophila larvae. Our experiments showed that the strain #10574 (without PCNA, but contained LacW) yielded a strong coloration of the irradiated specimens as well as in non-irradiated control. Thus we concluded that those lineages (#10574, #11192, #13285) were not suitable for mutagenic reporting. In further experiments was used strain #10361. Were carried out irradiation of larvae at a dose of 100 rad, fixing and staining the organs at 20, 60, 120 min after the treatment. We detected a weak expression of LacZ in the salivary glands after 120 min of incubation between irradiation and fixation in comparison with unirradiated controls.

Proliferating cell nuclear antigen (PCNA) is a DNA clamp that acts as a processivity factor for DNA polymerase δ in eukaryotic cells and is essential for replication. PCNA is a homotrimer and achieves its processivity by encircling the DNA, where it acts as a scaffold to recruit proteins involved in DNA replication, DNA repair, chromatin remodeling and epigenetics [11, 12].

Additionally, the irradiation of larvae was conducted at a dose of 100 rad, following that we performed fixing and staining of the salivary glands after 2, 4, 6, 10, 24 h. In the case of 24 h of incubation we observed a good signal in the cells nuclei. A repeated exposure to a dose of 200 rad and 2 h of incubation also showed good coloration of cell nuclei in the salivary glands.

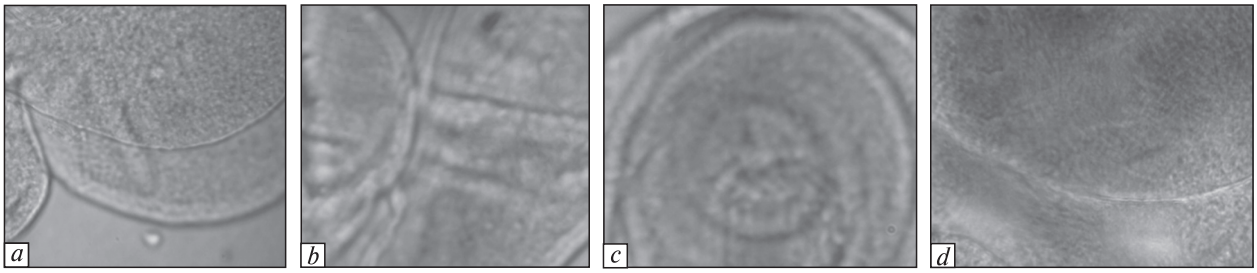

Fig. 2. Reporter induction in the wing imaginal discs of the larvae, irradiated at a dose of 300 (*a*) 500 (*b*) 700 (*c*), 900 (*d*) rad and fixed 10 h after irradiation. ×400

Our results demonstrate (Fig. 1) the dose dependence of the reporter induction in the range 200–1100 rad for the salivary glands. The degree of nuclei coloration reacting on the beta-galactosidase activity in the experiment is significantly higher than for the control.

We hypothesized that the level of the reporter expression would be directly proportional to the acquired dose. Yet from the Fig. 1 the proportionality of color intensity to the radiation dose is not obvious. An attempt to quantitatively analyze the coloration intensity via software also showed no linear relationship. This means that the salivary gland of the larvae, the insertion of the considered carriers, can be used only for qualitative assessment of the presence or absence of a mutagenic effect, starting from a dose of 200 rad or equivalent.

Fig. 2 shows the results of detection of the reporter activity in the wing imaginal discs of irradiated larvae after different doses of X-rays and being fixed after 10 h interval. It can be seen that a dose of 300 rad does not cause the reporter induction (as well as no coloration in the nonirradiated control) but irradiation at doses of 500, 700 and 900 rad causing visible changes in the activity of the reporter. Just like in the case of the salivary glands, the correlation of the color intensity to the irradiation is not obvious. However, there might be qualitative conclusion that irradiation had occurred at a dose of 500 rads or more.

Inducibility of the repair systems in response to irradiation is well known [13]. In this paper, we used the insertions of the *P{lacW}* element in the gene *mus209*, in which a beta-galactosidase reporter is induced by irradiation. Tht induction of reporter occured in both salivary glands and imaginal disc cells. Despite the fact that the correlation of the response to the treatment did not take place, the thresholds for the reporter responding were found different for these two tissues. Thus, the coloration in the salivary glands cells becomes apparent starting from the dose of 200 rad, whereas the cells from the wing imaginal discs respond after treating with 500 rad and above. Thus, multiple thresholds for induction of the reporter in the various tissues potentially allow to assess dose.

Dose of 500 rad numerically equals to the dispersion of 50 000 ergs per 1 g. Assuming that the cell density is close to the density of water ($-1 g/cm^3$), core diameter is 3 μm, and it is sufficiently ionized with 0.5 electron volts, it can be calculated that the visually detectable induction effect of *mus209* is observed when the nucleus encounters around $6 \cdot 10^4$ ionization events. This is a significantly large quantity.

Further development of assays for detecting the biological mutagenesis in *Drosophila* requires application of the methods aiming to enhance the expression of reporters. One such method would be to use the GFP-based reporter. Another option is to use insertions of *P{GawB}* into reparation genes and UAS-Gal4 enhancer of the reporter. We have had two insertions in gene *mus209*, only one of them turned out to be useful for research. The use of a wider range of insertions would possible be a better approach towards more sensitive biomarkers, compared with that we found.

ИСПОЛЬЗОВАНИЕ *LacZ* РЕПОРТЕРА АКТИВНОСТИ ГЕНА РЕПАРАЦИИ *mus209* В КАЧЕСТВЕ ТЕСТА НА МУТАГЕНЕЗ У *DROSOPHILA MELANOGASTER*

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Исследован ряд линий *D. melanogaster*, потенциально пригодных для тестирования различных факторов на их мутагенность. Линии содержат в своих геномах встройки энхансерной ловушки *P{lacW}*, в которой активность гистохимического репортера

LacZ находится под контролем регуляторной зоны генов репарации. Продемонстрировано, что репортерная бета-галактозидаза, кодируемая встройкой *P{lacW}* элемента в ген *mus209*, индуцируется облучением в клетках слюнных желез и имагинальных дисков. Несмотря на то, что пропорциональность степени окрашивания дозе облучения не установлена, оказалось, что пороги индукции репортера в этих тканях различаются. Так, окраска в клетках слюнных желез заметна, начиная с дозы 200 рад, в то время как в имагинальных дисках – с 500 рад. Таким образом, различные пороги индукции репортера в разных тканях позволяют оценивать дозу облучения.

ВИКОРИСТАННЯ *LacZ* РЕПОРТЕРА АКТИВНОСТІ ГЕНА РЕПАРАЦІЇ *mus209* ЯК ТЕСТА НА МУТАГЕНЕЗ У *DROSOPHILA MELANOGASTER*

Досліджено низку ліній *D. melanogaster*, потенційно придатних для тестування різноманітних факторів на їх мутагенність. Лінії містять конструкт, в якому активність гістохімічного репортера *LacZ* знаходиться під контролем регуляторної зони генів репарації. Продемонстровано, що репортерна бета-галактозидаза, яка є частиною вбудованого *P{lacW}* елемента в ген *mus209*, індукується опроміненням в клітинах слинних залоз та імагінальних дисків. Попри те, що пропорційність ступеня пофарбовування дозі опромінення не встановлена, виявилось, що пороги індукції репортера в цих тканинах різняться. Так, пофарбовування в клітинах слинних залоз помітно, починаючи з дози 200 рад, в той час як в імагінальних дисках – з 500 рад. Таким чином, різні порогі індукції репортера в різних тканинах дозволяють оцінити дозу опромінення.

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