ADAPTIVE RESPONSE TO ALKYLATING AGENTS
IN DROSOPHILA SEX LINKED RECESSIVE LETAL ASSAY
Nataliya Savina, Olga Dalivelya, Tatyana Kuzhir*

Institute of Genetics and Cytology, National Academy of Sciences
Akademicheskaya St., 27, Minsk, 220072, BELARUS
E-mail: antimut@biobel.bas-net.by; Kuzhir@biobel.bas-net.by

Keywords: Drosophila, germ cells, sex-linked recessive lethal (SLRL) mutation, adaptive response, ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS).

Abstract
The adaptive response to alkylating agents was studied by the sex linked recessive lethal (SLRL) test in Drosophila melanogaster under different treatment procedures revealing involvement of DNA repair and/or detoxifying pathways. Pretreatment of males as well as treatment of females with low EMS doses (0.05–0.1 mM) did not affect SLRL rates in sperm cells and decreased fly fertility. Pretreatment of males with MMS (0.1 and 1 mM) enhanced high dose mutagenesis induced by EMS at different spermatogenesis stages, potentiating effects exceeding additive ones. Physiological indices were inhibited too. Contrary to this experimental series, larval pretreatment with adaptive EMS dose (0.05 mM) resulted in high protective effect in both SLRL and fertility assays. Thus, the adaptive response to alkylating agents was found by Drosophila SLRL test under conditions indicating involvement of detoxifying mechanisms rather than DNA repair, and the latter was likely to be mediated by constitutive enzymes in Drosophila germ cells.

1. Introduction
The protective effects of low (nontoxic and non- or weakly mutagenic) dose pretreatment against high dose mutagenesis called “the adaptive response” have been studied in bacteria, plant, human and mammalian cells [1–5]. The current data in this field allow suggestion that this response is caused by a range of inducible enzymes depending on environmental factors, DNA adducts and mutagen biotransformation pathways in organism. For example, alkyltransferases are well known to be responsible for the adaptive response as well as for removal of O6-alkylguanine from DNA in bacterial and mammalian cells [5–9]. Some specific N-glycosylases seem to be involved in the cellular response under oxidative stress or ionizing irradiation [10]. Besides, the adaptive response to oxidative damage was assumed to be due to increase in expression of superoxide dismutase [11] or by induction of other endogenous antioxidant defences [12]. There is a point of view that the adaptive response to ionizing radiation or chemical mutagens in plants can be due to the process of “stimulated re-population” when accelerated cell division leads to fast elimination of injured cells imitating an “antimutagenic” effect [13, 14]. Probably, the adaptive response involves multiple protective mechanisms including non-specific ones, which can be realized at molecular (via DNA repair), intra- and extracellular levels (e.g. via systems controlling xenobiotic biotransformation or cellular population renewal).

In spite of the extensive investigations of this problem, as well as isolation and characterization of some appropriate enzymes, the adaptive response has been imperfectly studied in vivo (especially in germ cells), although it appears to play the key role in
individual fitness to environmental contamination. Therefore our objective was to study the adaptive response to alkylating agents in *Drosophila* germ cells.

2. Materials and methods

2.1 Chemicals

Monofunctional alkylating agents ethyl methanesulfonate (EMS, CAS No. 62-50-0) and methyl methanesulfonate (MMS, CAS No. 66-27-3) of Sigma production were used. The required mM concentrations were prepared by dissolving chemicals in 1% sucrose solution, which was also used as a negative control.

2.2. *Drosophila* strains

Flies of wild type (*Berlin wild*) and from the strain \(\text{In}(1) \; sc^{s1L}sc^{8R} + S, sc^{s1L}sc^{8}w^aB (\text{Basc})\) were used. In one set of experiments, males *Berlin wild* (adults or larvae) were exposed to mutagens at different doses and then mated with 3–4-day-old virgin *Basc* females to test for sex-linked recessive lethal (SLRL) mutations [15, 16]. In the other set, females *Berlin wild* were treated with low mutagen doses whereas males *Basc* were exposed to the challenger EMS dose. In the latter case, lethal mutations were determined by lack of males \(w^aB\) in \(F_2\) cultures.

2.3. Treatment procedures

Only males were exposed to challenge EMS doses (a) mainly by feeding adults according to Lewis’s and Bacher’s method [17], and (b) by larval treatment according to B. Kaya’s et al. recommendation [18]. In the absence of other procedures, this exposure corresponded to the positive control. When studying the adaptive response, three approaches were applied: (1) pretreatment of adult males with low doses of EMS or MMS; (2) larval pretreatment with a low EMS dose; (3) female treatment with low EMS doses.

In the first run, males of wild type received low mutagen doses during 12 h and immediately or 12 h after they were exposed to the challenge EMS dose (10 mM). Then males were mated individually with virgin intact females *Basc* for scoring SLRL in the \(F_2\). Five 2-day broods were made to sample germ cells treated at the different spermatogenesis stages [15, 19, 20].

In the second run, two-day-old larvae were washed and placed into vials with medium containing the adaptive EMS dose (0.05mM). After 24 h feeding, three-day-old larvae were washed and removed into vials with medium containing the challenge EMS dose (1 mM). This treatment lasted until pupation. Upon eclosion, males were sampled and mated with virgin females *Basc* in individual cultures. Besides, a part of larvae exposed to the adaptive dose was placed into vials with “clean” medium and remained there till fly eclosion. Adult males were treated with the challenge EMS dose (1 mM) and mated with females *Basc* for scoring SLRLs in the \(F_2\). Control flies were raised on “clean” medium, but larvae were twice washed, like variants of their adaptive and challenge treatments.

In the third run, two- or three-day-old virgin females of wild type were fed with EMS at low doses for 12 h whereas males *Basc* were exposed to the challenge mutagen dose (10 mM). Females were mated with males immediately after their treatment or 12 h later, i.e. there was or not an interval between “adaptive” and main treatments, like the first experimental run.
2.4. Accountable indices, and statistical analysis.

For estimation of the induced genotoxic effects, the SLRL frequencies were calculated in different experimental sets. It should be noted that mating for the F_1 was carried out strongly keeping the ratio \(1\sigma \times 5\varphi\) per culture, and progeny of every mutagenized male was analyzed individually. Simultaneously, some physiological indices were scored in the F_1. Fly sterility was determined by the frequency of cultures without offspring and fly fecundity was evaluated by the offspring quantity per culture. The SLRL frequencies in the series of adaptive treatments were compared to the negative (without chemicals) and positive (exposure to challenge doses) controls. The data obtained were computed for statistical significance by \(\chi^2\)-kriterion and Cochran-test for frequencies and t-Student test for absolute values.

3. Results

3.1. Effects of adaptive adult pretreatments on EMS-mutagenesis depending on repair processes in Drosophila germ cells

In this experimental series, both EMS and MMS were used to induce the adaptive response in *Drosophila*. Effects of low mutagen doses on high dose mutagenesis in sperm cells were studied taking into account DNA repair activity in paternal (Table 1, 2), and maternal (Table 3) germ cells.

Table 1  
Effects of male pretreatment with low EMS doses on high dose mutagenesis in sperm cells

<table>
<thead>
<tr>
<th>Exposure to EMS</th>
<th>Number of analysed</th>
<th>SLRL frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose (0,1 mM)</td>
<td>Interval between treatments, h</td>
<td>Challenger dose (10 mM), h</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>21</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>21</td>
</tr>
<tr>
<td>–</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>+</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

z (Cochran-test) = 1.73

The data in Tables 1 and 3 show that pretreatment of adults (both males and females) with low EMS doses did not affect the SLRL rates induced by high EMS doses in sperm cells, whether or not the 12-h interval was maintained between “adaptive” and challenge treatments. The analysis for physiological indices has indicated deleterious effects produced by an additional low dose treatment since fly sterility increased and their fertility appreciably decreased as compared to the positive control (Fig. 1). These tendencies were more evident under male pretreatment. Thus, low EMS doses used for adult “adaptive” treatment were inefficient in protection of males as well as in stimulation of maternal repair of primary DNA lesions induced by challenge doses in sperm cells.
Table 2

*Effects of male pretreatment with MMS on high dose EMS mutagenesis at different spermatogenesis stages*

<table>
<thead>
<tr>
<th>Exposure to mutagens</th>
<th>Brood No.</th>
<th>Number of analysed SLRL frequency, %</th>
<th>Number of analysed</th>
<th>SLRL frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>males</td>
<td>chromosomes</td>
<td>real</td>
</tr>
<tr>
<td>Negative control</td>
<td>1</td>
<td>259</td>
<td>2798</td>
<td>0.32</td>
</tr>
<tr>
<td>– – 10</td>
<td>1</td>
<td>77</td>
<td>910</td>
<td>20.11</td>
</tr>
<tr>
<td>– – 4–5</td>
<td>1</td>
<td>63</td>
<td>1158</td>
<td>8.29*</td>
</tr>
<tr>
<td>0.1 – –</td>
<td>1</td>
<td>78</td>
<td>877</td>
<td>0.57</td>
</tr>
<tr>
<td>4–5</td>
<td>72</td>
<td>1408</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>1 – –</td>
<td>1</td>
<td>75</td>
<td>945</td>
<td>5.07</td>
</tr>
<tr>
<td>4–5</td>
<td>69</td>
<td>1424</td>
<td>2.11*</td>
<td></td>
</tr>
<tr>
<td>0.1 – 10</td>
<td>1</td>
<td>78</td>
<td>796</td>
<td>25.87</td>
</tr>
<tr>
<td>4–5</td>
<td>72</td>
<td>1266</td>
<td>16.03*</td>
<td>8.69**</td>
</tr>
<tr>
<td>0.1 12 10</td>
<td>1</td>
<td>78</td>
<td>795</td>
<td>26.41</td>
</tr>
<tr>
<td>4–5</td>
<td>69</td>
<td>1198</td>
<td>17.11*</td>
<td>8.69*</td>
</tr>
<tr>
<td>1 – 10</td>
<td>1</td>
<td>77</td>
<td>813</td>
<td>31.73</td>
</tr>
<tr>
<td>4–5</td>
<td>69</td>
<td>1002</td>
<td>20.86*</td>
<td>10.38*</td>
</tr>
<tr>
<td>1 12 10</td>
<td>1</td>
<td>77</td>
<td>832</td>
<td>29.20</td>
</tr>
<tr>
<td>4–5</td>
<td>72</td>
<td>1002</td>
<td>15.17*</td>
<td>10.38*</td>
</tr>
</tbody>
</table>

P < 0.01 (by χ² criterion)

Investigation of germ cell mutagen sensitivity depending on spermatogenesis stages seemed to be more interesting and informative (Table 2). MMS at the dose of 0.1 mM was practically nongenotoxic whereas the dose 1 mM increased SLRL rates up to 5 and 2% in sperm and premeiotic cells respectively that allowed us to consider the first dose as “the adaptive” and the second dose as “the additive” treatment. It is typical that SLRL frequencies induced by EMS and MMS (1 mM) in spermatozoa were much higher than those at premeiotic stages where DNA primary lesions could be removed in the majority due to active repair processes [20–22]. Male pretreatment with MMS at both doses enhanced EMS mutagenesis. Potentiating effects greatly exceeded the additive ones being pronounced at the premeiotic spermatogenesis stages. Fig. 2 demonstrates the same tendencies for fly fertility and sterility, and contrast of all brood means revealed significant differences between offspring production resulting from additional paternal exposure to the lower MMS doses as opposed to the positive control.
Table 3

Effects of female treatment with low EMS doses on high dose mutagenesis in sperm cells

<table>
<thead>
<tr>
<th>Exposure to EMS</th>
<th>Number of analysed</th>
<th>SLRL frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose (0.1 mM)</td>
<td>Challenger dose (10 mM), h</td>
<td>males</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>0.05</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>–</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

\[ z \text{ (Cochran-test)} = 0.70 \]

Fig. 1. Effects of additional treatment of adults with low EMS doses on fly fertility

Sterility (proportion of sterile $F_1$ cultures, %) and fertility (average amounts of progeny per culture, absolute value) in the cases of “adaptive” pretreatment of males (3) and females (4) were compared to the negative control (1) as well as to the positive control, i.e. challenger EMS dose (2).
Fig. 2. Effects of additional treatment of adults with MMS on fly fertility

Sterility (proportion of sterile F\textsubscript{1} cultures, %) and fertility (average amounts of progeny per culture, absolute value) in the cases of male pretreatment with MMS 0.1 mM (4) and 1 mM (5) were compared to the positive control, i.e. the challenger EMS dose (1), as well as to MMS 0.1 mM (2) and MMS 1 mM (3), acting solely.

Thus, the used MMS doses including 0.1 mM did not induce the adaptive response against EMS mutagenesis in \textit{Drosophila} sperm and premeiotic cells, moreover SLRL rates increased and fertility decreased as compared with the challenge EMS treatment.

3.2. The adaptive response to ethyl methanesulfonate in \textit{Drosophila} larvae.

In this experimental set, EMS at the dose of 0.05 mM was used for larval pretreatment whereas the challenge dose was administered to larvae 24 h after (the positive control I) or to eclosed males (the positive control II). SLRLs were scored in sperm cells (Table 4 and Fig. 3, curve), and previously male sterility was analyzed (Fig. 3, bars). One can see that only larval treatment with EMS at the dose of 1 mM led to male sterility and the adaptive pretreatment much reduced this deleterious effect, with mutation frequencies altering in parallel. The highest SLRL rate was found in the variant of the larval treatment with the challenge EMS dose, and the adaptive pretreatment inhibited high dose mutagenesis more than 3 times. When treating adults, the challenge EMS dose induced minor mutation frequency. Against this background the protective effect of adaptive larval pretreatment was not observed. Thus, the adaptive response manifested itself under well-defined experimental conditions, namely in \textit{Drosophila} larvae.
Fig. 3. Effects of EMS on male sterility (Y1, %) and germ cell mutability (Y2, %) under different treatment procedures:

- the negative control corresponding to spontaneous endpoint frequencies (1);
- adaptive larval treatment (2);
- adaptive larval treatment and exposure of adult males to the challenger EMS dose (3);
- exposure of adult males to the challenger EMS dose, i.e. the positive control II (4);
- adaptive larval treatment and their exposure to the challenger EMS dose (5);
- larval exposure to the challenger EMS dose, i.e. the positive control I (6).

4. Discussion

The effects of low dose pretreatment on high dose mutagenesis in *Drosophila* were studied by using different treatment procedures and approaches. Our prior findings on this problem were published in 1998 – 1999 [23, 24], presented here are the results revealing possible relationships between the adaptive response and DNA repair, on the one hand, and mutagen detoxifying pathways, on the other hand.

First of all it should be mentioned, that EMS-induced SLRLs represent predominantly intra-locus endpoints [25], which do not accumulate during 14-day storage of mutagenized sperm in females in contrast to chromosome breaks [26]. Mutations were supposed to arise from alkylation at oxygen sites of DNA bases, and O\(^6\)-alkylguanine is indeed the principal lesion transformed into the predicted by A. Loveless GC-to-AT transition both *in vitro* and *in vivo* [27–30]. The close correlation between SLRL yield and O\(^6\)-alkylguanine induction in *Drosophila* was shown at first by comparing cytotoxicity, mutagenicity and clastogenicity of some monofunctional alkylating agents with their Swain-Scott factors [31, 32], and then by molecular dosimetry [33–35]. Alkyltransferases are the main enzymes repairing these DNA adducts, O\(^6\)-methylguanine-DNA methyltransferase (MGMT) being identified owing to discovery of the adaptive response in *E. coli* [1, 6, 36]. Demethylating activities have also been found in mammalian and human cells [5], and similar repair pathway seems to exist in *Drosophila*.

We would like to emphasize that germ cells essentially alter their physiological and biochemical status during development and division. There is evidence that DNA lesions induced in mature spermatozoa persist in a premutational form until egg fertilization
whereupon extremely condensed chromatin of male pronucleus gradually takes on an interphase structure and becomes accessible to maternal repair enzymes [20, 37]. The influence of mei-9L1 and mei-41D5 female phenotypes on EMS-induced chromosome breakage was shown in our previous work [38, 39]. These results along with the other findings [21, 40] corroborate the maternal repair contribution to chemical muta- and clastogenesis in Drosophila germ cells.

The mutagen sensitivity of spermatogenesis stages is determined by expression of the metabolic and repair processes [20, 22]. In general, the capacity for DNA repair is higher in premeiotic cells than in postmeiotic ones. As to EMS, a marked difference in SLRL rates between mature sperm and premeiotic cells was found to depend on DNA ethylation [25, 41]. The 5-fold reduction in SLRL frequency at premeiotic stages as opposed to spermatozoa in males of wild type [21, 39] points to involvement of error-free DNA repair in processing EMS-induced primary lesions. There is no doubt that excision repair is involved in chemical mutagenesis [42, 43]. As was proved in Drosophila germ cells, relative systems are the most efficient against DNA lesions induced by agents of the category I preferably alkylating N-sites [22]. In accordance with the proposed classification, EMS occupies an intermediate position [31] or rather belongs to this category [22]. The excision-defective mei-9L1 as well as mus(2)201D1 mutants are 2.4 – 2.0 times more sensitive to EMS than repair-proficient flies revealing the excision repair contribution, but hypermutability indices in SLRL rates for EMS are much lower than for MMS, a typical mutagen of this chemical group [21, 22]. One should not ignore mismatch repair [44], the more so as this repair pathway is quit pertinent to the cellular response to alkylating agents in mammalian cells [5, 45–48].

We proceeded from the assumption that alkyltransferases could be involved in alkylated DNA repair and provide the adaptive response in Drosophila like other organisms. However low dose pretreatments of adults were not efficient in protection against high dose mutagenesis in the experimental sets taking into account repair capacity of germ cells (Tables 2, 3). Moreover, in some cases such procedure resulted in hypermutability (Table 2) and fertility inhibition (Fig. 1, 2). It should be noted that fertility displays germ cell viability and may serve for estimation of DNA repair activity (or other protective processes favouring cell survival) as well as for the genotoxic effect correction. For example, offspring reduction corresponding to premeiotic stages principally results from cellular loss due to cytotoxicity or impossibility to overcome the meiotic barrier with chromosome aberrations. Minor portion of mutations easily penetrating through meiosis by themselves can be eliminated together with lost cells. In exactly the same way, high clastogenic effects closely associated with embryonic and postembryonic lethality permit elimination of individuals including potential mutation carriers. Hence, genotoxic effects can be diminished. The comparison of these indices and the data obtained in SLRL assay indicated the lack of the adaptive response as a result of DNA repair. Two causes seemed to underlie our failure: (1) necessary conditions were not provided, and/or (2) repair enzymes were not inducible. The latter conforms to described by Guzder et al. biochemical mechanism for repair of alkylated DNA in Drosophila [49]. The correction of O6-methylguanine was found to be due to transferring a methyl group from DNA to a protein cysteine residue. Two demethylating proteins were identified and both were reactive in adult flies and at other developmental stages (excluding embryos only). However, Drosophila alkyltransferases did not appear to be induced by sublethal exposures to alkylating agents [49]. So, mutagenesis potentiation pronounced at premeiotic stages can be explained assuming a major contribution of constitutive enzymes to DNA repair and their partial depletion in response to the first treatment of adult males with MMS. This situation closely resembles that simulated earlier in human cell cultures when pretreatment of normal fibroblasts and malignant cells with N-methyl-N\(^{\text{2}}\)-nitro-N-
nitrosoguanidine promoted their high susceptibility to certain chloroethylating crosslinking agents due to MGMT depletion [5, 50, 51].

Different mechanism is likely to be involved in the adaptive response to alkylating agents in Drosophila larvae. We found it following B. Kaya et al. [18] but using another test-system. Pretreatment of two-day-old larvae with the lowest EMS dose essentially decreased SLRL frequency induced by the challenge dose in three-day-old larvae but was unsuccessful in protection of adults. Intense larval growth and development predetermines the highest metabolic activity including both phases of xenobiotic biotransformation. EMS is a direct acting mutagen ethylating DNA, and at least in mammals, the main pathway of its detoxification is associated with glutathione and glutathione-S-transferases (GSTs) [52, 53], which are responsible for electrophile detoxifying mechanism [54–56]. Along with some other intra- and extracellular components, they form antioxidant defences against reactive oxygen species (ROS) generated in the course of normal metabolism and different pathological processes or produced by prooxidants [55–58]. Within the framework of this discussion, it is important that GST has been found in Drosophila melanogaster [59]. In different organisms, GSTs are induced by preliminary exposure to the lowest doses of electrophilic compounds or ionizing radiation [54, 56, 59], i.e. they are able to act in the manner of the adaptive response. Demonstration of low dose protective effect against high dose mutagenesis in larvae rather than in adults indicates that this detoxifying mechanism is likely to be involved in the adaptive response to alkylating agents observed in our experiments as well as in work of reference [18]. GSTs were shown to carry out multiple operations, e.g. they promote conjugation of electrophilic molecules with nucleophilic substrates, inhibit lipid peroxidation and even interfere in repair of oxidative DNA damage [55, 58]. So their induction seems to be more or less universal protective mechanism against different genotoxic agents that allows prediction of cross-adaptation both in vitro [11, 60–62] and in vivo.

Some other mechanisms involved in the adaptive response have been recently discussed [18, 63, 64]. We would like to draw attention to those, which could provide protection against different deleterious effects and factors. Among them, induction of antioxidant defences seems to inhibit many pathological processes and prevent DNA damage induced by internal ROS and environmental prooxidants. For example, metallothionein has been found to protect cells and organisms against oxidative stress [12, 57, 65], carcinogenesis, cytotoxicity and mutagenicity of certain metals [66–69] and alkylating anticancer drugs [70].

Generalized cell functions as well as signalling pathways could be of great importance for some protective mechanism initiation or realization. Thus, protein kinase-mediated signalling transduction has been supposed to be involved in radioadaptive response [63]. Poly(ADP-ribose) polymerase (PARP) responsible for posttranslational DNA and protein modifications has been shown to interfere in different molecular and cellular processes such as nick-detection [71], DNA replication [72], excision repair [73], apoptosis [74], and seems to cause the adaptation of eukaryotic cells to ionizing radiation [63]. It is interesting that PARP and DNA-dependent protein kinase cooperate to minimize genomic damage induced by DNA strand breaks in mammalian cells [75].

Apoptosis (programmed cell death) is the normal physiological process for elimination of unwanted or useless cells during eukaryotic development (including Drosophila embryos [76]), and creates the natural barrier against carcinogenesis [77–79]. Nevertheless its blocking was considered as a possible triggering mechanism for the radioadaptive response [63, 64]. Some regulatory genes encoding cellular susceptibility to apoptosis have been identified as oncogens and oncosuppressors [77–79]. One of them, p53 seems to be also involved in another protective mechanism. References to the alkyltransferase induction in
irradiated mammalian cells were an intriguing fact in this context [80, 81]. Inducible alkyltransferase activity in mouse tissue following whole body irradiation was completely dependent on the presence of functional p53 [81]. Hence, this gene appeared to control the alkyltransferase expression in response to in vivo irradiation suggesting cross-adaptation, at least against alkylated DNA damage.

Heat shock proteins (hsp) actively studied following description of specific puffing induced by a high temperature in Drosophila [82] are considered now as a system for the generalized response to any kind of stresses [83]. They are induced by multitude of factors, and some of them possess protective and chaperone functions. Their involvement in the radioadaptive response was discussed [63]. It has also been found that hsp70 present in Drosophila and mammalian cells [83, 84] inhibits apoptosis [85] and in that way could initiate the adaptive response to both ionizing radiation and other genotoxic agents.

All mentioned data together with ours allow supposition that some general mechanisms seem to underlie the adaptive response against DNA damage of different origin and nature as well as contribute to cross-adaptation in vitro and in vivo.

In conclusion, the adaptive response to alkylating agents was found in Drosophila SLRL assay. Low dose larval pretreatment (0.05 mM of EMS) provided their germ cell resistance to a high mutagenic dose (1mM) resulting in threefold reduction of mutation frequency and pronounced decrease in male sterility. By contrast, similar “adaptive” pretreatment of males and females was inefficient in inhibition of high dose mutagenesis in spermatozoa as well as at premeiotic spermatogenesis stages. Thus, under condition tested involvement of detoxifying mechanisms in the adaptive response seems to be preferable as compared to DNA repair, and the latter is likely to be mediated by constitutive enzymes in Drosophila germ cells, though it were excision repair or dealkylation with alkyltransferases.

References
11. Laval F. Pretreatment with oxygen species increases the resistance of mammalian cells to hydrogen peroxide and gamma-rays, Mutat. Res. 201 (1988) 73–79.
41. Jenkins J.B. Mutagenesis at a complex locus in Drosophila with the monofunctional alkylating agent, ethyl methanesulfonate, Genetics 57 (1967) 783–793.
45. Karran P., R. Hampson, Genomic instability and tolerance to alkylating agents, Cancer Surv. 28 (1996) 69–85.

82. Ritossa F. A new puffing pattern induced by heat shock and DNP in Drosophila, Experientia. 18 (1972) 571–573.

