Both the mus309 alleles used carry mutational changes that could potentially impair or abolish at least the helicase function of the MUS309 protein. In the mus309D2 allele, there is a stop codon between the sequence motifs encoding the third and fourth helicase motif of the protein. The mus309D3 allele for its part has a glutamic acid to lysine substitution in the conserved helicase II motif, as well as another amino acid substitution close to the C terminus (Kusano et al., 2001).

While constructing the stocks, I first constructed several lines carrying the w ct6 constitution in their X chromosomes and either the mus309/Tb or the +/Tb constitution in their 3rd chromosomes. These could not be distinguished on the basis of the morphological phenotype, because the mus309 mutations do not have any morphological effect. The lines were submitted to natural selection in order to follow whether the Tubby balancer was maintained in them and consequently were carrying the mus309 mutation, too.

The lines were checked for the first time after five generations of selection. It was observed that in five of the eight original D2 lines which had maintained the Tubby phenotype the cut phenotype had reverted, but in one line which also had maintained the Tubby phenotype the cut phenotype had not reverted. This line was preserved as a stock. Similarly, in one of the eight original D3 lines the cut phenotype had reverted and also the white phenotype had reverted. Subsequently, a double-revertant stock was constructed from this line.

Thus, it is highly likely that the mus309 mutations, defective, e.g., in P element excision, had mobilized the gypsy element residing in the ct6 allele of the cut locus, thus causing its reversion and also the reversion of the w mutation.

Both the D3 and the D2 stocks mentioned have since been stable now for approximately 20 generations.


Five mammalian carcinogens are active in a short-term tumorigenocity assay in Drosophila.

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Earlier we showed that mammalian carcinogens cause tumors in wts-heterozygous flies. We developed the only test capable to demonstrate tumor formation in Drosophila by carcinogenic compounds. The clone formation is based on heterozygosity loss in the imaginal disc cells as a result
of somatic mutation and mitotic crossing-over. Some mammal carcinogens, such as oxoplatin, benz(a)pyrene, aflatoxin B1, significantly increase tumor clone frequency (Sidorov et al., 2002). The purpose of the article is to present further information on the activity of five more mammalian carcinogens.

For the experiment we chose agents with different DNA damage mechanisms. They are Ethidium bromide (CAS#35322-47-5, EB), 7,12-dimethylbenz[a]anthracene (CAS#57-97-6, DMBA), 20-methylcholanthrene (CAS#56-49-5, MC), Sarcolysinum (CAS#531-76-0, Sarc), and Aranose (CAS#167396-23-8, Ara). Ethidium bromide (EB) is an intercalating agent usually used in molecular genetics and in structural studies of DNA and chromatin. It has been shown to inhibit cell growth in tissue culture (Heinen et al., 1978), induce frameshift mutations (after metabolic activation by liver microsomes) in bacteria (McCann et al., 1975), and induce petite mutants in Saccharomyces cerevisiae (Slominski et al., 1968). 7,12-dimethylbenz[a]anthracene and 20-methylcholanthrene are methylated polycyclic aromatic hydrocarbon carcinogens. They are known for their DNA bulk adduct forming ability. Sarcolysinum (4-(Bis(2-chloroethyl)amino)-DL-phenylalanine) acts as both a monofunctional and bifunctional alkylating agent. It forms mono-alkylated adducts located on the nucleobases as well as cross-links in DNA (Mohamed et al., 2008), and Aranose (Arabinopiranosilmethyl nitrosocarbamide) known to be a directly-acting alkylating agent.

We crossed the strains ♀ w; wts P4/TM3 × ♂ D-32 to obtain wts/+ heterozygotes. The 1st instar F1 larvae from the cross were treated either with distilled water or 10% aqueous dimethylsulfoxide (DMSO), negative control group, or with one of the mutagenic substances: Ethidium bromide (6.3 mM aqueous solution), Sarcolysinum (2 mM aqueous solution), Aranose (2 mM aqueous solution), 7,12-dimethylbenz[a]anthracene (7.8 mg/ml suspension in 10% aqueous dimethylsulfoxide (DMSO) solution), 20-methylcholanthrene (7.5 mg/ml suspension in 10% aqueous dimethylsulfoxide (DMSO) solution). In the F1, wts P4/+ flies selected by the absence of Sb and Ser markers were examined for wts tumorous clones. Clone frequency was calculated as (Number_of_clones / Number_of_flies) × 100%. The significance of differences between the series was calculated in Student’s t-test with Fisher’s correction \( \varphi = 2\arcsin\sqrt{\text{clone\_frequency}} \).

<table>
<thead>
<tr>
<th>Substance, concentration</th>
<th>EB 0.5-3.75 mM</th>
<th>EB 6.3 mM</th>
<th>Sarc 2 mM</th>
<th>Ara 2 mM</th>
<th>H2O (control)</th>
<th>DMBA 30 mM</th>
<th>20-MC 15 mM</th>
<th>10% DMSO (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approximate survival rate, %</td>
<td>100</td>
<td>60</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Number of flies</td>
<td>1161</td>
<td>404</td>
<td>298</td>
<td>337</td>
<td>820</td>
<td>273</td>
<td>269</td>
<td>531</td>
</tr>
<tr>
<td>Number of clones</td>
<td>35</td>
<td>21</td>
<td>25</td>
<td>30</td>
<td>20</td>
<td>116</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>wts clone frequency, %</td>
<td>3.0</td>
<td>5.2*</td>
<td>8.4*</td>
<td>8.9*</td>
<td>2.4</td>
<td>42.5 *</td>
<td>11.2*</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Legend: * - the wts tumor frequency is significantly higher than the control one, \( P < 0.01 \)

The results summarized in the Table 1 show that:

1) The test used is suitable for detection of mammal carcinogens with different DNA damage mechanisms;
2) All the five mammalian carcinogens, EB, Sarc, Ara, DMBA, and 20-MC, significantly increase tumor clone frequency in wts-heterozygous flies. Among the compounds tested, the
intercalating agent, ethidium bromide, acts as a weak mutagen, but a strong toxic chemical. It does not increase clone frequency up to the dose LD40. The strongest effect is registered with DMBA treatment. In comparison with benz(a)pyrene (11.7% clone frequency) (Sidorov et al., 2002), DMBA acts as more powerful carcinogen (42.5% clone frequency), that, probably, makes conditional upon methylated structure of DMBA.

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The annotation CG17337 of D. melanogaster is the gene Dipeptidase-A.

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Supporting Notes and Data

Dipeptidase-A (Dip-A) is one of a cohort of Dipeptidases (-A, -B and -C) which appear ubiquitously in the fly (Hall, 1983; Laurie-Ahlberg, 1982) and provide the final step of protein catabolism (Collett, 1989). The identification of the annotation CG17337 as the gene coding Dip-A reported here emerges from several kinds of information. Its chromosomal region has been defined by the associations of its electrophoretic variants and activities with chromosomal deficiencies (Voelker and Langley, 1978; Hall, 1983; and Table 1), while its physiological characterization in the large dipteran Calliphora erythrocephala (Collett, 1989) and in Drosophila (Collett, in prep.) has allowed discrimination among the putative proteinases in the annotations of this defined region. Further, these characteristics of Dip-A together with the coding of CG17337 correspond to those of the characterized human proteinase PepA. This information is compiled here as definitive evidence of the identification of the annotation CG17337 as the gene Dip-A.

To refine the earlier localization of a gene for Dip-A (Voelker and Langley, 1978) in the proximal region of 2R, Dip-A activity was measured in duplication and deficiency heterozygotes in the F1 progeny of a mating of Oregon R and the stock Df(2R)nap1 (Table 1). The activities of both Dip-A and Dip-C, as a standard within each sample, were measured in samples of adult males of the parental and progeny genotypes and are presented as the ratios of Dip-A and Dip-C. As may be seen in Table 1, these ratios are consistent with the haploid, diploid and triploid copy number of the region of 41D2 – 42B3 in the sampled genotypes. Thus the gene Dip-A is restricted to the chromosomal region bounded proximally by 41D2.