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 *mus309*^{D2} allele, there is a stop codon between the sequence motifs encoding the third and fourth helicase motif of the protein. The *mus309*^{D3} 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 ct*^{6} 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 *ct*^{6} 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.

References: Beall, E.L., and D.C. Rio 1996, Genes and Devel.10: 921-933; Boyd, J.B., J.M. Mason, A.H. Yamamoto, R.K. Brodberg, S.S. Banga *et al*., 1987, J. Cell Sci. Suppl. 6: 39-60; Boyd, J.B., M.D. Golino, K.E.S. Shaw, C.J. Osgood, and M.M. Green 1981, Genetics 97: 607-623; Brabant, A.J. van, R. Stan, and N.A. Ellis 2000, Ann. Rev. Genom. Hum. Genet. 1: 409-459; Ellis, N.A., J. Groden, T.-Z. Ye, J. Straughen, D.J. Lennon, *et al.*, 1995, Cell 83: 655-666; Heyer, W.-D., 2004, Current Biol. 14: R56-R58; Heyer, W.-D., K.T. Ehmsen, and J.A. Solinger 2003, Trends Biochem. Sci. 28: 548-557; Karow, J.K., R.K. Chakraverty, and J.D. Hickson 1997, J. Biol. Chem. 272: 30611-30614; Kusano, K., D.M. Johnson-Schlitz, and W.R. Engels 2001, Science 291: 2600-2602; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, San Diego; McVey, M., J.R. LaRocque, M.D. Adams, and J.J. Sekelsky 2004, Proc. Natl. Acad. Sci. USA 101: 15694-15699; Mohaghegh, P., J.K. Karow, R.M. Brosh Jr., V.A. Bohr, and I.D. Hickson 2001, Nucl. Acids Res. 29: 2843-2849; Wu, L., S.L. Davies, N.C. Levitt, and I.D. Hickson 2001, J. Biol. Chem. 276: 19375-19381.

Five mammalian carcinogens are active in a short-term tumorogenicity 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 B_1, 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-methylcholantrene (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-methylcholantrene 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 F_1 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-methylcholantrene (7.5 mg/ml suspension in 10% aqueous dimethylsulfoxide (DMSO) solution). In the F_1, *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 $\varphi = 2\arcsin\sqrt{clone\_frequency}$.

Table 1. The influence of the genotoxic agents treatment on the tumor clone frequency in wts/+ heterozygotes.

Substance, concentration	EB 0,5-3,75 mM	EB 6,3 mM	Sarc 2 mM	Ara 2 mM	H_2O (control)	DMBA 30 mM	20-MC 15 mM	10% DMSO (control)
Approximate survival rate, %	100	60	100	100	100	100	100	100
Number of flies	1161	404	298	337	820	273	269	531
Number of clones	35	21	25	30	20	116	30	14
wts clone frequency, %	3.0	5.2*	8.4*	8.9*	2.4	42.5 *	11.2*	2.6

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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References. Mohamed, D., M. Linscheid 2008, Anal. Bioanal. Chem. 392(5): 805; Siddique, Hifzur, R., D. Kar Chowdhuri, D.K. Saxena, and Alok Dhawan 2005, Mutagenesis 20: 285-290; Sidorov, R.A., E.G. Ugnivenko, E.M. Khovanova, and G.A. Belitsky 2002, Dros. Inf. Serv. 85: 121-122; Sidorov, R.A., E.G. Ugnivenko, E.M. Khovanova, and G.A. Belitsky 2001. Mut. Res. 498(1-2): 181-191; Gaivao, I., L.M. Sierra, and M.A. Comendator 1999, Mut. Res. 440: 139-145; McCann, J., E. Coi, E.Yamasaki, and B.N. Ames 1975, Proc. Natl. Acad. Sci.USA 72: 5135; Slominski, P.P., G. Perrodin, and J.N. Croft 1968, Biochem. biophys. Res. Commun. 30: 232; Heinen, E., 1978, Virchows Arch. B27: 79.

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.