into consideration the dramatic biotic contrasts in the Nahal Oren canyon, we can conclude that our data corroborate these cited results, but further direct tests are needed.

References: Derzhavets, E., A. Korol, and E. Nevo 1996, Dros. Inf. Serv., 77: 92-94; Derzhavets, E., A. Korol, and E. Nevo 1997a, Dros. Inf. Serv., 80: 73-75; Derzhavets, E., A. Korol, T. Pavlicek, and E. Nevo 1997b, Dros. Inf. Serv., 80: 53-54; Giesel, J.T., 1986, Evolution 40(3): 649-651; Giesel, J.T., 1988, Evolution 42(6): 1348-1350; Harry, M., E. Rashkovetsky, T. Pavlicek, S. Baker, E.M. Derzhavets, P. Capy, M.-L. Cariou, D. Lachaise, N. Asada, and E. Nevo 1999, Biologia, Bratislava 54/6: 685-705; Kilias, G., and S.N. Alahiotis 1985, Theor. Appl. Genet. 69: 645-650; Korol, A., E. Rashkovetsky, K. Iliadi, Y. Ronin, and E. Nevo 2000, Sympatric ecological speciation in action: microclimatic contrasts induce incipient premating isolation in *Drosophila* at the "Evolution canyon" microsite, submitted; Nevo, E., 1995, Proc. R. Soc. Lond. B262: 149-155; Nevo, E., 1997, Theor. Pop. Biol. 52: 231-243; Nevo, E., E. Rashkovetsky, T. Pavlicek, and A. Korol 1998, Heredity 80: 9-17; Pavlicek, T., V. Kravchenko, and E. Nevo 2000, Microclimatic differential stresses underlie biodiversity contrasts, submitted; Rashkovetsky, E., A. Korol, T. Pavlicek, and E. Nevo 1997, Dros. Inf. Serv., 80: 83-85.

# Tumor inducing *Drosophila*: resistance to hydroxyurea and methane sulfonic acid methyl ester.

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**Abstract:** Tumor induction lies in proliferative gene defects in *Drosophila* and humans. Mutant proliferative genes allow replication. Recent results obtained in human leukaemic cell lines point towards an involvement of the repair system: DNA damage response is inadequate in tumors. Hydroxyurea inhibits semiconservative replication but allows repair replication. Methane sulfonic acid methyl ester damages DNA and induces apoptosis. Proliferative gene mutants of *Drosophila* show intrinsic resistance to hydroxyurea and methane sulfonic acid methyl ester. This raises evidence, that repair replication is constitutively active and circumvents semiconservative replication, response to DNA damage is inadequate.

## Introduction

Tumor formation lies in gene defects that alter the normal program of cell proliferation. *Drosophila* stock Malignant Brain Tumor (MBT) has been genetically analyzed. Out of six mutant genes, those defects have been identified that are responsible for loss of control over cell proliferation. Tumor growth in flies is dependent on mutation of a proliferative gene. Proliferative genes are defined as the class of genes, that, when mutant, allow cell divisions in cell cycle competent cells. Replication allowance has been identified in cell cycle restricted cells, which aberrently polytenize DNA. Thus, the mutation of a proliferative gene could shortcut the cell cycle by onset of replication (Riede, 1996, 1997, 1998). Due to the phenotypic expression of proliferative gene defects in *Drosophila* – somatic pairing defect in combination with replication allowance – the involvement of the repair system has been anticipated. Recombination repair is dependent on homologous pairing of chromosomes and is able to initiate replication. Recent studies with human

leukaemic cell lines, containing mutant ALL-1, a proliferative gene, indicate that part of replication is constitutively performed by the repair system in tumor cells (Riede, Repair replication in tumor cells, submitted).

Hydroxyurea (HU) is used as an antimetabolite anticancer agent. It inhibits semiconservative DNA synthesis, whereas repair is largely unaffected by the drug (Smith and Hannawalt, 1976; Clarkson, 1978). Thus, this S-phase inhibitor can be used to distinguish repair replication from semiconservative replication. In leukaemic cell lines, part of replication is resistant to HU, *i.e.* performed by repair DNA synthesis (Riede, submitted). Thus, proliferative genes might be responsible for inducing the repair system, which might shortcut the cell cycle. Wild-type flies depend on semiconservative replication during the cell cycle. HU blocks the S-phase, thus developing animals are HU sensitive. Mutations with a shortcut in the cell cycle, allowing replication by the repair system, might not be dependent on semiconservative replication, might manage to synthesize DNA with repair DNA-synthesis, and thus might be HU resistant.

Methane sulfonic acid methyl ester (MMS) induces apoptosis in normal cells due to DNA damage. The biological sense of this response lies in conservation of the genome integrity (Duckett *et al.*, 1999; Jieang *et al.*, 1999). The response of leukaemic cell lines to MMS has been shown to be indadequate. Tumor cells either repair DNA instead of inducing apoptosis in the case of irrepairable DNA damage, or do not identify DNA damage and do not induce the repair system, possibly dependent on the type of mutation inducing tumor growth (Riede, submitted). By that defect, the signal to apoptosis remains uninduced, cells try to repair irrepairable DNA damage or are blind to DNA damage. If DNA damage control is due to proliferative gene defects, *Drosophila* mutants carrying those defects should reveal intrinsic resistance to DNA damaging drug MMS. DNA damage would not lead to apoptosis of cells, animals would survive DNA damage lethal for wild type.

**Results and Discussion**

Proliferative gene mutants have been obtained in *Drosophila* (Riede, 1997). Quantitatively interacting mutations in different genes show similar phenotypes, predominantly melanotic tumor formation. P-elements were used to tag the genes. Proliferative gene mutations destroy the somatic pairing process of homologous chromosomes. All P-element insertions used here show somatic pairing gaps (Table 1).

Whereas EMS induced mutations lead to melanotic tumor formation, none of the so far obtained P-element insertions induces melanotic tumors. Thus, a certain quality of the gene product is required for tumor induction. Partial activity of the gene product leads to tumors, whereas destruction of the gene confers lethality but not tumor formation. ALL-1, a proliferative gene involved in human leukaemias, shows a similar quality. Essentially, destruction of the gene in mice confers embryonic lethality but not tumor formation. Tumor inducing molecular constructs contain AT-hooks and a region homologous to methyltransferase fused to different polypeptides (Slany *et al.*, 1998; Dobson *et al.*, 2000).

Replication in human leukaemia cells with mutant ALL-1 is partially resistant to HU, indicating that the repair system constitutively replicates DNA. DNA damage response to MMS has been shown to be inadequate, the signal transduction cascade to apoptosis is broken. Human leukaemic cell lines carry a set of mutations, as tumor formation is always related to genome instability (Vessey *et al.*, 1999). Accumulation of secondary mutations is intrinsic. HU resistant replication or inadequate response to DNA damage could be due to a proliferative gene mutation or due to secondary mutations.

In *Drosophila*, single events can be analyzed. A proliferative gene mutation can be separated from secondary gene defects by genetic methods. HU or MMS resistance of mutants in proliferative

Table 1. Response to HU and MMS.

| | Tumor induction[a] | Somatic pairing defect[a] | HU[b] | MMS[c] |
|---|---|---|---|---|
| wildtype[d] | - | - | S | S |
| *stern*$^{88}$ | + | ++ | R | S |
| *stern*$^{p21}$ | - | ++ | R | S |
| *stern*$^{p18}$ | - | ++ | R | S |
| *Aus*$^{9}$ | +++ | ++ | R | R |
| *Aus*$^{p21}$ | - | ++ | R | S |
| *merlin*$^{14}$ | ++ | ++ | S | R |
| *merlin*$^{p19}$ *merlin*$^{14}$ | + | ++ | R | R |
| *merlin*$^{que}$ | - | nd | R | R |
| *mali*$^{27}$ | +++ | + | R | R |
| *efendi*$^{89}$ | +++ | + | R | R |
| *hexe*$^{77}$ | ++ | ++ | R | R |

a-Tumor induction of homozygous L3 larvae is indicated. Somatic pairing gaps were determined in allele/OregonR L3 larvae

b-Eggs of allele/TM3 or allele/TM6b parents were deposited on medium containing 5mM HU. More than 80% of allele/TM3 or allele/TM6b eclosed (dominant drug resistance, R) or died (sensitivity to drug, S) after 14 days.

c-Eggs of allele/TM3 or allele/TM6b parents were deposited on medium containing 1mM MMS. More than 80% of allele/TM3 or allele/TM6b eclosed (dominant drug resistance, R) or died (sensitivity to drug, S).

d-OregonR, OregonR e, ri/ri and w/w; TM3 Ser/ TM6b Tb are sensitive to MMS and HU.

nd- not determined.

genes indicates a primary relation rather than resistance due to secondary mutations. Flies carrying mutant proliferative gene defects had never been under selective pressure of chemotherapeutic agents, the human leukaemic cell lines might have been. Thus intrinsic resistance of the flies would be due to the proliferative gene mutation itself and not due to accumulation of secondary defects.

A set of proliferative gene mutants was exposed to HU and MMS, at a drug concentration tolerated by adult flies but interfering with development (Table 1). Most mutations provide dominant resistance. In homozygous larvae, formation of melanotic tumors is suppressed by MMS and enhanced by HU. EMS induced allele *stern*$^{88}$ and P-element insertions *stern*$^{p21}$ and *stern*$^{p18}$ are resistant to HU and sensitive to MMS. *stern*, 56 cM, is a proliferative gene with major interfering capability: it confers lethality in trans over *efendi*, 97F. Thus, most but not all proliferative gene mutations provide resistance to HU and MMS.

Some proliferative gene mutants, like *merlin* or *hexe* spontaneously revert lethality at a frequency of 1:10$^4$. This reversion, called quench, can be due to secondary mutations in different genes, because the original phenotype causing 100% lethality can sometimes be regained by either chromosome exchange or by recombination with a marked wild-type chromosome. Quench can be mimicked by P-element insertions. So far, no lethal P-element insertion over *merlin*$^{14}$ has been found. P-elements quenching lethality of *merlin*$^{14}$ were isolated. One of those alleles, *merlin*$^{pi9}$, and a spontanous quench mutation *merlin*$^{que}$ show resistance whereas the EMS induced allele *merlin*$^{14}$ is sensitive to HU (Table 1). This reveals that quench mutations increase resistance to HU.

Hypersensitivity of *Drosophila* to HU and MMS has been described (deBuendia, 1998; Banga *et al.*, 1986). Mostly repair enzymes and proteins that guarantee genome stability are involved. Resistance to those drugs, so far, has not been due to analysis in *Drosophila.*

Replication in leukaemic cell lines, which carry mutant ALL-1, is partially resistant to HU. Cells react inadequately to DNA damage induced by MMS. From these data, it was hypothesized that proliferative genes are involved in DNA damage control (Riede, submitted). Here I show that proliferative gene mutants reveal intrinsic resistance to HU and MMS.

HU inhibits semiconservative replication and allows repair replication. Resistance to HU implicates an S-phase independent from semiconservative replication, possibly involving the repair system to replicate the DNA. MMS induces DNA damage and cell death. Intrinsic resistance to MMS in flies carrying mutant proliferative genes points towards inadequate DNA damage control.

Proliferative genes destruct the somatic pairing of chromosomes, allow replication, and induce genome instability (Riede, 1996, 1997, 1998). HU resistance implies that the repair system might be involved in onset of replication, MMS resistance points towards a role of proliferative genes in management of the DNA damage response.

EMS induced *Aus*, *mali*, *efendi* and *hexe* mutations are resistant to HU and MMS. Thus, those gene defects that allow tumor growth of cells might use repair synthesis during S-phase, and coincidentially DNA damage control is defective. *stern* alleles are HU resistant and MMS sensitive; thus, *stern* mutations allow circumvention during S-phase with repair synthesis, but MMS specific DNA damage control remains intact. *merlin*$^{14}$ shows sensitivity to HU, but secondary alleles that quench lethality enhance resistance to HU. Thus, proliferative gene mutations themselves and secondary mutations provide resistance to chemotherapeutics.

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**Methods**

Drosophila *stocks*: Proliferative genes mutations *stern*$^{88}$ *e*, *Aus*$^{9}$ *e*, *merlin*$^{14}$ *ri*, *hexe*$^{77}$ *e*, *mali*$^{27}$ *e*, and *efe*$^{89}$ *e*, are EMS induced alleles (Riede, 1997), lethal and tumor inducing over MBT. P-element insertions were obtained as described (Bier *et al.*, 1989). *stern*$^{p21}$ *ri*, and *stern*$^{p18}$ *ri* are P-element insertions lethal over *stern*$^{88}$. *Aus*$^{p21}$ *ri* is a P-element insertion lethal over *Aus*$^{9}$. *merlin*$^{que}$ *ri* is a spontaneous reversion of *merlin*$^{14}$ having lost lethality as homozygote. *merlin*$^{pi9}$ *ri* is a P-element insertion which suppresses the lethality of *merlin*$^{14}$ in a quantitative manner: *merlin*$^{14}$ / *merlin*$^{14}$ is L3-lethal, *merlin*$^{14}$ *merlin*$^{pi9}$ / *merlin*$^{14}$ *merlin*$^{pi9}$ is L2-lethal and *merlin*$^{14}$ *merlin*$^{pi9}$ / *merlin*$^{14}$ is viable as an adult. *merlin*$^{pi9}$ was mapped to 56 cM, the location of *merlin*$^{14}$. *merlin*$^{pi9}$ could not be separated from *merlin*$^{14}$ by recombination, screening a progeny of 200 animals. Alleles were kept over TM3 or TM6b. Neither stock, that had been used to cross alleles is HU or MMS resistant (*ri* / *ri*, Oregon-R *e*, *w* / *w*; ; TM3 / TM6b). *Drosophila* stocks, containing proliferative gene mutations, have never been exposed to HU or MMS.

*Drugs*: HU was prepared as 1M stock solution prior to use. Medium containing 5mM HU is lethal for Oregon-R *e* and *ri* development, whereas adult flies tolerate this concentration. Wild type grown on 5mM HU reaches pupariation after 10 days at 25°C and dies. At 10mM HU, embryonic lethality occurs (60%) in Oregon-R, some L1 larvae hatch (40%).

1mM MMS was applied to the medium prior to use. This concentration is tolerated by wild-type adult flies, but interferes with the development. Wild type grown on 1mM MMS reaches pupariation after 12 days at 25°C, 50% of progeny reaches pupariation, 10% eclose. 2mM MMS is lethal for adult Oregan-R within 48 hours. At 0.5mM MMS, 50% of the progeny ecloses.

*Tumor induction and somatic pairing defect*: Melanizing tumors in third instar larvae are characteristic for proliferative gene mutants, however melanoma formation varies. Few and small melanomas (+) or many and large melanotic tumors (+++ in Table 1) might occur.

Salivary glands were prepared in 45% acetic acid from stage 3 larvae (allele / Oregon-R) grown at 18°C. The tissue was fixed in 1 N HCl for one minute and washed with 50% lactic acid / 30% acetic acid. The salivary glands were stained with 2% orceine / 30% lactic acid / 30% acetic acid for 10 minutes, rinsed in 45% acetic acid, and squashed. The chromosomes reveal somatic pairing gaps. + indicates shorter regions, up to three sections of a chromosome being unpaired in Oregon-R / allele hybrid larvae; ++ indicates longer unpaired regions, comprising five to ten sections of a chromosome or unpaired half chromosomes in Table 1.

References: Banga, S.S., R. Shenkar, and J.B. Boyd 1986, Mutat. Res. 163:157-165; Bier, E., H. Vaessin, S. Shepherd, K. Lee, K. McCall, S. Barbel, L. Ackermann, R. Carretto, T. Uemura, E. Grell, L.Y. Jan, and Y.N. Jan 1989, Genes and Development 3:1273-1287; Clarkson, M.M., 1978,

Mutat. Res. 52:273-284; deBuendia, G., 1998, Mutat. Res. 407:67-84; Dobson, C.L., A.J. Warren, R. Pannell, A. Forster, and T.H. Rabbitts 2000, EMBO J. 19:843-851; Duckett, S.T., M.M. Morris, M. Illand, H.J. McKay, C. McCormick, G.L. Hirst, R.H. Borts, and R. Brown 1999, Curr. Biol. 9:51-54; Jiang, M.C., H.J. Liang, C.F. Ciao, and F.G. Lu 1999, Toxicol. Lett. 106:201-208; Riede, I., 1996, Cancer Genet. Cytogenet. 90:135-141; Riede, I., 1997, Cancer Genet. Cytogenet. 97:143-154; Riede, I., 1998, Cencer Detection and Prevention 22:5-61; Slany, R.K., C. Lavan, M.L. Cleary 1998, Mol. Cell. Biol. 18:122-129; Smith, C.A., and P.C. Hannwalt 1976, Biochim. Biophys. Acta. 432:336-347; Vessey, C.J., C.J. Norbury, and I.D. Hickson 1999, Prog. Nucleic Acid Res. Mol. Biol. 63:189-211.

## Cell size is a factor in body size variation among Hawaiian and nonHawaiian species of *Drosophila*.

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Adult body sizes in the genus *Drosophila* are remarkably diverse, especially among the species endemic to Hawaii, which hosts the giants and the dwarfs of the *Drosophila* world. Body lengths among these species vary over a greater than five-fold range, encompassing flies that are much smaller than *D. melanogaster*, and others that are more than four times as large. This size variation raises questions as to the adaptive significance of these astounding differences within one evolutionary lineage, and the developmental factors that underlie this phenotypic variation. Size in multicellular organisms depends on both cell number and cell volume; but, are larger body sizes in related species due solely or primarily to increases in the number of cells, or do cell size differences contribute to this variation? To address this question, we compared cell sizes in six Hawaiian and two nonHawaiian species, selected to represent a range of adult body sizes. We used flies from stock cultures rather than field-collected individuals in order to minimize effects on body size due to variations in temperature or nutrition (Robertson, 1959; Partridge *et al.*, 1994).

The Hawaiian species included one member of the modified-mouthparts group, *D. mimica* (strain K85P1), and five picture-winged flies: *D. silvestris* (strain U34B4) and *D. heteroneura* (strain W48B6) of the *planitibia* species group, and *D. grimshawi* (strain G1), *D. disjuncta* (strain U59G44), and *D. hawaiiensis* (strain Y17P5) of the *grimshawi* species group. All were reared by standard methods for Hawaiian *Drosophila* on yeastless Wheeler-Clayton medium (Wheeler and Clayton, 1965) at 17°C, 75% humidity, and a 12:12 light/dark cycle. The continental species were *D. melanogaster* and *D. paulistorum* (Orinocan), kindly provided by Dr. Lee Ehrman. These species were maintained at room temperature on Carolina Instant Drosophila medium, supplemented with yeast. Using a stereomicroscope fitted with an ocular micrometer, four indices of adult body size – body length, thorax length, wing length, and tibia length of the right prothoracic leg – were measured for twelve individuals of each sex (see Kacmarczyk, 1999, for details of measurement points). Mean lengths and standard errors were calculated for males and females of each species. Body mass (average wet weight per fly) was estimated by weighing groups of sexed flies sedated by chilling. To estimate cell sizes, we used computerized image analysis of mounted right wings of five individuals of each sex and species, to make trichome counts in a 0.01 $mm^2$ area of the posterior cell of the wing. The selected region, just slightly anterior to the intersection of the posterior cross vein and the fifth