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FUNCTIONAL GENETIC CHARACTERIZATION OF THE *DROSOPHILA [l(3)k3.13/2]* GENE

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ABSTRACT

Recently, the [*l(3)k3.13/2*] gene of *Drosophila melanogaster* was generated in a specific genetic screen for lethals on the third chromosome. It was shown to be the *Drosophila* homolog of one of the mouse proteinases genes which its expression is required to cut the protein (amino acid chain) once at a specific site. The latter is always very near to the C-terminus of the polypeptide chain thus affecting a distinct regulatory pathway. Soon it was revealed that the [*l(3)k3.13/2*] gene is a tumor suppressor gene displaying malignant development of the hematopoietic organs and in some other body organs. This massive tissue overgrowth finally leads to the death of the animal as late larvae or pupae. Based on the above mentioned facts, the present investigation was designed at the aim of trying to find out the suggested functional genetic role played by this gene in *Drosophila* tumorigenesis. Especially, in a concert either with previously known oncogenes like $Ras^{v/2}$ or tumor suppressor genes like *oho31* and *Df(21) dp-38a* in *Drosophila.*

For this purpose, a large scale experiment including a series of different genetic crosses was carried out. The results could be summarized as following. The analyses of the lethal phase of [*l(3)k3.13/2*] as well as the other strains used in this study showed that lethality always took place during late larval stage for [*l(3)k3.13/2*], *oho-31* and *Df(2L)dp-38a.* In *(UAS-*Ras^{v12}*)* and *(N14)* strains, however, lethality took place during early pupal stage of *Drosophila* development. The lethality percentages raised up in all the (1:1) combinations of [*l(3)k3.13/2*] with the other four stocks used suggesting that the [*l(3)k3.13/2*] gene expression is required in its normal case for the function of other genes in *Drosophila*.

Interestingly enough, the presence of the homozygous third chromosome [*l(3)k3.13/2*]/ [*l(3)k3.13/2*] side by side with the heterozygous second chromosome *UAS-*Ras^{v12}*/N14* in the same genotype, dropped the viability to the lowest percentage at all and lethality took place during the embryonic stage (instead of being during late larval-early pupal stage), specially that was counted for *y* Tb^{+} larvae. This result may indicate that there is a synergistic effect of [*l(3)k3.13/2*] and Ras^{v12} genes. Dissecting *y* Tb^{+} larvae revealed that their inside organs were deleteriously more affected than the control larvae. These findings confirm, at the genetic level, the idea that the tumor suppressor gene [*l(3)k3.13/2*] is required for the inactivation of Ras^{v12} oncogene as well as for the proper genetic functions of both *oho-31* and *Df(2L)dp-38a* tumor suppressor genes in *Drosophila melanogaster*. This kind of study opens a new dimension for understanding the process of tumorigenesis both in insects and in human cancer.

INTRODUCTION

The fruit fly, *Drosophila melanogaster* has been proved to be an ideal model system for genetically dissecting tumorigenesis. More than 60 genes in which homozygous mutations cause tumors in various body tissues were identified up to date (Gateff and Schneiderman, 1969; Gateff, 1978; Mechler,

1990; Torok *et al*., 1993; Ollmann *et al*., 2000). Examination of the mutant larvae showed that tumors in most of the animal organs give rise to massive tissue overgrowth during larval development. Accordingly, leading to the death of the animal as late larvae or pupae. The recessive lethal technique was powerful in selecting most of the mutations affecting genes that control tissue overgrowth; now called tumor suppressor genes (Torok *et al*., 1993).

Advanced genetic and molecular studies suggested that tumorigenesis in *Drosophila* may result from disruption of distinct regulatory pathways. That is because most of these mutants were involved in vital functions such as encoding for *importin*-like protein (*oho-31*) Tick *et al*., 1999], glycogen phosphorylase protein [*Df(2L)dp-38a*) Torok *et al*., 1995]. Also many other types of proteins like serine proteinases, serpin protein, nudel protein, masqurade protein were shown to have functional regulatory pathways in *Drosophila* development (Huang *et al*., 2000; Han *et al*., 2000; Le Mosy *et al*., 2000; Le Mosy and Hashimoto, 2000). Previous investigations reported that mutations in more than 30 genes in *Drosophila* can cause overgrowth of hematopoietic organs during larval development (Gateff and Mechler, 1989; Torok *et al*., 1993; Torok *et al*., 1995; Weinkove and Leevers, 2000). The uncontrolled growth and differentiation of these organs' cells are disrupted giving rise to overgrowth of the hematopoietic organs. One example of the latter type of mutants is the third chromosome mutation *l(3)k3.13/2* which was recently recovered in a genetic screen designed for identifying genes controlling cell proliferation and tumorigenesis in *Drosophila melanogaster* (Torok *et al*., 1993).

Yet, no available informations are known about the developmental genetic effects of this mutation on other *Drosophila* genes involved in controlling cell proliferation and tumorigenesis as well. Hence, the aim of this present work was to try to explore the suggested functional genetic role exhibited by this gene, through a concert either with previously known oncogenes like Ras^{v12} or tumor suppressor genes like *oho-31* and *Df(2L)dp-38a*, in *Drosophila* tumorigenesis. The *Drosophila* homolog of *Ras* oncogene, on the other hand, was found to regulate cell proliferation through promoting growth during G1/S period in the animal wing cells (Prober and Edgar, 2000). In addition, it has a vast range of effects through the so called *Ras*/Mitogen-activated protein kinase signalling pathway on the differentiation of multiple cell types (Rebay *et al*., 2000). Besides to being involved in tumor formation, *Drosophila Ras* homolog has been reported to cause learning defects (Guo *et al*., 2000) and is necessary in adherens junctions for development of a regular array of ommatidia in the *Drosophila* compound eye (Matsuo *et al*., 1999). Activated *Ras1* was found to induce hyperplastic growth and increased cell death in *Drosophila* imaginal tissues (Karim and Rubin, 1998).

The present investigation throws the lights on the functional involvement of the [*l(3)k3.13/2*] gene in such mentioned mechanisms both in regulating cell proliferation and in causing tumors in *Drosophila*.

MATERIALS AND METHODS

I. *Drosophila* culture and stocks

a, *Drosophila* culture

All *Drosophila* stocks used in this study and the genetic crosses cultures were reared on standard cornmeal- yeast-agar medium. Molasses or sugar was added as a source of carbohydrate. Propionic acid was also added to control media from mites and molds. The suitable temperature was 25°C±1°C if not stated otherwise. Standard fly techniques were carried out as described by Ashburner, 1989.

b., *Drosophila* stocks

Most of *Drosophila* stocks used in the present investigation was kindly provided by Prof. Dr. Istvan Kiss, Szeged, Hungary. Table 1 shows a description of these strains.

Table 1: Morphological description for *Drosophila* stocks.

N.	Stock	Fll Genotype	Description
1	*l(3)k3.13/2*	* $\frac{yw}{yw}$; $\frac{+}{+}$; $\frac{l(3)k3.13}{TM6Tb}$	3rd chromosome lethal mutation has *Tubby* phenotype and *yellow* color.
2	*oho-31*	* $\frac{yw}{yw}$; $\frac{oho-31}{y^{+}CyO}$; +/+	2nd chromosome tumor suppressor mutation carried on *Curly* balancer chromosome marked with y^{+} phenotype.
3	*Df(2L)dp-38a*	* $\frac{yw}{yw}$; $\frac{Df(2L)dp38a}{y^{+}CyO}$; +/+	2nd chromosome deficiency represents a tumor suppressor gene carried on *Curly* balancer chromosome y^{+} marked.
4	*Ras^{v12}*	* $\frac{yw}{yw}$; $\frac{UAS-Ras^{V12}}{y^{+}CyO}$; +/+	*Ras^{v12}* represents the mutant allele of the *Drosophila homolog of the human Ras* oncogene driven by *UAS* for *GAL4* binding.
5	*N14*	* $\frac{yw}{yw}$; $\frac{N14}{y^{+}CyO}$; +/+	2nd chromosome mutation having *GAL4* source.
6	*N14; l(3)k3.13/2*	* $\frac{yw}{yw}$; $\frac{N14}{y^{+}CyO}$; $\frac{l(3)k3.13}{TM6Tb}$	A constructed double heterozygous mutation for *N14* and *l(3)k3.13/2* genes, dosage ratio is (1:1).
7	*UAS-Ras^{v12}; l(3)k3.13/2*	* $\frac{yw}{yw}$; $\frac{UAS-Ras^{V12}}{y^{+}Cy}$; $\frac{l(3)k3.13}{TM6Tb}$	A constructed double heterozygous mutation for *UAS- Ras^{v12}* and *l(3)k3.13/2 genes, dosage ratio is (1:1).*

*** For all the stocks the male *X* chromosome is *yw/Y.***

The *y⁺CyO* balancer chromosome carries a *P(y⁺)* insertion and *y⁺* marker is particularly useful for selecting homozygous yellow larvae. More details about the genetic symbols used here and balancers could be found in Lindsley and Zimm, 1992. This work was performed at the Department of Genetics,Faculty of Agriculture,Mansoura University.

II- Genetic crosses

A series of different genetic crosses was done according to the dosage ratio of the wild type allele of each mutant.

a; Crosses for (1:1) ratio

1- $\frac{yw}{Y};\frac{+}{+};\frac{\ell(3)k3.1}{TM6Tb} \otimes \frac{yw}{yw};\frac{oho-31}{y^{+}\ CyO};\frac{+}{+}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{+}{oho-31}+\frac{+}{y^{+}\ CyO}\right);\left(\frac{+}{\ell(3)k3.13/2}+\frac{+}{TM6Tb}\right)$$

Look for *y Cy⁺ Tb⁺* larvae or flies!

2- $\frac{yw}{Y};\frac{+}{+};\frac{\ell(3)k3.1}{TM6Tb} \otimes \frac{yw}{yw};\frac{Df(2L)-dp8a}{y^{+}\ CyO};\frac{+}{+}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{+}{Df(2L)-dp8a}+\frac{+}{y^{+}\ CyO}\right);\left(\frac{+}{\ell(3)k3.13/2}+\frac{+}{TM6Tb}\right)$$

Look for *y Cy⁺ Tb⁺* larvae or flies.

3- $\frac{yw}{Y};\frac{+}{+};\frac{\ell(3)k3.1}{TM6Tb} \otimes \frac{yw}{yw};\frac{UAS-RaS^{V12}}{y^{+}\ CyO};\frac{+}{+}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{+}{UAS-RaS^{V12}}+\frac{+}{y^{+}\ CyO}\right);\left(\frac{+}{\ell(3)k3.13/2}+\frac{+}{TM6Tb}\right)$$

Look for *y Cy⁺ Tb⁺* larvae or flies.

4- $\frac{yw}{Y};\frac{+}{+};\frac{\ell(3)k3.1}{TM6Tb} \otimes \frac{yw}{yw};\frac{N14}{y^{+}\ CyO};\frac{+}{+}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{+}{N14}+\frac{+}{y^{+}\ CyO}\right);\left(\frac{+}{\ell(3)k3.13/2}+\frac{+}{TM6Tb}\right)$$

Look for *y Cy⁺ Tb⁺* larvae or flies.

b., Crosses yielding homozygous form of *l(3)k3.13/2 but heterozygous for the second chromosome*

1- $\frac{yw}{yw};\frac{N14}{y^{+}\ CyO};\frac{\ell(3)k3.1}{TM6Tb} \otimes \frac{yw}{Y};\frac{UAS-RaS^{V12}}{y^{+}\ CyO};\frac{\ell(3)k3.1}{TM6Tb}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{N14}{UAS\text{-}Ras^{v12}}+\frac{N14}{y^{+}\,CyO}+\frac{UAS\text{-}Ras^{v12}}{y^{+}\,CyO}\right);\left(\frac{\ell(3)k3.13}{\ell(3)k3.13/2}+\frac{\ell(3)k3.13}{TM6Tb}+\frac{TM6Tb}{TM6Tb}\right)$$

dies

2- $\frac{yw}{yw};\frac{N14}{y^{+}\,CyO};\frac{\ell(3)k3.1}{TM6Tb}$ ⊗ $\frac{yw}{Y};\frac{UAS\text{-}Ras^{v12}}{UAS\text{-}Ras^{v12}};\frac{\ell(3)k3.1}{TM6Tb}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{N14}{UAS\text{-}Ras^{v12}}+\frac{y^{+}\,CyO}{UAS\text{-}Ras^{v12}}\right);\left(\frac{\ell(3)k3.13}{\ell(3)k3.13/2}+\frac{\ell(3)k3.13}{TM6Tb}+\frac{TM6Tb}{TM6Tb}\right)$$

dies

3- $\frac{yw}{yw};\frac{N14}{N14};\frac{\ell(3)k3.1}{TM6Tb}$ ⊗ $\frac{yw}{Y};\frac{UAS\text{-}Ras^{v12}}{y^{+}\,CyO};\frac{\ell(3)k3.1}{TM6Tb}$

$$\left(\frac{yw}{Y}+\frac{yw}{yw}\right);\left(\frac{N14}{UAS\text{-}Ras^{v12}}+\frac{N14}{y^{+}\,CyO}\right);\left(\frac{\ell(3)k3.13}{\ell(3)k3.13/2}+\frac{\ell(3)k3.13}{TM6Tb}+\frac{TM6Tb}{TM6Tb}\right).$$

dies

* From all these crosses look for *y Tb*$^{+}$ larvae and/or flies (if any).

c., Crosses for maternal effect

Three reciprocal crosses of that mentioned above in (b,), were carried out to check if there was any maternal effect of the *l(3)k3.13/2* gene.

III- Analysis of the lethal period

The analyses of lethal periods for all the stocks and crosses were carried out according to Ashburner, 1989 with some modifications mentioned by Torok *et al*., 1993.

IV- Dissecting the lethal phenotype

l(3)k3.13/2 homozygous larvae showed to survive for a long period of time as late third instar larvae and die without puparium formation. Because the malignant phenotype develops gradually during the prolonged survival period, *y Tb*$^{+}$ mutant larvae were selected and kept on fresh medium in humidified atmosphere. The overgrowth phenotype of the different organs was examined by dissecting the aged larvae, usually 17-22 days after egg laying in Ringer's solution (Torok *et al*., 1995).

RESULTS AND DISCUSSION

I- Analysis of the lethal period for mutants used in the present study

As shown in Table 2, there is some embryonic lethality in all the mutant stocks used in this study. It was calculated as (14.3%, 12.2%, 16.2%, 11.8% and 14%) for *l(3)k3.13/2, oho-31, Df(2L)dp-38a, Ras*^{v12} and *N14*

stocks, respectively. It is clear that the *Ras^{v12}* mutant stock has the lowest percentage of embryonic lethality. At the early pupal (EP) stage, the viability of *l(3)k3.13/2, oho-31* and *Df(2L)dop-38a* dropped severely down to less than 50%. This finding cleared that the lethal period for these three stocks is the late larval stage (LL) where the lethality reached the highest percentage (53.2%, 51% and 51.5%, respectively). In the *Ras^{v12}* and *N14* mutant stocks, on the other hand, the lethal period is early pupal (EP) because viability of each mutant was dropped down to less than 50% in late pupal stage (LP). There is also some lethality in the successive stages but not so important. This finding is in accordance with the results obtained by (Torok *et al*., 1993; Torok *et al*., 1995 and Tick *et al*., 1999).

Table 2: Lethal phase period for *Drosophila* stocks used in this study.

Stock*	E	EL	LL	EP	LP	PA	AD
1	4565 (100)	3912 (85.7)	3396 (74.4)	2136 (46.8)	1940 (42.5)	1903 (41.7)	1881 (41.2)
2	3840 (100)	3372 (87.8)	2882 (75.1)	1880 (49.0)	1767 (46.0)	1672 (43.5)	1663 (43.3)
3	3235 (100)	2710 (83.8)	2437 (75.3)	1570 (48.5)	1514 (46.8)	1465 (45.3)	1457 (45.0)
4	2870 (100)	2530 (88.2)	2210 (77.0)	2140 (74.6)	1380 (48.1)	1277 (44.5)	1268 (44.2)
5	3140 (100)	2700 (86.0)	2471 (78.7)	2386 (76.0)	1492 (47.5)	1422 (45.3)	1416 (45.1)

*** For stocks, see Materials and Methods**
*** For crosses, see Materials and Methods**
*** E: Total No of eggs, EL: early larva, LL: late larva, EP: early pupae, LP: late pupae, PA: pharate adult, AD: adult.**
*** Numbers in brackets show percentages.**

II- Lethal phase for the (1:1) genetic interaction ratio

In general, the genetic interaction of the *l(3)k3.13/2* with the other four *Drosophila* mutant stocks led to the increase of lethality percentages as shown in Table 3. It was ranging from 58.8% in the early pupal stage for *l(3)k3.13/2/oho-31* combination to 60.2% in the late pupal stage for the *l(3)k3.13/2/UAS-Ras^{v12}* combination.

The lethal phase for the *l(3)k3.13/2/oho-31* and *l(3)k3.13/2/Df(2L)dp-38a* combinations was shown to take place during the late larval stage (LL). Whereas, the lethal phase for the *l(3)k3.13/2/Ras^{v12}* and *l(3)k3.13/2/N14* combinations was shown to take place during early pupal stage (EP) of *Drosophila* development.

The high percentages of lethality during the lethal phase of each combination suggest that the *l(3)k3.13/2* gene expression is required in its normal dose for the proper function of other developmental genes in *Drosophila*. Also suggest that the *Ras^{v12}* gene is the most affected locus with respect to the dosage of the *l(3)k3.13/2* gene. This result is in agreement with some investigators like (Torok *et al*., 1995; Tick *et al*., 1999; Han *et al*., 2000, Rebay *et al*., 2000).

Table 3: Lethal phase for the (1:1) genetic interaction ratio.

Cross*	E	EL	LL	EP	LP	PA	AD
II_a 1	5978 (100)	4985 (83.4)	4286 (71.7)	2397 (40.1)	2379 (39.8)	2303 (38.5)	2283 (38.2)
II_a 2	6763 (100)	5518 (81.6)	4932 (72.9)	2786 (41.2)	2711 (40.1)	2657 (39.3)	2630 (38.9)
II_a 3	7254 (100)	6129 (84.5)	5520 (76.1)	5259 (72.5)	2889 (39.8)	2858 (39.4)	2837 (39.1)
II_a 4	6310 (100)	5356 (84.9)	4846 (76.8)	4644 (73.6)	2549 (40.4)	2525 (40.0)	2511 (39.8)

*** For crosses, see Materials and Methods**

*** E: Total No of eggs, EL: early larva, LL: late larva, EP: early pupae, LP: late pupae, PA: pharate adult, AD: adult.**

*** Numbers in brackets show percentages.**

III- Synergistic and maternal effects of *l(3)k3.13/2* and *UAS-Ras^{v12}* genes

As it was revealed from Table 3, *Ras^{v12}* gene showed a strong genetic interaction with the *l(3)k3.13/2* locus. In Table 4, accordingly, data showed the results of three different crosses all were designed for testing the synergistic and/or maternal effects of the *l(3)k3.13/2* when combined with the *UAS-Ras^{v12}* gene. In the first cross (b_1), the source of the *GAL4* i.e. (*N14*) is coming from the mother in a heterozygous state. Whereas the *UAS-Ras^{v12}* is from the father and is in heterozygous state also. In(b2) cross, the male has *UAS-Ras^{v12}* in a homozygous state while in (b3), the female has *N14* in a homozygous one. *y Tb^{+}* larvae is the most interesting phenotype from all crosses.

As presented in Table 4, the combination of *l(3)k3.13/2* and *UAS-Ras^{v12}* in the presence of *GAL 4* source has very deleterious effects on the animals carrying this genotype. Strangely, they led to the death of most of the hatched embryos giving rise to a high percentage of embryonic lethality in all the three crosses. This embryonic lethality percentage ranged from 75.5% to 76.7%. Although, the lethal phase for the (1:1) combination was shown in Table 3 to take place during the early pupal stage of *Drosophila* development, here it was revealed to take place during the embryonic stage. This could be explaiend by assuming such a synergistic effect of *l(3)k3.13/2* on the *UAS-Ras^{v12}*. This synergism was able to increase the lethality percentage and force it to take place earlier in the development. Also, the synergism significantly affected the sex ratio of the eclosed flies as it was tested by Chi-square test (χ^2).

In the reciprocal crosses [cb1; cb2 and cb3] while the *UAS-Ras^{v12}* was coming from the female, the same effect was obtained but in a strong way. The embryonic lethality percent was counted as 80%, 80.4% and 80.1% for cb1, cb2 and cb3 crosses, respectively. Also, sex ratio for the offspring flies was strongly affected in the reciprocal than in the crosses above. So, this could be taken as there are some maternal effects also for the *UAS-Ras^{v12}* gene. These results are in agreement with Rebay *et al*., 2000 and Guo *et al*., 2000).

Table 4: Synergistic and maternal effects of *l(3)k3.13/2* and *UAS Ras^{v12}* genes.

Cross		E	EL	LL	EP	LP	PA	AD*	
								F	M
B_1	N	4898	1200	1171	1160	1155	1146	466	665
	%	100	24.5	23.9	23.7	23.6	23.4	9.5	13.6
b_2	N	5020	1170	1145	1109	1103	1089	439	635
	%	100	23.3	22.8	22.1	22.0	21.7	8.8	12.6
b_3	N	4287	1033	1008	969	960	951	368	570
	%	100	24.1	23.5	22.6	22.4	22.2	8.6	13.3
Cb1 Rec.	N	5188	1038	1022	944	929	918	327	586
	%	100	20.0	19.7	18.2	17.9	17.7	6.3	11.3
Cb2 Rec.	N	4974	975	960	940	935	930	375	540
	%	100	19.6	19.3	18.9	18.8	18.7	7.5	10.9
Cb3 Rec.	N	5365	1067	1057	1030	1019	1014	422	576
	%	100	19.9	19.7	19.2	19.0	18.9	7.9	10.7

1- For crosses: See Materials and Methods.
2- M = males, F = females
3- E = Total number of eggs, EL: early larva, LL: late larva, EP: early pupa, LP: late pupa, PA: Pharate adult, AD: Adult

IV- Effect of temperature on different genetic combinations of *l(3)k3.13/2* and *UAS-Ras^{v12}* genes

Data from Table 5 represents the developmental effects of three different temperatures (18°C, 25°C and 29°C) on the *l(3)k3.13/2* and *UAS-Ras^{v12}* genes as found in different genetic combinations. Four categories of the offspring flies are expected from each of the genetic crosses. They are: (*y Cy$^+$ Tb$^+$*), (*y Cy$^+$ Tb*); (*y$^+$ Cy Tb$^+$*) and (*y$^+$ Cy Tb*). The most interesting category of them is the (*y Cy$^+$ Tb$^+$*) which is yellow in the body colour, straight winged and normal in length and size of the body.

The (*y Cy$^+$ Tb$^+$*) individuals represent *l(3)k3.13/2* in homozygous third chromosome and *UAS-Ras^{v12}* in a heterozygous condition with *N14* on the second chromosome. The other three categories are taken as inner controls in these experiments, especially (*y$^+$ Cy Tb*) class which had no *GAL4* source for the *UAS-Ras^{v12}* activation.

In general, the three different temperatures affected the sex ratio of the eclosed flies from the control category as well as affecting the earlier developmental stages of the other three cateogries. 29°C showed to have the

strongest effect in this respect especially in the reciprocal cross (cb1) when only 120 (29.48) females were counted against 287 (70.52) males. It seems that females are more sensitive to temperature than males may be because of its increased body size containing more moisture than the other individuals. It is very important to mention that no offspring flies of the (*y Cy*$^{+}$ *Tb*$^{+}$) category were obtained in this temperature experiment. This coincides with the results from the previous experiment and insures the synergistic effect of *l(3)k3.13/2* with *UAS-Ras*^{v12} genes. On the other hand there were some very rare escaper flies which had the chance to hatch (eclose) from both (*y Cy*$^{+}$*Tb*) and (*y*$^{+}$ *Cy Tb*$^{+}$) categories. Most of the eclosed flies are belonging to the (*y*$^{+}$ *Cy Tb*) control flies.

V- Dissection of the lethal phenotype

As mentioned previously in Materials and Methods, some of the *y Tb*$^{+}$ mutant larvae were selected (making use of yellow marker) and kept on fresh medium in humidified atmosphere. These very long aged, slowly motioned larvae were dissected under a streomicroscope. Looking for overgrowth phenotype of the different organs of the animal, all the tested larvae showed affected and distorted hematopoietic organs (lymph glands, aorta, …etc). Also, the imaginal discs and brain seemed to be affected.

In case of embryonic lethality, dead embryos were chosen, used for making microscopic preparations after dechorionating in hypochlorite. Most of these lethal embryos showed distorted phenotype especially in head and mouth parts, halters and in its thoracic and abdominal segments in number and polarity.

All the above mentioned results confirm, "at the genetic level", the idea that the tumor suppressor gene [*l(3)k3.13/2*] is required in its normal phase for the inactivation of *Ras*^{v12} oncogene. Also, it is needed for the proper genetic functions of both *oho-31* and *Df(2L)dp-38a* tumor suppressor genes in *Drosophila melanogaster*. Finally, it is hopped to use oncogene and tumor-suppressor mutations to build a *Drosophila* model of step-by-step carcinogenesis by making genetic combinations of the mutants and monitoring their effects on the phenotype.

Because most of the gene functions important for *Drosophila* viability and development show a high degree of evolutionary conservation from the fruit fly to man, this makes a direct comparison of the two systems possible in many cases.

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التوصيف الوراثى الوظيفى للجين *l(3)k3.13/2* فى الدروسوفيلا

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فى أحدى تجارب المسح الوراثى الخاصة بالبحث عن العوامل المميته على الكروموسوم الثالث فى حشرة دروسوفيلا ميلانوجاستر تم اكتشاف الموقع الوراثى *l(3)k3.13/2* والذى بينت الدراسات أنه جين الدروسوفيلا المثيل لأحد جينات البروتينيز فى الفأر والمسئولة عن إتمام بعض المسارات الانزيمية التى تنشط عمليات إنقسام وتشكل الخلايا فى الكائن الحى. وسرعان ماتم اكتشاف أن هذا الجين *l(3)k3.13/2* له خصائص الجينات الكابحة للسرطان حيث أن غيابه أو طفوره يتسبب فى تكوين ونمو الأورام السرطانية فى الأنسجة الليمفاوية وأنسجة تكوين الدم وأجزاء أخرى من أجسام يرقات حشرة الدروسوفيلا ميلانوجاستر. وهذا النمو الكثيف والغير عادى لتلك الأنسجة يؤدى فى النهاية الى موت الكائن المحتوى عليه وهو فى مرحلة الطور اليرقى المتأخر أو حتى فى مرحلة العذراء.

وبناء على ماتقدم فقد صمم هذا البحث بغرض محاولة فهم الدور الوراثى الوظيفى الذى يلعبه جين الدروسوفيلا *l(3)k3.13/2* فى عملية تكوين الأورام فى هذه الحشرة وخاصة تفاعله الوراثى مع الجين الورمى المعروف (Ras^{v12}) وكذلك مع اثنين من الجينات الكابحة للسرطان فى الدروسوفيلا وهما *oho-31* و *Df(2L)dp-38a* . ولهذا الغرض فقد صممت تجربة على نطاق واسع ويمكن تلخيص النتائج المتحصل عليها كما يلى:

* بينت نتائج تحليل الطور المميت للسلالة المحتوية على *l(3)k3.13/2* وكذلك السلالات الأخرى تحت الدراسة أن الموت دائما كان يحدث فى مرحلة الطور اليرقى المتأخر وذلك للسلالات الثلاث *l(3)k3.13/2), (oho-31), (Df(2L)dp-38a)* فى حين أظهرت النتائج ان الموت للسلالتين *N14* و $UAS\text{-}Ras^{v12}$ كان يحدث دائما فى مرحلة العذراء المبكرة من تطور الدروسوفيلا.

* فى حالة تواجد الجرعة الوراثية (١:١) من *l(3)k3.13/2* مع الأربع سلالات الأخرى ارتفعت النسبة المئوية للموت فى جميع هذه التوليفات مما يمكن معه الاقتراح بأن التعبير الجينى للجين *l(3)k3.13/2* لابد من توافره فى حالته الطبيعية من أجل إتمام الجينات الأخرى لوظائفها.

* ومما يثير الدهشة أن وجود *l(3)k3.13/2* بصورة أصيلة على الكروموسوم الثالث جنبا الى جنب مع التركيب الخليط $UAS\text{-}Ras^{v12}/N14$ على الكروموسوم الثانى فى نفس الفرد الواحد من حشرة الدروسوفيلا قد أدى الى أقل نسبة حيوية على الاطلاق. بل ظهرت نسبة الموت العالية جدا هذه فى مرحلة الجنين وذلك بدلا من وقوعها فى مرحلة الطور اليرقى المتأخر أو طور العذراء المبكر كما هو الحال فى حالة الجرعة (١:١) وخاصة عندما درست بالنسبة لليرقات من النوع $Tb^{+}\ y$. وهذه النتائج تشير الى أن هناك تأثيرا تشاركيا للجين *l(3)k3.13/2* مع الجين Ras^{v12} من أجل إحداث هذا التأثير والذى كانت نسبته دائما عالية جدا فى التهجينات التى كانت الأم فيها Ras^{v12} مما يشير الى وجود تأثير أمى لمثل هذا الجين فى حالة تفاعله مع الجين *l(3)k3.13/2*.

* وبتشريح عدد معقول من اليرقات الناتجة من التهجينات السابقة تبين أن أعضاءها الداخلية كانت مشوهة ومختلفة التركيب بنسبة عالية اذا ماقورنت بالأعضاء الداخلية ليرقات المقارنة.

* جميع هذه النتائج المتحصل عليها تؤدى الى الاقتراح بأن التعبير الجينى للجين الكابح للسرطان *l(3)k 3.13/2* ، مطلوب توافره لتثبيط فعل الجين المسرطن Ras^{v12} ، كما أنه مطلوب من أجل قيام الجينين *oho-31* و *Df(2L)dp-38a* بوظائفهما الطبيعية فى حشرة الدروسوفيلا. ولاشك أن هذا النوع من الدراسة يفتح الباب واسعا أمام محاولة فهم عملية التسرطن وتكوين الأورام سواء فى الحشرات أو فى الأورام السرطانية البشرية.

Table 5. Effect of temperature on different genetic combinations of *l(3)k3.13/2* and *UAS-Ras^{v12}* genes.

Cross	°C	OFFSPRING FLIES								Total No. of flies
		y Cy$^+$ Tb$^+$		*y Cy$^+$ Tb*		*y$^+$ Cy Tb$^+$*		*y$^+$ Cy Tb*		
		Females	Males	Females	Males	Females	Males	Females	Males	
b_1	18	-	-	-	-	-	-	254 (41.50)	358 (58.50)	612 (100)
	25	-	-	-	-	-	-	207 (40.75)	301 (59.25)	508 (100)
	29	-	-	-	-	-	-	169 (39.21)	262 (60.79)	431 (100)
b_2	18	-	-	-	5 (0.87)	-	2 (0.35)	242 (42.09)	326 (56.69	575 (100)
	25	-	-	2 (0.41)	3 (0.61)	-	-	196 (40.08)	288 (58.90)	489 (100)
	29	-	-	-	-	-	-	149 (39.31)	230 (60.69)	379 (100)
b_3	18	-	-	-	-	-	3 (0.49)	259 (41.30)	365 (58.21)	627 (100)
	25	-	-	-	-	-	-	213 (39.23)	330 (60.77)	543 (100)
	29	-	-	-	-	-	-	153 (38.15)	248 (61.85)	401 (100)
Cb_1 Rec.	18	-	-	-	-	-	-	255 (35.92)	455 (64.08)	710 (100)
	25	-	-	-	-	-	-	203 (35.00)	377 (65.00)	580 (100)
	29	-	-	-	-	-	-	120 (29.48)	287 (70.52)	407 (100)
Cb_2 Rec.	18	-	-	1 (0.17)	2 (0.34)	2 0.34)	4 (0.68)	241 (40.99)	338 (57.48)	588 (100)
	25	-	-	-	-	-	-	198 (40.41)	292 (59.59)	490 (100)
	29	-	-	-	-	-	-	148 (37.28)	249 (62.72)	397 (100)
Cb_3 Rec.	18	-	-	-	1 (0.16)	-	3 (0.49)	258 (42.23)	349 (57.12)	611 (100)
	25	-	-	-	-	-	-	217 (41.97)	300 (58.03)	517 (100)
	29	-	-	-	-	-	-	133 (37.15)	225 (62.85)	358 (100)

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