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SHORT COMMUNICATION

Characterization of a Unique Lethal Tumorous Mutation in *Drosophila* *

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Spontaneous and induced mutations producing melanotic tumors in *D. melanogaster* have been reported by several different investigators [1]. These tumors, except for variations with respect to the type of tissues that house the tumors, display a common set of characteristics. Normally, the growth pattern of the tumor is restricted to small growths that, depending upon the specific genetic and environmental parameters, reach predictable sizes and patterns of distribution throughout the abdomen of the fly [2, 3]. The genetic component controlling these tumors are generally polygenic in structure. By examining the importance of the different activities of the genes making up the polygenic system, it is possible to subdivide the genes into major and minor genes. The major genes are responsible for the development of the tumor while the minor genes influence the frequency of the tumors. In an article surveying a large number of *Drosophila* tumors, Barigozzi presents data demonstrating that, for the tumors examined, genetic control consists of at least two polygenic systems [2]. One polygenic system apparently triggers the proliferation of the lamellocytes and the other system controls the initiation of melanization. Almost without exception, these polygenic systems were located on the second chromosome with a few residing on the third chromosome. In Barigozzi's study and for those tumors reported in Lindsley and Grell [4], the tumorous condition has been found to be recessive visible, having only occasional mild effects in the heterozygous condition. Finally, almost all of the effects of tumor causing genes have been shown to be temperature sensitive. The effect that temperature has on the frequency of occurrence of tumors differs with different strains of *Drosophila*. However, generally higher temperatures decrease the incidence of tumors [3]. In contrast to the above set of attributes, a melanotic tumor was induced with ethylmethane sulfonate that displayed unique and experimentally useful qualities. The following report describes the isolation, genetic localization and preliminary phenotypic description of the tumor.

The technique used to isolate the *Tum*^{1} mutation utilized a breeding scheme designed to isolate mutations capable of surviving in the mosaic condition, i.e., when the organism is composed of both mutant and wild type tissue. The analysis began by feeding males an ethylmethane sulfonate (EMS) solution for 24 h (EMS and sucrose were dissolved in a pH 7.0 phosphate buffer until a 0.01 M EMS and a 5% sucrose solution was obtained). The treated males were mated to females containing an attached-X chromosome (*yf*:=), homozygous for the recessive markers yellow body (*y*; 1, 0.0) and forked bristles (*f*; 1, 54.4). The EMS treated X-chromosomes of the males were recovered in the hemizygous condition among the males of the F_1 progeny. After scoring the F_1 males for phenotypic abnormalities, all abnormal males and a sample of 10 normal males were mated individually of two *yf*:= virgin females and two *Basc* virgin females that were homozygous for the X-chromosome markers Bar eye (*B*, 1, 57), apricot eye (w^a, 1, 1.5), and major scute (sc) inversions. The progeny of the F_2 crosses were scored for the presence of abnormal *non-Basc* males. If abnormal *non-Basc* males were found, they were mated to *yf*:= virgin females to verify the transmission of a visible mutation. In addition, a maximum of five heterozygous *Basc* virgin females were selected from each vial and individually mated to *Basc* males. The F_3 vials produced by the heterozygous *Basc* females were scored for the presence of *non-Basc* males. Those vials which did not contain *non-Basc* males were scored as lethals.

Examining the specific case from which the *Tum*^{1} mutation was isolated, the F_1 male was phenotypi-

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Table 1. The data report the number of male progeny expressing the parental and recombinant phenotypes resulting from the cross of *Tum*^{l}/*y v f car* females with *y v f car* males. The presence of reciprocal viable products in region II establishes the location of the *Tum*^{l} mutant to be between vermilion (*v*) and forked (*f*). The number of recombinations (15) falling between *v* and *Tum*^{l} (expressed as viable v^{+}f phenotypes), divided by the total number of recombinations in region II (124) provides a means of calculating the map location of *Tum*^{l} to the right of vermilion

Genotypes	Parental		Single crossovers						Double crossovers						Triple crossovers	
Crossover regions			I		II		III		I & II		II & III		I & III		I, II & III	
Genetic markers	+	y	+	y	+	y	+	y	+	y	+	y	+	y	+	y
	+	v	v	+	+	v	+	v	v	+	+	v	v	+	v	+
	+	f	f	+	f	+	+	f	+	f	f	+	f	+	+	f
	+	car	car	+	car	+	car	+	+	car	+	car	+	car	car	+
Number of each class	0	489	302	0	12	104	0	17	18	1	0	1	10	0	1	2

Table 2. The above data reports the viability of 6 genotypes at restrictive (29° C) and permissive (18° C) temperatures. The percentages reflect the distribution of the tumorour and non-tumorous phenotypes in those flies containing a *Tum*^{l} gene. Since *Tum*^{l} is essentially lethal in the hemizygous condition, male percentages are based upon the number of surviving heterozygous *Basc* females as an estimate of the expected number of *Tum*^{l} males at the two temperatures. At 29° C, only 0.6% of the expected males survive, while at 18° C, 62% survive

Temperature	Male progeny			Female progeny		
	Basc	Non-Tum^{l}	Tum^{l}	$\frac{\text{Basc}}{\text{Basc}}$	$\frac{\text{Tum}^{l}}{\text{Basc}}$	$\frac{\text{Tum}^{l}}{\text{Basc}}$ (tum)
29° C	532	1 0.2%	2 0.4%	533	6 1.5%	402 98.5%
18° C	456	215 (75)* 47.1%	68 14.9%	455	576 93.7%	39 6.3%

* These 75 males displayed leg, wing, and bristle abnormalities but not the tumor expression

cally normal. The F_1 male, when crossed to the *yf:=* females produced 16 *yf:=* females and no wild type males. This suggested that the F_1 male contained a mosaic recessive lethal mutation. All of the twenty F_1 heterozygous *Basc* females contained large tumors within the abdomen. All twenty females were remated to *Basc* males. The results of this cross indicated that the lethal effect and the tumorous effect were the result of the same mutant chromosome. Of the 125 heterozygous *Basc* females, all contained large dark abdominal growths, whereas all of the homozygous *Basc* females contained no such growths. In addition, a single *non-Basc* male was found. This male was extremely weak and contained large growths within it's abdomen.

In light of the above results, further crosses were carried out designed to determine (1) if the lethal and tumorous phenotypes were controlled by the same mutational event and (2) the approximate map position(s) for the mutant(s). Mapping of the EMS induced mutational event was accomplished by employing the standard four point recombinational analysis. Virgin females, heterozygous for the *Tum*^{l} containing chromosome and the *Basc* chromosome were crossed to males carrying an X-chromosome marked with the following markers; yellow body (*y*, 1, 0.0), vermillion eyes (*v*, 1, 33.0), forked bristles (*f*, 1, 56.7), and carnation eyes (*car*, 1, 62.4). The *non-Basc* heterozygous females which carried both the *Tum*^{l} and marker chromosomes were crossed to males carrying the marker chromosome. The progeny from this cross were scored for the presence of recombinant males displaying cross over combinations between the four recessive visible markers on the test chromosome and the lethal and/or tumorous marker(s) from the *Tum*^{l} chromosome. From Table 1, it can be seen that the lethal and tumorous phenotypes never assort independently, i.e., no viable tumorous males were found. This supports the hypothesis that a single gene mutation controls both

the lethal and tumorous phenotypes. In addition, since 15 out of the 124 recombinants that fell within the *v* to *f* region arose to the left of the *Tum*^{1} gene, it can be calculated that the mutant gene resides at 35.8 map units on the X-chromosome.

Because the majority of genetically controlled tumors in *Drosophila* have been shown to be temperature sensitive and since EMS induces a high frequency of temperature sensitive mutations [5] both the lethal and phenotypic expressions of *Tum*^{1} were examined for temperature sensitive effects. The expression of the lethal and tumorous phenotypes were evaluated in cultures grown at 18° C and 29° C. The cultures arose from crossing virgin females heterozygous for the *Tum*^{1} and *Basc* chromosomes with Basc hemizygous males. The F_1 progeny produced under the two different temperatures were screened for the presence of (1) *non-Basc* males and (2) melanized growths within the heterozygous females. The results of these crosses are presented in Table 2.

From the data, it is obvious that lethality and the dominant tumorous phenotypic effect are temperature sensitive. However, unlike other *Drosophila* melanotic tumors, 29° C enhances the expression of the phenotypes while 18° C suppresses their expression. At the restrictive growth condition of 29° C, the tumorous genotype is lethal in the hemizygous males and triggers the formation of tumors in essentially 100 percent of the heterozygous females. Dropping the temperature to the permissive growth condition of 18° C, allows 62 percent of the hemizygous males to survive (using the number of *Basc* males as an indication of the expected number of the *Tum*^{1} males). In addition, only 6.2 percent (39/615) of the heterozygous females and 24 percent (68/283) of the surviving hemizygous males displayed detectable tumors at 18° C.

Larva and pupa, displaying the tumorous phenotype, were isolated from cultures produced by mating females heterozygous for the *Tum*^{1} mutation and the balancer chromosome *Basc* to *Basc* males. These cultures contained both adult heterozygous *Basc* females displaying large abdominal tumors, and numerous larvae and pupae with melanotic growths. There are superficially two classes of tumorous larvae and pupae; those that show limited tumor growth and those with extensive multiple tumors. These two larval and pupal phenotypic classes may represent heterozygous females and hemizygous males. Gynandromorphs were produced by mating females heterozygous for the *Tum*^{1} mutation coupled to the markers white eye (*w*, 1, 1.5), and singed bristles (*sn*, 1, 21.0), and the balancer chromosome *Binscy* to males containing the Catcheside ring-X chromosome (R(1)2, $w^{vC)}$. The progeny of the cross were examined for the presence of gynandromorphs with singed tissue. Numerous gynandromorphs were found which contined large patches of singed tissue, indicating that the *Tum*^{1} mutation is viable in the mosaic condition. The expression of the *Tum*^{1} mutation in hemizygous tissue in gynandromorphs is extremely severe, often displaying multiple growths in the abdomen and occassionally in the thorax.

Due to the unique aspects of this mutation, work is now in progress to define the temporal and spacial action of the mutant gene and to characterize the histological and developmental consequences of the tumorous lethal mutation.

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