Abstract

In 2001, researchers at UCSD found that over 77% of human disease genes had a homologous *Drosophila* sequence. More recent research shows that the actual figure may be even more significant. Cancer, Down's Syndrome, and hemophilia are all diseases whose genes have been found within the Drosophila genome, and the implications are astounding: by studying Drosophila, more effective gene therapy could be developed. This project furthered our knowledge of six more Drosophila genes whose functions have not yet been elucidated. Drosophila provides us with the means to study disease genes that are too complicated to analyze within the human body, and this experiment demonstrated one way to explicitly probe the role of a gene in a particular pathway. P-element mutagenesis was used both to disrupt a certain gene on the left arm of the second chromosome and to induce mitotic recombination in the Drosophila eye, and the resulting phenotype was examined. Whether a gene partakes in eye development is thus determined, and its specific effects (if any) are visualized. The recombination distances of these genes were also calculated and compared with theoretical values, and there were some clear discrepancies.

Introduction

Drosophila melangaster is an ideal model organism because it has been the subject of extensive genetic research for almost a century. Its entire genome has been sequenced, many aspects of its genetic structure, function and regulation are known, and there are numerous striking similarities between *Drosophila* genes and human genes. Functional analysis demonstrates that developmental processes are highly conserved between invertebrates and vertebrates. Thus, by studying genetic pathways in *Drosophila*, one can gain valuable insight into corresponding mechanisms in human beings. Furthermore, many tools geneticists need (i.e. balancers, P-element stocks, etc.) are already available for *Drosophila*. One could simply contact a stock center to obtain some of the flies one needs and save much valuable time. The rapid life cycle of *Drosophila* compared to that of other model organisms such as mice is another asset to scientists; desired crosses can be completed in weeks, and abundant progeny is available. The latter characteristic of *Drosophila* reproduction is particularly important in this project, as potentially thousands of flies have to be screened in order to obtain recombinants.

Mitotic recombination is induced in certain progeny after the introduction of several factors: flippase, FRT, and the P-element. The P-element originally carries a sequence that encodes for an enzyme, transposase, that allows it to excise (and then reinsert) itself throughout the genome. One can modify the P-element and replace the sequence with those of one's choice. This enables one to track the P-element (and therefore, a particular gene one is interested in) as marker genes (usually a color such as rosy or mini-white) can be inserted into it. The transposase would then have to be provided because the P-element can no longer produce it. However, what actually controls mitotic recombination is the flippase/FRT mechanism. Flippase, an enzyme first isolated in yeast, causes recombination to occur at two FRT sites (one needs to be present on each chromosome in a corresponding pair for crossing-over to take place). The goal of this experiment is to express the phenotype in the eye. Therefore, the flippase-encoding sequence, FLP, is fused to the enhancer of the eyeless gene, which is activated only in the eye. Balancers, which "reduce or eliminate recombination between a mutation-bearing chromosome and a homolog that carries the wild-type allele" are incorporated into some of the crosses to avoid undesired mitotic recombination.

A mutation is defined as a change in DNA structure that influences protein function. For the purposes of this project, mutations are caused by P-element insertional mutagenesis. A P-element is a transpodon that is capable of jumping around the genome and inserting itself into other sequences. When a P-element is inserted into a gene, it disrupts the expression of that gene; if it is placed in the exon or enhancer region of a gene, it directly affects coding activity and if it is placed in the intron of a gene, it affects splicing activity. Mutations result in visible phenotypes that allow one to track the presence of a gene. P-element insertional mutagenesis is used because it is more efficient and

more traceable than EMS/X-ray induced mutagenesis. Another kind of mutation, known as the Minute mutation, is also used in the experiment to preferentially express the homozygous lethal in the eye. A Minute mutation adversely affects the function of ribosomal proteins, and thus leads to slower development in heterozygotes, and death in homozygotes.

Drosophila melangaster has large compound eyes each containing about 800 similar facets (or ommatidia). The eye forms from the imaginal disc, which consists of a small cluster of cells fated to become photoreceptors. Differentiation occurs with the formation of a morphogenetic furrow. The furrow moves from posterior to anterior, and stimulates the cells both to divide and to take on particular roles. Cells ahead of the furrow continue to proliferate, while those in the furrow do not, and those behind the furrow begin to differentiate. Ommatidia are formed by the differentiation of the photoreceptor cells; each ommatidium is comprised of twenty cells falling into twelve cell types: eight photoreceptor cells of three types (the outer cells: R1 - R6, the apical central R7 and the basal central R8), and twelve accessory cells of six types (see Figure 1). The disruption of a gene that participates in any step of this process will thus lead to mutant eye phenotypes. For instance, the rough phenotype represents a loss of ommatidia organization, and the gene disrupted can therefore be inferred to be involved in the development of ommatidia and the construction of the eye (see Figure 2)

One could determine the location of these genes quickly and effortlessly through BLAST, and find the functions of the transcribed proteins in a matter of seconds on a public database such as NCBI. Yet, the data provided by these sources are often extrapolated and/or not completely conclusive. For instance, one may know that judging from the functions of homologous genes in other organisms, the *vas* gene encodes for ATP-dependent helicases. However, this observation does not give explicit information on how or when this gene affects the pathway(s) it's involved in. By expressing a visible phenotype in the eye, one could clearly see the actual effects of a mutation on the *vas* gene. Deeper insight into the specific functions of *vas* may be gained by closely following the development of the flies, as well as examining the kinds of phenotype that are produced. This approach is especially valuable when

Figure 1. Top: The wildtype Drosophila eye. Bottom: The rough phenotype.

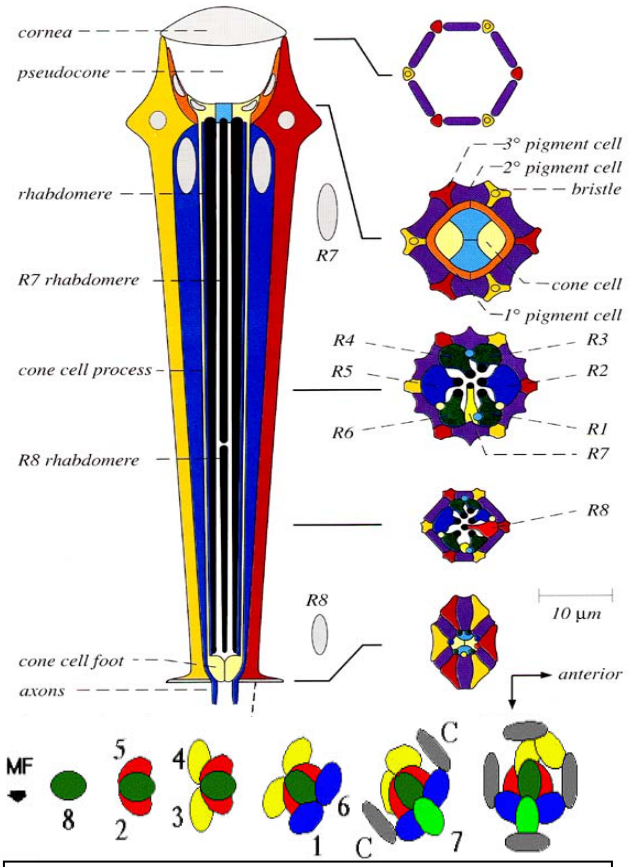


Figure 2. Top: Components of an ommatidium. Bottom: Differentiation of photoreceptor cells lead to the formation of an ommatidium.

studying genes that participate in vital processes in the organism; homozygous mutations of these essential genes in normal scenarios would lead to death. However, when localized in the eye, the function of these lethal genes can be probed.

This project also serves as a model for research on genetic diseases. Acquiring a better understanding of lethal genes is crucial to developing more effective gene therapy techniques for diseases such as cancer. Frequently, cancer is caused by heterozygosity followed by loss-of-function of the remaining allele. In this project, this process is emulated by disrupting a gene using P-element mutagenesis. The analogy is not at all far-fetched because some of the genes studied by the project have human homologs that have been shown to be oncogenes. Furthermore, many mutations caused by P-element insertions are loss-of-function mutations.

Materials and Methods

My project studied genes on the left arm of the second chromosome (2L) over a 10-week span. Every two weeks, a new factor was introduced, until all those required for mitotic recombination to occur in the eye were present.

In the first four weeks, the eyeless-gene bound FLP sequence, FRT, and the P-element were all introduced into the stocks. Flies needed for this cross was ordered from the stock center in Bloomington, Indiana.

During the fifth and sixth weeks, mitotic recombination was induced in the eye, and the resulting progeny had mosaic eyes; their eyes consisted of a large (non-recombinant and heterozygous) orange clone, and two smaller twin spots. The red spot is made up of the homozygous mutant recombinant cells, while the white spot is made up of the homozygous wild-type recombinant cells.

In weeks 7 and 8 the white spot is preferentially expressed through the coupling of a Minute mutation with the non-lethal allele. The presence of the Minute mutation caused both the heterozygous (dark red) cells to grow slower than the homozygous lethal (orange-red) cells, and the homozygous wild-type (red) cells to die. The progeny of this cross thus had a large white clone in their eyes, and some exhibited mutant phenotypes.

During the last two weeks, a balanced stock was be set up. The purpose of this cross is to retain the phenotype; the previous cross can be easily recovered from this one. (Figure 3)

Adult flies were mounted on glass slides with clear nail polish. Pictures were digitally acquired. Light microscope pictures were taken of all the small clones and large clones, while SEM (scanning electron microscope) pictures were taken of only small clones and large clones displaying a mutant phenotype. A light microscope picture sufficed as documentation of wildtype alleles, but mutant alleles needed to be examined more closely for structural features that might explain what factors caused the deformity. Light microscope pictures were taken with the Nikon D100 camera, and SEM pictures were taken with the Hitachi S-2460N scanning electron microscope.

Results

Nine stocks were examined in this study, and six were successfully crossed; 14985, 15094, 15106 all failed to produce small clone (and hence large clone) progeny. The presence of a background mutation was detected in stocks 14926 and 14985, and contributed at times to a false rough phenotype. 14956 and 15096 demonstrated a considerable variability in both small clone and large clone phenotypes. The recombination distances of each stock were calculated by counting both the number of mosaic flies and the total number of flies (Stuyvesant method). The results were then compared with theoretical data, and were found to be mostly consistent (See Appendix).

14956 and 15096 were the only stocks among those examine to display a mutant phenotype (Figure 4). The mutant clones are cell-lethal, and hence are not present; the eye is shrunken as a result. The severity of lethality varies, with the progeny of male 14956B being the most affected. Light microscope/SEM pictures show considerable distortion of eye due to cell lethality. Either only these genes partook in the eye-development pathway, or the P-element was inserted into a redundant gene.

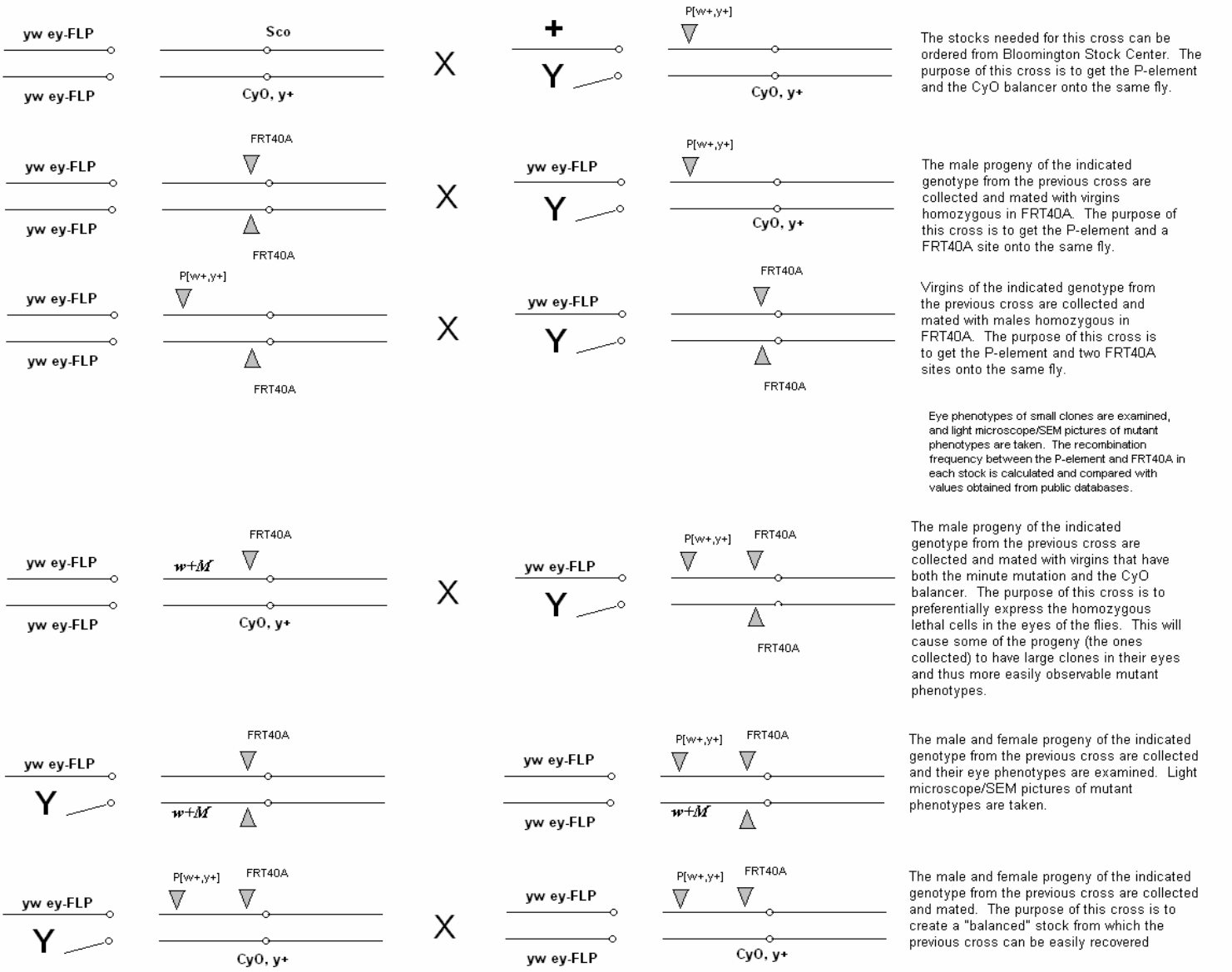


Figure 3. The crossing scheme used for the experiment.

A redundant gene is one that codes for a similar product as another gene, and thus is not vital. The disruption of a redundant gene still results in a wildtype eye because another gene will continue to make proteins that satisfy the same need.

The range of phenotypes obtained from 14956 and 15096 could be explained by the presence of a background mutation on the 2L chromosome arm. This mutation bestows a rough phenotype on non-recombinant flies, and was detected in many stocks (but frequently only in 14898 and 14926). The mutation caused by P-element mutagenesis resulted in a cell-lethal phenotype, and if a background mutation was also present, the phenotype is especially severe. This hypothesis is consistent with the fact that the background mutation generates a rough phenotype – which implies disruption of ommatidia development/organization (Figure 5).

14985 had a theoretical recombination distance of 0.15 map units. Thus, it was not particularly surprising that no small clones hatched out; the P-element was inserted too close to the FRT40A site for frequent recombination to occur. However, recombination took place in other stocks with small recombination distances (e.g. 14970) and so it can be assumed that if more flies were collected, recombinants might have been found.

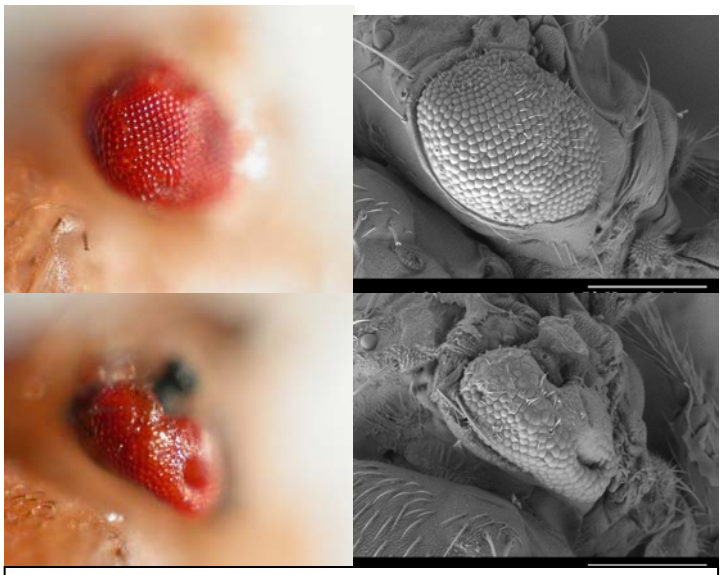


Figure 4. Top: 14956B Small Clone. Bottom: 14956B Large Clone. (Cell-lethal)

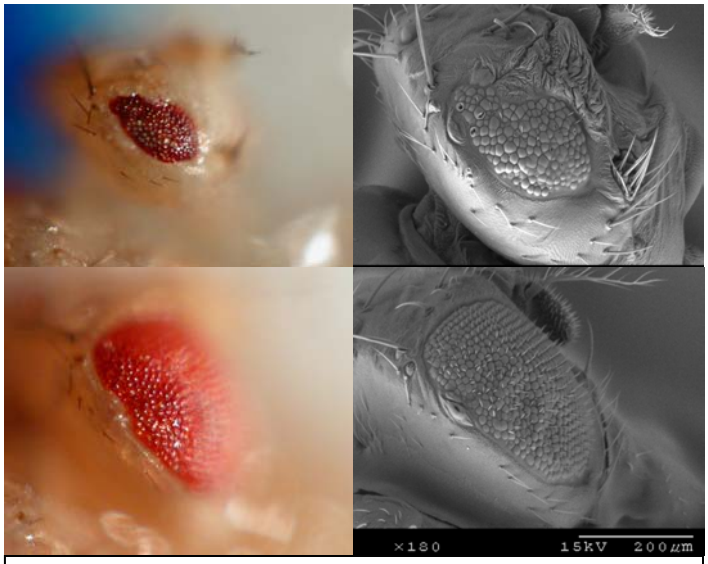


Figure 5. Top: 15096A Large Clone. Bottom: 15096B Large Clone. (Cell-lethal) Note the variability of phenotype.

15094 represents a significant deviation of empirical data from theoretical projections. According to information gathered from Flybase, the two P-element insertions in 15094 are 47.30 map units, and 47.28 units away from the FRT40A site, respectively. Though large recombination distances usually correspond to high recombination frequencies, no small clone flies were found for this stock. This occurrence suggests that perhaps the P-element insertion occurred on another chromosome arm, and not 2L as indicated by Bloomington. It is also possible that the two P-element insertions, being so close together, each affected the other's recombination events.

The genotype of the progeny of 15106 in the earlier crosses did not match expected results. Thus, the presence of some inherent mutation and/or mislabeling is suspected, and the stock was abandoned.

Discussion

One of the goals of this project was to provide a foundation for future studies. The experiment identified genes that are essential for the eye-development pathway, and more in-depth functional analysis could be performed on them at a later date. The project also disproved some data gathered from public databases, which would probably have gone unquestioned otherwise. For instance, 15094 now seems like a stock very worthy of further analysis because it had an effective recombination distance of 0.00 map units rather than 47.30 map units.

Though studying mutant phenotypes expressed in the adult eye of *Drosophila melangaster* yields valuable insight into the function of certain genes, the intricate effects of the genes on the molecular level can only be probed in the larval stage; the development of the eye starts with the 3rd instar eye disc, and the workings of the gene must be traced to that phase. Moreover, many mutations actually occur during the early stages of *Drosophila* development. It is therefore critical that the genes of interest uncovered by this project be studied at different periods of the *Drosophila* life cycle.

The span of this project was limited to ten weeks. Consequently, some of the more difficult stocks (e.g. 14985) were not crossed. An extension of this project would thus be to successfully induce mitotic recombination in the stocks that did not work. This endeavor is worthwhile because sometimes the P-element is inserted into a gene of interest. In the case of 14985, the P-element was placed into the Grp-1 (grapes) gene, which is responsible for encoding a product with serine/threonine kinase activity that is involved in DNA damage response.

Wildtype Stocks:

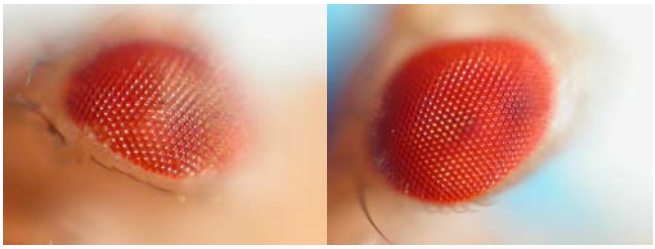
14898

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG01651

Insertion Site: 035C01

Molecular Information: vas (vasa). Also known as cgt and fs(2)ltoRJ36. The P-element is inserted in the 2nd intron of vasa. Vasa is thought to encode a product with RNA helicase activity and involved in pole plasm assembly which is localized to the cytoplasm; it is expressed in the adult (egg chamber and testis), embryo (pole cell and pole granule), larva (pole cell), ovary (female germline stem cell , germarium , nurse cell , oocyte and 2 other listed tissues), cyst cell , male germline stem cell and spermatocyte) and pole granule). Vasa has 34 recorded mutant alleles. Loss-of-function mutations have been isolated which affect the egg and the embryonic abdominal segment and are maternal effect male sterile, maternal effect female sterile and maternal effect recessive lethal. Twenty-four functional analysis studies have been done on vasa.

Description of Phenotype: Red/White mosaic eyes are observed for small clone analysis. Light microscope pictures denote a wildtype phenotype. Red/Red mosaic eyes are observed for large clone analysis. The homozygous mutant twin spot clones are difficult to visualize due to little difference in colors (they are of only a slightly darker red color). Light microscope pictures denote a wildtype phenotype.



14970

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG08808

Insertion Site: 037F01

Molecular Information: CG10337. The P-element is inserted in the 1st exon of CG10337. There are no recorded mutant alleles for CG10337. No functional analysis studies have been done on CG10337.

Description of Phenotype: Red/White mosaic eyes are observed for small clone analysis. Light microscope pictures denote a wildtype phenotype. Red/Red mosaic eyes are observed for large clone analysis. The homozygous mutant twin spot clones are difficult to visualize due to little difference in colors (they are of only a slightly darker red color). Light microscope pictures denote a wildtype phenotype.

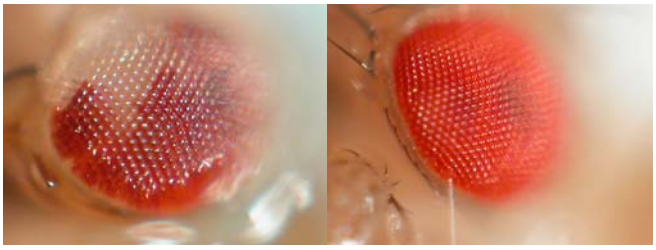
14962

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG08607

Insertion Site: 039C01

Molecular Information: CG31988. Also known as CG17401. The P-element is inserted in the 1st exon of CG31988. CG31988 is thought to encode a product with putative structural constituent of cytoskeleton putatively involved in cell motility. CG31988 has no recorded mutant alleles. Two functional analysis studies have been done on CG31988.

Description of Phenotype: Red/White mosaic eyes are observed for small clone analysis. Light microscope pictures denote a wildtype phenotype. Red/Red mosaic eyes are observed for large clone analysis. The homozygous mutant twin spot clones are difficult to visualize due to little difference in colors (they are of only a slightly darker red color). Light microscope pictures denote a wildtype phenotype.



14926

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG31678[KG07890]

Insertion Site: 038E01

Molecular Information: CG31678. Also known as CG17465 and CG17466. The P-element is inserted in the 1st exon of CG31678. CG31678 is thought to encode a product with putative structural molecule activity. CG31678 has one recorded mutant allele.

Three functional analysis studies have been done on CG31678.

Description of phenotype: Red/White mosaic eyes are observed for small clone analysis. Light microscope pictures denote a rough phenotype. Suspected background mutation. Red/Red mosaic eyes are observed for large clone analysis. The homozygous mutant twin spot clones are difficult to visualize due to little difference in colors (they are of only a slightly darker red color). Light microscope pictures denote a wildtype phenotype.

14985

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG09493

Insertion Site: 039F01

Molecular Information: Grp-1 (Grapes). The P-element is inserted in the second intron of grapes. Also known as Chk1, Pk36A and lemp. Grapes is thought to encode a product with protein serine/threonine kinase activity involved in DNA damage response, signal transduction resulting in cell cycle arrest which is a component of the nucleus ; it is expressed in the ovary (oocyte). Grapes has twenty-nine recorded mutant alleles. Loss-of-function mutations have been isolated which affect the maternal effect embryonic cycle 11 to 14 , the maternal effect mitotic telophase , the maternal effect mitotic metaphase and 3 other listed tissues and are maternal effect recessive lethal, mitotic, recessive female sterile and viable. Among findings on grp mutants, molecular characterization of grp and cellular analysis of grp mutants. Among findings on grp function, mutations in grp block the morphological and biochemical changes that accompany the midblastula transition (MBT), lead to a continuation of the maternal cell-cycle programme and disrupt DNA-replication checkpoint control of cell-cycle progression. Seven functional analysis studies have been done on grapes.

Description of Phenotype: N/A

15094

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9894[KG00761a] P{SUPor-P}CG3117[KG00761b]

Insertion Site: 023B01, 023B03

Molecular Information: CG9894, CG3117. The P-element is inserted in the 1st exon of CG9894. There are ten recorded mutant alleles for CG9894. No putative conserved domains have been detected and no significant similarities have been found in a protein-protein BLAST of the products of CG9894. The function of CG9894 is unknown. One functional analysis study has been done on CG9894. The P-element is inserted in the first exon of CG3117. CG3117 has no recorded mutant alleles. The function of CG3117 is unknown. No functional studies have been done on CG3117.

Description of Phenotype: N/A

15106

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9894[KG06482a] P{SUPor-P}CG18558[KG06482b]

Insertion Site: 023B01, 023B03

Molecular Information: CG9894, CG18558. The P-element is inserted in the first exon of CG9894. There are ten recorded mutant alleles for CG9894. No putative conserved domains have been detected and no significant similarities have been found in a protein-protein BLAST of the products of CG9894. The function of CG9894 is unknown. The P-element is inserted in the first exon of CG18558. CG18558 is thought to encode a product with putative transferase activity putatively involved in protein amino acid glycosylation. CG18558 has one mutant allele. One functional study has been done on CG18558.

Description of Phenotype: N/A

Cell-lethal Stocks:

14956:

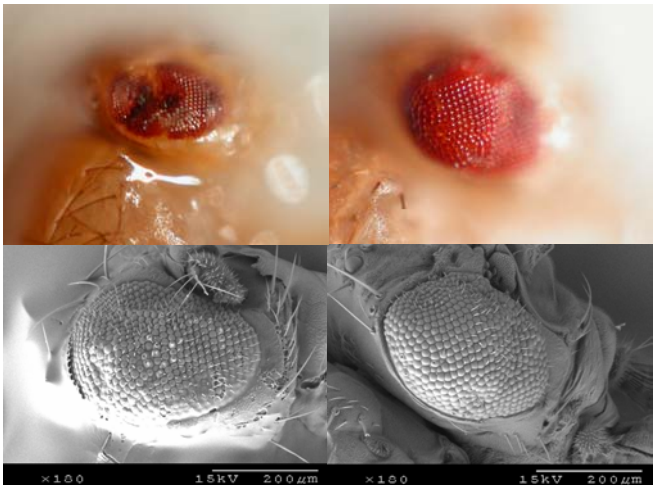
Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG08523

Insertion Site: 021B04

Molecular Information: kis (kismet). Also known as BEST:GM02209, CG18326, CG3660 and l(2)07812.

The P-element is inserted into the 2nd intron of kismet. Kismet is thought to encode a product with DNA helicase activity putatively involved in regulation of transcription from Pol II promoter which is a component of the nucleus. Kismet has 67 recorded mutant alleles. Loss-of-function mutations have been isolated which affect the adult somatic clone cell autonomous abdominal segment 5 , the somatic clone metathoracic leg , the somatic clone metathoracic tarsal segment 1 to 5 and 16 other listed tissues and are recessive lethal and homeotic recessive somatic clone visible. Five functional analysis studies have been done on kismet.

Description of Phenotype: Red/White mosaic eyes are observed for small clone analysis. Light microscope/SEM pictures denote a rough phenotype. Red/Red mosaic eyes are observed for large clone analysis. The mutant clones are cell-lethal, and hence are not present; the eye is shrunken as a result. The severity of lethality varies, with the progeny of male 14956B being the most affected. Light microscope/SEM pictures show considerable distortion of eye due to cell lethality. Variability of phenotype in both small clones and large clones.



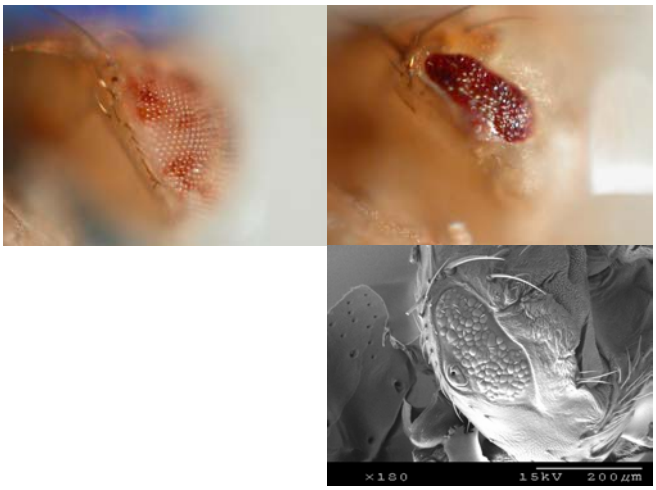
15096

Genotype: P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG01610

Insertion Site: 025F02

Molecular Information: No genes known. The P-element is 3654 bases upstream from the 1st exon of CG14010. Cg14010 is thought to encode a product putatively involved in cell adhesion. CG14010 has no recorded mutant alleles. No functional analysis studies have been done on CG14010.

Description of Phenotype: White/Red mosaic eyes are observed for small clone analysis. Male progeny consistently emerge after female progeny; this reversal suggests that the gene disrupted is involved in the development of female flies. Light microscope pictures denote a wildtype phenotype. Red/Red mosaic eyes are observed for large clone analysis. The mutant clones are cell-lethal, and hence are not present; the eye is shrunken as a result. Male progeny consistently emerge after female progeny; this reversal suggests that the gene disrupted is involved in the development of female flies. Also, only a few large clone flies hatched out for each single male cross - the mutation may be pupa lethal as well. Variability of phenotype in both small clones and large clones.



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Utpal Banerjee (UCLA)

Jiong Chen (UCLA)

Gerald Call (UCLA)

C. Elegans II, Donald L. Riddle, Thomas Blumenthal, Barbara J. Meyer, James R. Priess

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A Systematic Analysis of Human Disease-Associated Gene Sequences in Drosophila melangaster, Lawrence L. Reiter (UCSD)

Human Disease Genes, Gerardo Jimenez-Sanchez (Johns Hopkins University, HHMI)

From Sequence To Phenotype: Reverse Genetics In Drosophila Melanogaster, Melissa D. Adams and Jeff J. Sekelsky

Untitled Document, Kevin Moses (Emory University)

Drosophila Eye Development, Suja Spanaval (George Mason University)

Appendix

Stock Number and detailed genotype (eg. 13945 on 3L y^{1}; P{$y^{+mDint2}$ $w^{BR.E.BR}$=SUPorP}$CG7986^{KG03090}$ ry^{506}/TM3,Sb^{1} Ser^{1})	Cytological location of P-element insertion site (eg. 66B13)	Recombination map location of P-element insertion site (eg. 27.6)	Theoretical recombination map distance between P[w+] and FRT (for FRT80B (3L), its recombination position is at 47) (eg. 47 – 27.6 = 26.4 m.u.)	If the total progeny will be 500, what will be the number of FRT-P[w+] recombinant progeny? (eg. (26.4% X 500) / 2 = 66)	#Mosaics	#Total	Empirical Recombination map distance between P[w+] and FRT.
14898 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG01651/SM6a; ry[506]	035C01	51	54.75 – 51.00 = 3.75 m.u.	(3.75% X 500)/2 = 9.375 **10**	11	1061	2.073516
14970 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG08808/CyO; ry[506]	037F01	54.10	54.90 – 54.10 = 0.80 m.u.	(0.80% X 500)/2 = **2**	4	1307	0.612089
14962 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG08607/CyO; ry[506]	039C01	54.50	54.90 – 54.50 = 5.00 m.u.	(5.00% X 500)/2 = 12.5 **13**	5	1151	0.86881
14985 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG09493/CyO; ry[506]	039F01	54.75	54.90 – 54.75 = 0.15 m.u.	(0.15% X 500)/2 = 0.375 **1**	0	917	0

14926 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG31678[KG07890]/CyO; ry[506]	038E01	54.60	54.90 – 54.60 = 0.30 m.u.	(0.30% X 500)/2 = 0.750 **1**	5	1019	0.981354
14956 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG08523/CyO; ry[506]	021B04	0.2	54.90 – 0.2 = 54.70	(54.90% X 500)/2 = 137.25 max is 50%, so **125**	132	803	32.87671
15094 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9894[KG00761a] P{SUPor-P}CG3117[KG00761b]/In(2LR)Gla	023B01, 023B03	7.60 7.62	54.90 – 7.60 = 47.30 54.90 – 76.2 = 47.28	(47.30% X 500)/2 = 118.5 **118** (47.22% X 500)/2 = 118.05 **119**	0	641	0
15096 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}KG01610/SM6a; ry[506]	025F02	17.5	54.90 – 17.5 = 37.40	(37.40% X 500)/2 = 93.5 **94**	106	1046	20.26769
15106 on 2L y[1]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG9894[KG06482a] P{SUPor-P}CG18558[KG06482b]/In(2LR)Gla	023B01, 023B03	7.60 7.62	54.90 – 7.60 = 47.30 54.90 – 76.2 = 47.28	(47.30% X 500)/2 = 118.5 **118** (47.22% X 500)/2 = 118.05 **119**	N/A	N/A	N/A

15094: See Data for May 20th, 2004