Mutation Notes

Cell lethal mutations associated with the *Drosophila* homolog of CRK-7.

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Cdc2-related kinase 7 (*CRK7*) is part of the ErbB2 amplicon in many breast cancers (Neve *et al*., 2006; Chin *et al*., 2006). CRK7 is known to be overexpressed in breast cancer cells and may be a marker of aggressive cancers (Capra *et al*., 2006). In addition, CRK7 colocalizes with the spliceosome and may be a novel, conserved link between the transcription and splicing complexes (Ko *et al*., 2001). We identified CG7597 (4,773 bp) as the *Drosophila melanogaster* ortholog of the human CRK7 gene using *blastp* (Pearson, 1990). The *Drosophila* cognate has 266 amino acid identities (63%) and 321 amino acid similarities (76%) in a 419 amino acid region at the terminal end of the *Drosophila* protein when compared to its human cognate. To determine the structure of the *Drosophila* gene we isolated and sequenced a cDNA (SD04681) from a cDNA library made from the Schneider cell line. The *Drosophila* gene has at least seven exons. Using the cDNA as a probe to determine the embryonic expression pattern, we detected maternally deposited RNA followed by zygotic ubiquitously-expressed transcription throughout embryogenesis. Using a modified pWiz vector (Lee and Carthew, 2003), we made an RNAi construct for CG7597 containing 750 bp (from bp 1,866 to bp 2,615, primarily exon 4 of CG7597-RA) in the direct and inverted orientation. We obtained 27 RNAi lines: 12 on the 2nd, 10 on the 3rd, and 5 on the X chromosome.

We mated CRK7-pWiz RNAi males to virgin females of two Gal4 drivers and raised the progeny at 25ºC. When mated to an en-Gal4 driver (BL-8860), seven of nine tested lines were lethal. When mated to MS1096 (BL-6356), which expresses Gal4 in the dorsal wing pouch (Capdevila and Guerrero, 1994), nine tested lines had blackened, atrophied wings; nine more had reduced, crumpled wings; and four lines had curled wings. One line had no phenotype. In all cases, wings of male progeny were more strongly affected than wings of female progeny. A CRK7-UAS overexpression construct driven by MS1096 resulted in a curled wing phenotype similar to that of the weaker RNAi lines.

In order to create a knockout mutation in CG7597, we imprecisely excised the P-element KG05512 (Bellen *et al*., 2004). We have 27 putative excisions, 11 of which are lethal. Characterization of the lethal excisions is forthcoming.

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T**wo new mutants from an EMS screen of *D. erecta*.**

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Procedure

We used 600 male *D. erecta* flies that were 3-4 days old to perform an EMS mutagenesis screen as described by Lewis and Bacher (1968). Two important changes were made to the method. First, the dehydration step was shortened to 90 minutes, because the *D. erecta* flies died after a few hours of dehydration. Second, we fed the flies the EMS/sucrose solution for six hours before allowing them to recover on standard cornmeal food. We set up mass matings in bottles for the first cross of EMS-treated males with WT virgins and then mated the progeny pair-wise for the F1 cross.

Results

We found a curly-winged fly that appears to be dominant and autosomal, which we have designated Cy[1]. We found a yellow body mutation that appears to be recessive and X-linked, which we have designated y[1]. Both mutants have been deposited in the Tucson Stock Center. Other mutations found in this screen are currently being analyzed.

References: Lewis, E.B., and F. Bacher 1968, Method of feeding ethyl methane sulfonate (EMS) to *Drosophila* males. Dros. Inf. Serv. 43: 193.

New wing and eye mutations in *D. subobscura*.

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To study the viability in homozygous condition of O chromosomes of *D. subobscura* from Tibidabo (Barcelona), appropriate crosses using the lethal balanced strain *Va*/*Ba* were carried out (Sperlich *et al.*, 1977; Mestres *et al.*, 1990). One of the chromosomal lines obtained in homozygous condition presented a wing mutation: wings were longitudinally undulated like roofing material. The mutation is recessive and located in the O chromosome. We have called this mutation *ur* (*uralita*). It can be qualified as rank RK 1.

In another chromosomal line, several individuals appeared with an eye mutation. The eyes were dramatically reduced and the remaining facets were necrosed. The places where the eyes must

be located were substituted by other tissues of the head (Figures 1, 2 and 3). This mutation resembles Pax 6 mutants of *D. melanogaster*. Ectopic ocelli could be observed. It was impossible to obtain a mutant line, because these flies were blind and *D. subobscura* species needs light for being active (Krimbas, 1993). Thus, these mutant flies cannot intercross. For this reason it was impossible to carry out the genetic analysis of this mutation. We have named it *un* (*ulls necrossats*) and its rank is RK 1.

Neither of these mutations has been previously described in the *D. subobscura* species (Krimbas, 1993).

Figure 1. General view of a *D. subobscura* female with the *un* mutation

Figure 2. Dorsal-lateral view showing that the place of the eye has been substituted by another head tissue. Ocelli can be observed.

Figure 3. Ventral view of the *Drosophila* head. It allows observing the remains of necrosed eyes and the substitution of eye by other head tissues.

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Thirty-three new mutations in *D. simulans*.

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About fifty visible markers across the *D. simulans* genome are currently available at the Tucson Drosophila Species Stock Center. We generated six new visible mutations by EMS mutagenesis of the *D. simulans st, e* strain (Tucson Drosophila Species Stock Center strain # 14021-0251.034) and of the *D. simulans* strain collected in Nueva **(**#14021-0251.006**)**. These new mutations are described in Table 1. The three *white* alleles and the *forked* allele failed to complement the *D. simulans white*^{1} allele and the *D. simulans forked*^{1} allele, respectively. We did not obtain progeny from a cross between our *D. simulans crossveinless*^{2} line and *D. melanogaster crossveinless*^{1} which we performed to test allelism to *D. melanogaster crossveinless*. To our knowledge, the *D.*

simulans crossveinless^{1} allele isolated by Sturtevant from a natural population (Sturtevant, 1929) has been lost. Our *crossveinless*^{2} allele is temperature-sensitive: homozygotes display the *crossveinless* phenotype at 17ºC and are wild-type at 25ºC. The new *forked*^{4} allele is strong and resembles the *D. melanogaster forked*^{36a} allele. The *Enhancer of Ubx* allele produces enlarged and flat halteres when transheterozygous with the In(3R)Ubx inversion, but it does not give any phenotype in the absence of the In(3R)Ubx inversion.

Table 1. List of the new *D. simulans* mutations.

Allele name	Chromosome	Strain used for mutagenesis	Phenotypic class	Mutant phenotype
white-apricot2 (*w*^{a2})	X	14021-0251.006	visible recessive	Eye color: light orange
white-3 (*w*^{3})	X	14021-0251.006	visible recessive	Eye color: white
white-4 (*w*^{4})	X	14021-0251.006	visible recessive	Eye color: white
forked-4 (*f*^{4})	X	14021-0251.034	visible recessive	Macrochaetes and microchaetes shorter and bent
crossveinless-2 (*cv*^{2})	X	14021-0251.006	visible recessive, temperature-sensitive	Anterior and posterior crossveins missing
Enhancer of Utrabithorax (*E(Ubx)*)	3	14021-0251.034	dominant visible with In(3R)Ubx, homozygote viable	No phenotype on its own. In combination with In(3R)Ubx: enlarged and flat haltere
l(3)39 kkv?	3	14021-0251.034	lethal recessive	Cuticle phenotype: head skeleton malformed, denticle bands narrower
l(3)207 kkv?	3	14021-0251.034	lethal recessive	Cuticle phenotype: head skeleton malformed, denticle bands narrower
l(3)106	3	14021-0251.034	lethal recessive	Cuticle phenotype: anterior and posterior regions abnormal
24 lines named as l(3)* with * being a number between 6 and 209 {*l(3)6, l(3)8, l(3)11, l(3)22, l(3)35, l(3)52, l(3)89, l(3)91, l(3)107, l(3)111, l(3)118, l(3)121, l(3)125, l(3)130, l(3)143, l(3)162, l(3)169, l(3)186, l(3)187, l(3)189, l(3)191, l(3)200, l(3)203, l(3)209*}	3	14021-0251.034	lethal recessive	-

We also generated 27 homozygous lethal mutations on chromosome three by EMS mutagenesis of the *D. simulans st, e* strain # 14021-0251.034. Mutagenized chromosomes were balanced over the *In(3R)Ubx* inversion (described in Coyne and Sniegowski, 1994) to prevent recombination events in the region covered by the inversion. 222 independent lines carrying a mutagenized chromosome 3 and a chromosome with the *In(3R)Ubx* inversion were generated and screened for recessive lethality, *i.e*. absence of *ebony* progeny (the *ebony* locus is located in the region covered by the inversion). We obtained 27 lines that produced no *ebony* progeny after at least five generations, and we infer that each of these lines carries at least one lethal mutation balanced by *In(3R)Ubx*, which corresponds to regions 81F1-84F and 93F-89E1 in the *D. melanogaster* nomenclature (Coyne and Sniegowski, 1994). Three lines displayed a first-instar larva cuticle

phenotype. In line *l(3)106*, anterior and posterior regions are abnormal. This phenotype is not caused by a mutation in the *huckebein* gene, because line *l(3)106* complements the *D. melanogaster huckebein*^{2} mutation. Lines *l(3)39* and *l(3)207* failed to complement each other and displayed the same cuticle phenotype, with narrow ventral denticle bands and a malformed head region. This phenotype is characteristic of the *D. melanogaster krotzkopf verkehrt* mutation. Unfortunately we did not obtain progeny from a cross of these *D. simulans* lines with *D. melanogaster krotzkopf verkehrt*^{1} mutants.

All of these stocks, except for w^{3} and w^{4}, are available from the Tucson Drosophila Species Stock Center (http:// stockcenter.arl.arizona.edu).

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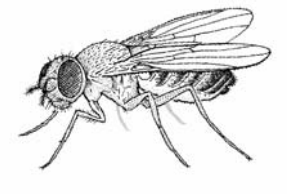

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