Mutation Notes

Rediscovery and characterization of mus309[D1].

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The *mus309* locus of *Drosophila melanogaster* was first identified in 1981 by Prof. Boyd and coworkers (Boyd *et al.*, 1981) and was originally characterized by three different mutant alleles; they came from a pool of third chromosome, EMS-induced, mutations conferring high sensitivity to mutagens ('mus' meaning mutagen sensitive). Specifically, *mus309* lines are hypersensitive to methyl methanesulfonate (MMS) and nitrogen mustard. It is one of the best characterized *mus(3)* genes, and it is the *Drosophila* homologue of the human gene mutated in Bloom's Syndrome (BS; OMIM entry: #210900). Bloom's Syndrome protein (BLM) is a member of the RecQ family of helicases and its function is to resolve recombinational intermediates, either in meiosis (Walpita *et al.*, 1999) or during somatic DNA repair (Kusano *et al.*, 2001). During a revision of the *Drosophila* stocks of the collection of Emer. Prof. Gatti (Sapienza, Università di Roma), we found three copies of the original *mus309* stocks coming from Prof. Boyd's laboratory. Of them, two were labeled as *mus309[D2]* and *mus309[D3]*, while the third was unreadable as for the allele, but the label clearly stated that it was a *mus309* stock. A complementation test with *mus309[D3]* confirmed the sterility of heterozygous females. Thus, to identify the allele, we sequenced the coding region of both *mus309[D3]* and the undefined line, and compared these sequences with that of the mRNA available in

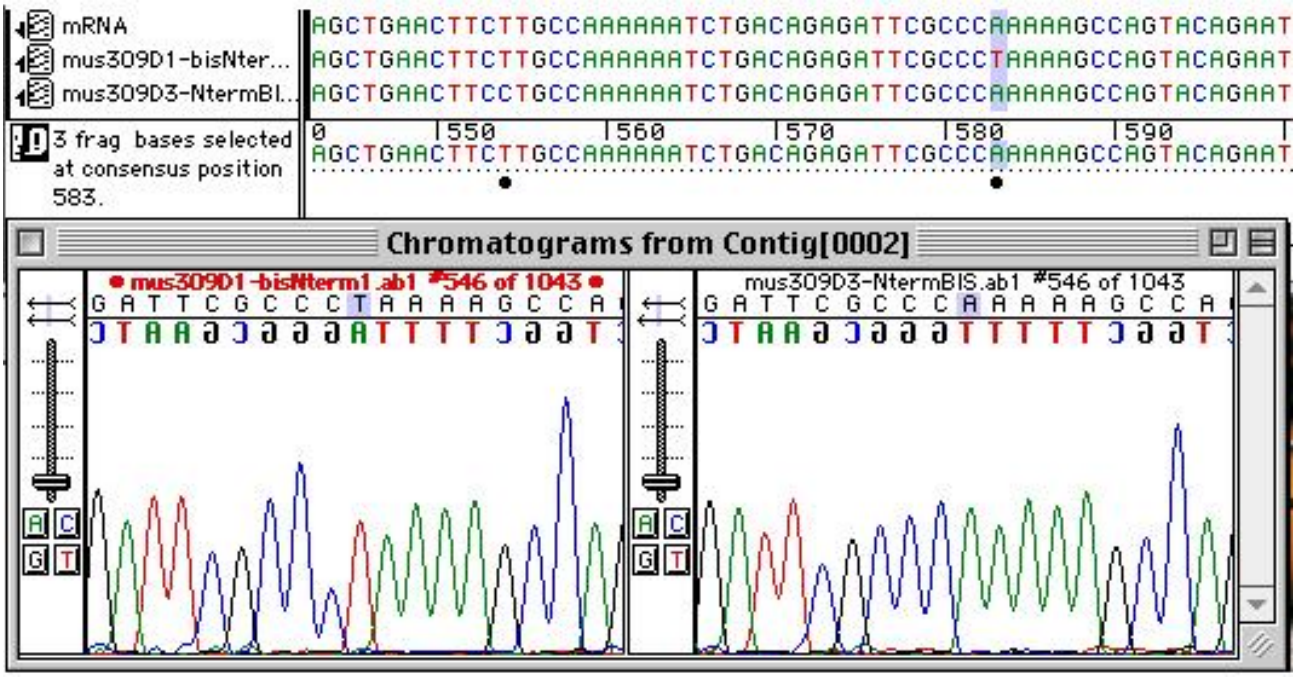

Figure 1. The *mus309[D1]* line presents a precocious stop codon at position Lys-110 inside the dmBLM gene. Top: alignments of mRNA (from FlyBase), *mus309 [D1]*, and *mus309[D3]* in the region of the mutation; the last two sequences were obtained in our laboratory, using external resources. Bottom: chromatograms of the same region from both *mus309* alleles used in the present study.

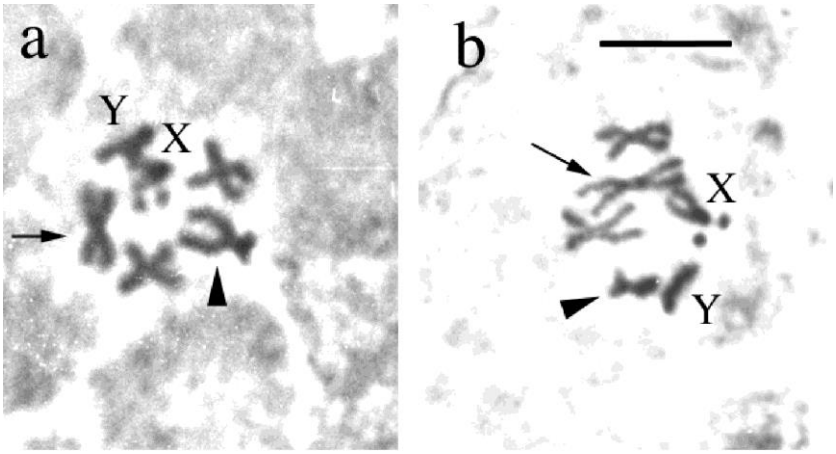

Figure 2. Cytology of *mus309[D1]/ Df(3R)M-kx1* cells. (a) Cells having this genotype show that *mus309* bearing chromosome has a peri-centromeric inversion (arrowhead), if compared to its deficiency bearing homologue (arrow). (b) With a frequency much higher than expected (see also Table 2), a translocation between these two chromosomes occurs, leading to the formation of one big chromosome (derived from the normal one, arrow) plus a small one (derived from the inverted one, arrowhead). The translocations between these two chromosomes always show such a configuration, suggesting that the aberrant recombination always occurs in the same chromosomic region. Both cells in panels come from the same, male brain squash. Bar: 5µm.

FlyBase. As expected, in the *mus309[D3]* sequence both mutations reported in literature are present (Kusano *et al.*, 2001). Instead, the other line showed an A/T transversion changing K110 into a stop codon (Figure 1); this change was confirmed twice, by sequencing both DNA strands.

This mutation is not reported in literature, and due to the age and nature of the stock we found, we are confident that this line indeed represents the *mus309[D1]* line, which was believed lost (McVey *et al.*, 2007). Consequently, from now on, we propose to name this line as *mus309[D1]*. To better characterize the stock, we analyzed the karyotype of the line. Since homozygous flies are now early lethal, we analyzed them in the combination *mus309[D1]/Df(3R)M-kx1*, for which both larvae and adults are available. We found that the *mus309[D1]*-bearing chromosome is characterized by a pericentromeric inversion, clearly recognizable by the shift of the centromeric region towards one chromosome end (Figure 2A). We verified that this inversion is not in the deletion-bearing stock, by crossing it with an Oregon-R line (data not shown).

Table 1. Spontaneous chromosome aberrations in *mus309[D1]* hemizygotes, compared to control.

Line	Total cells scored	Chromatid breaks (%)	Isochromatid breaks (%)	Exchanges (%)	Total aberrations (%)
control (Oregon-R)	1000	0.11	0.38	0.01	0.5
mus309[D1] / Df(3R)M-kx1	605	0.17	7.27	3.3	10.74

Table 2. Distribution of the exchanges inside *mus309/Df(3R)M-kx1* cells.

U-type asymmetric	U-type symmetric	X-type asymmetric	X-type symmetric	More complex exchanges
3	2	4	10	1

Since the *mus309* locus is involved in DNA repair, we also analyzed the line for the presence of spontaneous chromosome damage. For this purpose, we prepared standard brain squashes with colchicine, without adding any chemical compound (MMS, HN2), and stained them with orcein. As illustrated in Table 1, we found that the hemizygous larvae, despite their viability, show a very high number of spontaneous chromosome breaks, compared to controls (overall, more than 20×). As in controls, the class of the

isochromatid breaks (both sister chromatids broken at the same point) is the most represented, but interestingly this genetic combination also shows an excess of chromosome translocations. It is long known that mutated BLM protein in man typically induces an increase in sister chromatid exchanges (Schroeder and German, 1974). Accordingly, we hypothesize that the high number of isochromatid breaks is due to the initiation and subsequent failure of the recombination-mediated repair of single chromatid breaks, which indeed have a frequency comparable to controls. The scored excess of chromosome translocations (> 300× compared to control) further support this idea.

As shown in Table 2, the exchanges scored are not equally distributed among all categories. There is an enrichment in the class of X-type exchanges versus U-type ones. Moreover, X-type symmetric exchanges involve only the inverted chromosome and its homologue (Figure 2B). It is intriguing to conjecture that, because of the imperfect pairing of the two homologues, the inverted chromosome is detected by the cell as something 'unusual'; this in turn would activate the recombination-mediated repair machinery, inducing a high level of somatic recombination between these homologues. The failure to resolve this somatic recombination in a *mus309* mutated background would lead to the over-production of both isochromatid breaks and X-type symmetric exchanges scored.

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References: Boyd, J.B., M.D. Golino, K.E. Shaw, C.J. Osgood, and M.M. Green 1981, Genetics 97: 607-623; Kusano, K., D.M. Johnson-Schlitz, and W.R. Engels 2001, Science 291: 2600-2602; McVey, M., S.L. Andersen, Y. Broze, and J. Sekelsky 2007, Genetics 176: 1979-1992; Schroeder, T.M., and J.B. German 1974, Humangenetik 25: 299-306; Walpita, D., A.W. Plug, N.F. Neff, J. German, and T. Ashley 1999, Proc. Nat. Acad. Sci. 96: 5622-5627.

A new mutation in *Drosophila parabipectinata.*

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D. parabipectinata was described by Bock in 1971 and is one of the members of the *D. bipectinata* complex, which is comprised of four closely related and morphologically very similar species: *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana*, and *D. pseudoananassae*. This complex is part of the *ananassae* subgroup of the large *melanogaster* species group (Bock and Wheeler, 1972) of the subgenus *Sophophora*. These species occur throughout Southeast Asia, extending into north eastern Australia, the Indian subcontinent and South Pacific. However, *D. parabipectinata* has restricted geographical distribution as compared to the other three species of the complex. All the four species are sympatric over most of their geographic ranges. All the four species hybridize with each other in the laboratory, and hybrid females are fertile but males are sterile (Mishra and Singh, 2006). *D. parabipectinata* shows incomplete sexual isolation with other members of the *bipectinata* complex (Bock, 1978; Singh *et al*., 1981; Banerjee and Singh, 2012). It shows asymmetrical sexual isolation with *D. bipectinata* and *D. malerkotliana* (Banerjee and Singh, 2012). Results based on interspecific crosses and behavioral studies provide evidence that *D. bipectinata* and *D. parabipectinata* are very closely related species (Bock, 1978; Hegde and Krishnamurthy; 1979, Crossley, 1986; Singh and Singh, 2013, 2014).

A large number of stocks of *D. parabipectinata* established from flies collected from different geographical localities are being maintained in our laboratory. This note describes an x-ray induced mutation in *D. parabipectinata*. For irradiation experiments, the males were taken from a wild type stock collected from Mysore, India, and reared for numerous generations in the laboratory. The newly hatched and two days old wild type males were collected, and these males were kept in a gelatine capsule and were exposed to X-rays under following conditions:-