



Rediscovery and characterization of *mus309*[D1].

Piergentili, Roberto. Istituto di Biologia e Patologia Molecolari del CNR and Dipartimento di Biologia e Biotecnologie, Sapienza Università di Roma; Rome, Italy.

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 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.

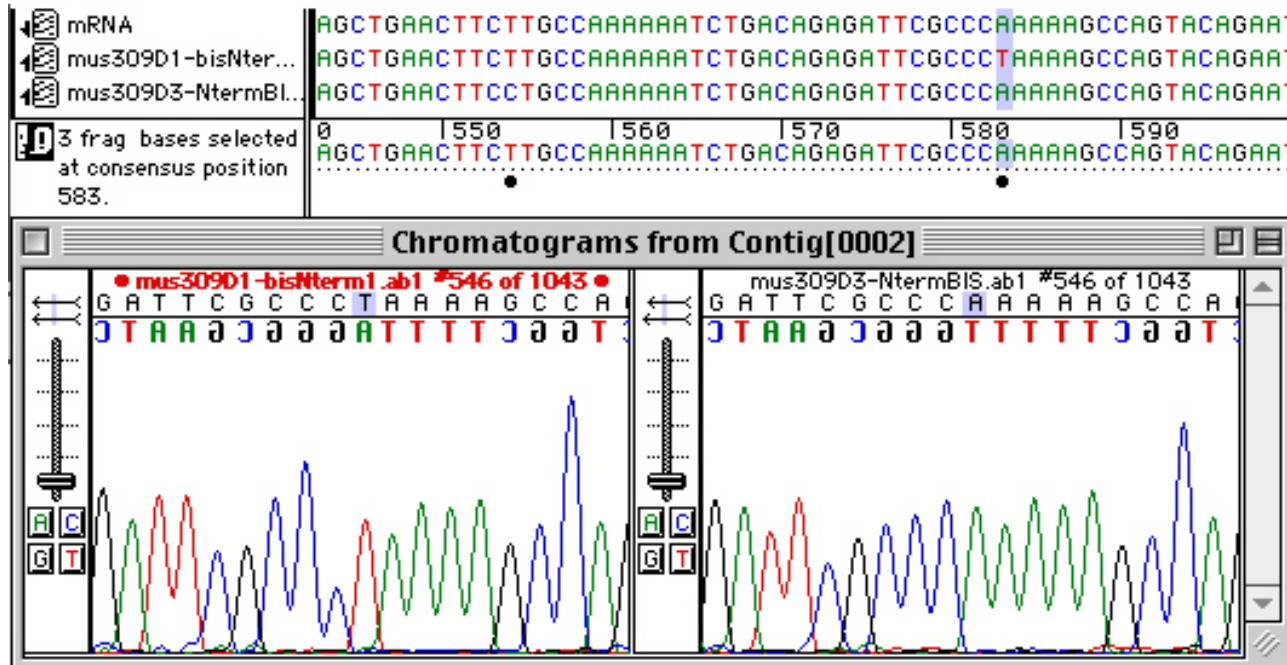


Figure 1. The *mus309*[D1] line presents a precocious stop codon at position Lys-110 inside the dmBLM gene. Top: alignments of mRNA (from FlyBase), *mus309* [D1], and *mus309*[D3] in the region of the mutation; the last two sequences were obtained in our laboratory, using external resources. Bottom: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

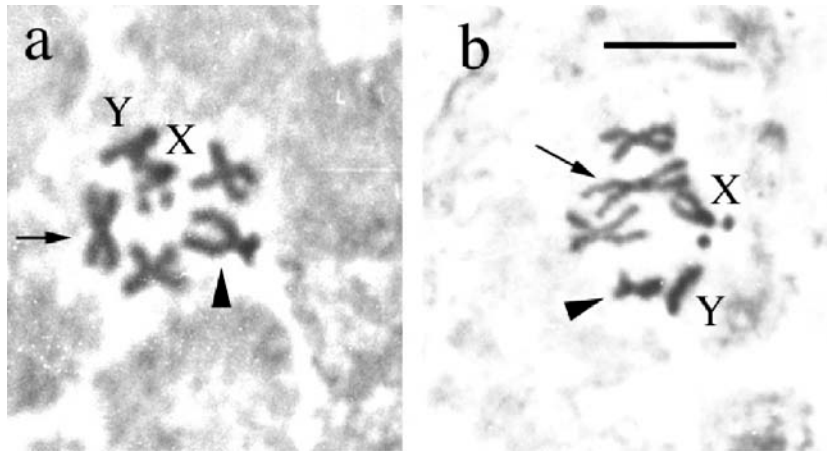


Figure 2. Cytology of *mus309[D1]/Df(3R)M-kx1* cells. (a) Cells having this genotype show that *mus309* bearing chromosome has a pericentromeric inversion (arrowhead), if compared to its deficiency bearing homologue (arrow). (b) With a frequency much higher than expected (see also Table 2), a translocation 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 chromosomal region. Both cells in panels come from the same, male brain squash. Bar: 5µm.

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
<i>mus309[D1]/Df(3R)M-kx1</i>	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.