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Post-replicative repair of an X-ray damaged chromosome following fertilization in Drosophila melanogaster.

During the course of an X-ray mutagenesis screen designed to find mutations in the scarlet region, we recovered a chromosome rearrangement whose properties suggested the occurrence of an unusual mutational event. In that screen, males of the genotype Ki roe pP were given an X-ray dose of 4000R and mass mated to stB2C3e virgin females.

After three days the bottles were cleared of parents, and the resulting progeny were subsequently scored for scarlet-eyed flies. Among the mutations obtained was an allele, stB18, recovered in a scarlet-eyed F1 male. This male, when mated to tester females carrying a deficiency for the scarlet region, Df(3L)stB17(2) (73A2-73D1-2), balanced over TM6b, Hu Tb e ca, yielded no st Ki progeny. The lack of st Ki flies among the F2 progeny could mean that the irradiated Ki roe pP chromosome, in addition to being mutant for st, is also mutant for an essential gene (or genes) that is not complemented by the Df(3L)stB17(2) chromosome (for example, stB18 could be a deficiency that deletes not only st but also other essential loci in the 73A2-73D1-2 region). The appearance of st+ Ki progeny in the F2 suggested that the F1 male was a st+/st+ mosaic in which the eyes were st+ and at least part of the germ line was st+.

While the production of mosaic flies from X-irradiated sperm is not that uncommon an occurrence, our cyto genetic observations on the stB18 chromosome indicated that the mutational event giving rise to the stB18/stB18 F1 male was, indeed, unusual. Salivary gland chromosome squashes were prepared from stB18/+ larvae. Figure 1a shows the bizarre pairing configuration that was observed in the 72E-75A region. Chromosome spreads in which the homologues were well-stretched and asynapsed revealed that this rearrangement is an inversion of region 72E1-2 to 74F4-75A1 that is tandemly duplicated. Since mature sperm (i.e., haploid) had been irradiated, the insertion of this duplicated material must have occurred after zygotic chromosome replication and, thus, could be related to the same event that generated the st+ tissue in the F1 mosaic male. This explanation is consistent with evidence demonstrating that chromosome breaks induced in spermatozoa do not rejoin before fertilization (Muller 1940; Helfer 1940; Kaufmann 1941). Under our hypothesis the material duplicated in one chromatid was donated at the expense of the other chromatid, implying that the genotype of the recovered mosaic male was:

Dp(3L)stB17(2)/stB2C3e e/Df(3L)stB18 stB18/stB2C3 e

The existence of this deficiency chromosome was proven by the subsequent recovery of st+ chromosomes from some of the Ki Hu Tb flies that were saved from the F2 generation. The F1 mosaic male must have therefore had a st+/st+ mosaic germline. Cytological analysis showed that this stB18 chromosome is deleted for the same region (72E1-2 to 74F4-75A1) that is tandemly duplicated in Dp(3L)stB18 (Figure 1b).

The induction in mature sperm and recovery of both a deficiency and the complementary duplication chromosomes from the same individual can be explained by the occurrence of post-replicative repair of a broken chromosome in the zygote (Figure 2). In this case, the four broken ends were ligated back together in the

Figure 1. Photomicrographs of orcein-stained salivary gland chromosomes from: (a) Dp(3;3) stB18+, and (b) Df(3L) stB18+ larvae. Arrows point to the 72E-75A region of chromosome arm 3L.
Figure 2. Model for the generation of the duplication and deficiency chromosomes shown in Figure 1.

wrong order, resulting in one chromatid with an inverted tandem duplication, and another chromatid with a deletion. Segregation of these sister chromatids at mitotic anaphase resulted in an individual that was mosaic for these chromosome rearrangements. The occurrence of post-replicative repair is also evident from the observation that the Df(3L)stg18 chromosome carries an inversion between regions 65A1-2 and 99A1-2, whereas the Dp(3L)stg18 chromosome carries no such inversion (data not shown).

A similar interpretation invoking chromatid exchange occurring after replication in the zygote was proposed in 1969 by Leigh & Sobels (cited in Sankaranarayanan & Sobels 1976) to explain their recovery of homo-isochromosomes following irradiation of post-mitotic male germ cells.

Flies homozygous for the Dp(3L)stg18 chromosome can survive to the adult stage, although their viability is low. The observation that these flies exhibit normal sexual phenotypes and are fertile is noteworthy, since they should carry four wild-type doses of the transformer (tra, 3-45) locus, a sex determination regulatory gene whose function is required in females, but not in males, for normal sexual development (Sturtevant 1945; Baker & Ridge 1980).

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