Figure 1. Morphology of Df(3R)P9 complete and incomplete GBS embryos. Df(3R)P9 embryos of the same age were dechorionated and observed by microscope. The majority of embryos (A) completed GBS, while approximately one quarter (B) exhibited incomplete GBS. (x 960)

Table 1. Assortment of embryos with respect to germ band shortening and ventral belt pattern.

Class	Ventral Belt Pattern	
	Wild Type	Df(3R)P9
Complete GBS	197	2
Incomplete GBS	4	55

type. Those that failed to hatch, as well as some of the hatched larvae, were mounted for phase contrast microscopy in lactic acid and ethanol (Lewis 1978), and their denticle belt pattern examined. Only 2 of the complete GBS class examined had the Df(3R)P9 phenotype; these may have been overlooked in the bulk screening procedure. We also mounted all of the incomplete GBS animals. All but 3 of these embryos failed to hatch. 55 out of 59 expressed the Df(3R)P9 phenotype; the other 4 animals had a wild type belt pattern. These could have been incorrectly identified during the screening procedure if they were younger than the other embryos.

This screening method should only be performed on embryos incubated at 18°C, since the results are not as clean when they have been incubated at 25°C. However, incomplete GBS has also been observed in another Df(3R)P9 stock that is marked with multiple wing hair. This indicates that the incomplete GBS phenotype may be used to screen for Df(3R)P9 homozygotes in all Df(3R)P9 stocks.

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Gerasimova, T.I.[1] and Yu.V.Ilyin[2] [1]Institute of Molecular Genetics, USSR Academy of Sciences, [2]Institute of Molecular Biology, USSR Academy of Sciences. The role of the mobile element mdg4 in the formation of unstable cut mutations in Drosophila melanogaster.

Earlier, an unstable ct^{MR2} allele was obtained in a cross of Oregon-R females and MRh12/Cy males under hybrid dysgenesis (Gerasimove 1981) (the MRh12/Cy genome contains multiple copies of the P-element). This mutation was characterized in the homozygous stock by a high frequency of reversions, the occurrence of new unstable visible and lethal mutations and super-unstable ct mutations, as well as the emergence of new mutations at other loci in the X-chromosome (Gerasimova 1981, 1982). In the present study, we look into the molecular nature of the mobile element integrated at the cut locus in the ct^{MR2} mutant. To this end, we have carried out in situ hybridization on crushed salivary-gland chromosomes of ct^{MR2} larvae, using the standard procedure (Ilyin et al. 1978). In our hybridization assays, we used plasmid DNA (labeled with ^{3}H and ^{125}I) containing the following elements: mdg1, mdg2, mdg3, mdg4 (Tchurikov et al. 1981), copia (Finnegan et al. 1978) obtained from D.Finnegan, fb elements obtained from S.Potter (Potter et al. 1980), and P-element from G.M.Rubin (Rubin & Spradling 1982). The cut locus is known to be located in the 7B region of the X-chromosome. Mdg4 is the only one of the above-listed elements that hybridizes with the 7B region in the ct^{MR2} mutant. This is a typical mdg which contains direct and inverted repeats at the edges. The total length of mdg4 is 7 kb. Its structure has been revealed earlier by Yu.V.Ilyin. In situ hybridization was performed with different subfragments of mdg4. The picture was the same: the label was invariably found in 7B. At the same time the original Oregon stock, whence ct^{MR2} was derived, does not contain mdg4 in the 7B region (or anywhere in the X-chromosome).

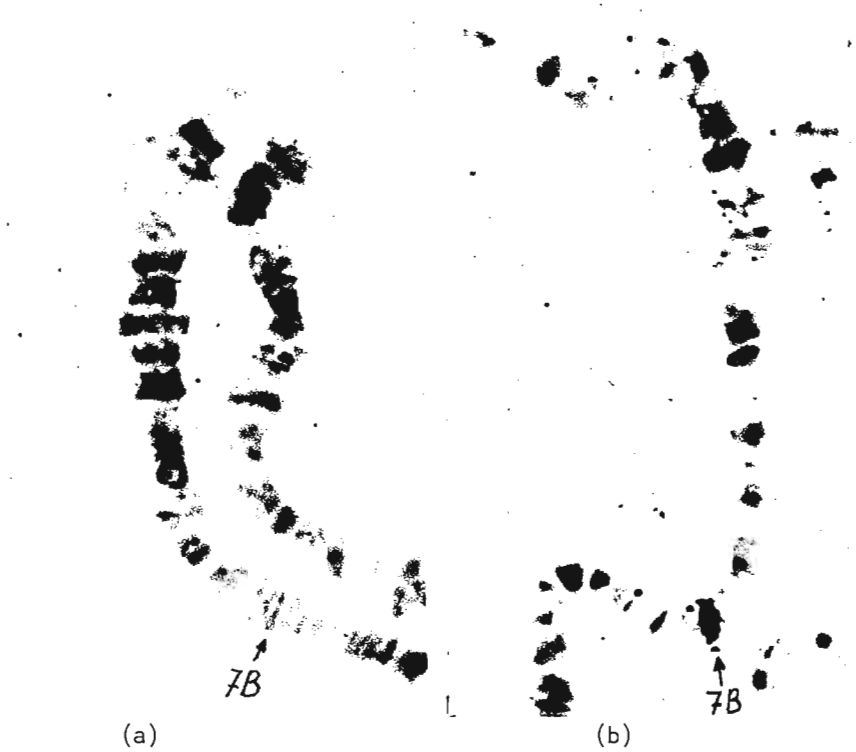

Figure 1. Results of the in situ hybridication DNA mdg4 with polytene chromosomes: (a) stable revertant $ct^{+}sn^{17}$, (b) unstable ct mutant - ct^{pn10}.

To test the connection between mdg4 and the instability of the ct^{MR2} allele, we carried out the hybridization of mdg4 and a wide range of different mutants and revertants derived from ct^{MR2}. In all our tests (nine altogether) of stable reversions, mdg4 proved to have disappeared from the 7B region (Fig. 1a), whereas in unstable ct alleles differing from ct^{MR2} in phenotype, including the unstable ct lethals (a total of 12 different mutations were analyzed) mdg4 was retained in the 7B region (Fig. 1b). These results prove the connection between the unstable ct^{MR2} mutation and the integration of mdg4 at the cut locus. The emergence of new ct mutations is probably due to the displacement of mdg4 within the cut locus. The point is that the new mutations affect different parts of the locus, including its "regulatory" and "structural" regions (Gerasimova 1981, 1982). The occurence of new mutations cannot be attributed to legitimate recombination, since all types of unstable ct mutations and ct^{+} revertants emerged in clusters, i.e., at the premiotic stage. Never in all our assays did we observe mdg4 in other remote loci of the X-chromosome, even when it "left" the 7B region. Mdg4 is probably transferred to long distance with a lower probability. Another possibility would consist in the existence of a limited number of sites preferred by mdg4. Indeed, mdg4 is an mdg of few copies. In Oregon-R, it is only present in two copies in the autosomes, and in the chromocentre.

The instability of the ct^{MR2} mutation was maintained in a homozygous stock for 1.5 years, 50 generations. The frequency of reversions to the wild type remained the same: 1.5×10^{-3}. Then, however, the reversions died down dramatically to a near-zero level. One of the possible explanations would be the loss of full P-elements, which seem to be responsible for the synthesis of transformation enzymes. Therefore, we crossed ct^{MR2}/ct^{MR2} females (two copies of the P-element) to MRh12/Cy males (multiple copies of the P-element). The reversion frequency went up as far as 8×10^{-3} in the progeny. Here, again mdg4 disappeared from the 7B region. Thus, the migration of mdg4 in the ct^{MR4} allele can be controlled.

The existence of such a genetically chracterized system along with a cloned mobile element offers good opportunities for the study of mdg transposition mechanisms and their effects on the target genes.

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