A POSSIBLE ROLE OF REPETITIOUS DNA IN RECOMBINATORY
JOINING DURING CHROMOSOME REARRANGEMENT
IN *DROSOPHILA MELANOGASTER*

C. S. LEE

Department of Zoology, University of Texas, Austin, Texas 78712

Manuscript received July 1, 1974

ABSTRACT

It is postulated that certain repetitious DNA components play a role in the recombination processes during chromosome rearrangements. When the distribution of silver grain densities after the *in situ* hybridization of repetitious DNA (RUDKIN and TARTOF 1973) and the distribution of chromosome breaks due to X-irradiation (KAUFMANN 1946) are compared, a strong correlation is found for the euchromatic portion of the *D. melanogaster* salivary X chromosome. These observations justify the postulate above that certain repetitious DNA provides homologous regions in the DNA of broken chromosome ends necessary for proper recombinatory joining.

GENOMES of all the eukaryotes examined so far contain highly and immediately repetitious DNA components, the amounts of which vary according to the organism. Various hypotheses have been proposed on the role(s) of these DNA components (see recent reviews by BOSTOCK 1971; by WALKER 1972; and by FLAMM 1972). However, none of them has yet been proven satisfactorily, except for a few cases such as the ribosomal RNA genes, 5S RNA genes and histone genes. It is postulated *a priori* in this communication that a certain type of repetitious DNA plays an important role in the recombination processes of chromosome breakage and rejoining.

Chromosome breaks due to X-irradiation and other mutagens have been studied for several decades. The breaks produced have, in general, been determined by cytological examination of resulting chromosome rearrangements such as deletions, insertions, inversions and translocations. Thus, the data collected are based primarily on the results of the rejoining of broken chromosomes, not on the actual breaks produced. A number of breaks may not have been detected simply because broken chromosomes can join back to produce the original intact chromosomes, while others may not join at all and thus result in lethality. Therefore, it is reasonable to assume that the reported frequency of chromosome breaks can be considered as the frequency of the detectable rejoining of broken chromosomes.

An important part of the postulate that repetitious DNA plays a role in the rejoining of broken chromosomes is that this process requires a recombination mechanism. It follows that in order to recombine two broken ends there should exist some homologous regions between the DNA of broken ends. That is, the

base sequence of one broken end should be identical or sufficiently similar to that of the other broken end in order for recombination to occur. This reasoning, by necessity, invokes the presence of certain repetitious sequences rather widely distributed throughout the genome. If this postulate is correct, then the frequency distribution of chromosome "breaks" should be correlated with that of the amounts of repetitious DNA and/or the degree of repetition throughout the genome. This notion is supported by the fact that about one-fourth of the breaks observed are in the proximal heterochromatic regions (KAUFMANN 1939). The DNA in this region is now known to be largely repetitious according to *in situ* hybridization studies (JONES and ROBERTSON 1970; RAE 1970; GALL, COHEN and POLAN 1971; BOTCHAN *et al.* 1971). Indeed KAUFMANN (1946) already suggested a possible correlation between the intercalary heterochromatin and the chromosome breakage.

KAUFMANN (1939, 1946) accumulated extensive data on the distribution of breaks in the euchromatic portion (division 1 through 19 in Bridge's map) of the salivary gland X chromosome of *D. melanogaster*. Very recently RUDKIN and TARTOF (1973) reported data on the distribution of silver grains on the X chromosome (division 1 through 13) of *D. melanogaster* in their *in situ* hybridization studies. Since the hybridization of H³-cytidine labeled complementary DNA was done up to C₀t of 5, the silver grains which appeared in the autoradiographs should be due to the hybridization of both highly and intermediately repetitious DNA. Thus, a high silver grain density in a certain region reflects a large amount of repetitious DNA or a high degree of repetition or both.

The data of both KAUFMANN (1946) and RUDKIN and TARTOF (1973) were analyzed and compared. The distributions of breakage frequency and grain counts are shown in Figure 1. As can be seen, the correlation between the two distributions is remarkably good. Only three regions, 1A-B, 7A-C and 9C-F, do not seem to follow the correlation. The deviation for the region 1A-B is understandable in that the rearrangements in this region may not be as easily detectable as in the others. Furthermore, LEFEVRE (1973) states that this region is thought to contain heterochromatic elements. It should be emphasized here that the heights of two frequencies need not be the same. For instance, a high silver grain density with a relatively low rejoining frequency in a region could easily be due to the large amount of repetitious DNA having many different kinds of repeating sequences. A similar plot (not shown) using the amounts of DNA per region given by RUDKIN and TARTOF (1973; Table 3, column 2) does not show as good a correlation as the one seen in Figure 1. The excellent agreement observed immediately suggests that the hot spots for chromosome breakage-rejoining are the hot spots for the hybridization of repetitious DNA. Therefore, within the limitation of cytological observations the indication is quite clear that certain repetitious DNA, if not all, provides homologous regions in the DNA of broken chromosome ends for proper recombinatory joining during chromosome rearrangements in *D. melanogaster*.

This suggestion is further supported by the experimental findings of LEFEVRE (1973) that about 70% of detectable rearrangements are non-lethal and that a

FIGURE 1.—The distributions of chromosome breaks (dashed line) and grain counts (solid line) on the Bridge's map of the euchromatic portion of the *D. melanogaster* X chromosome. The data of chromosome breaks are from KAUFMANN (1946; Table 2, column 3) and the grain counts are from RUDKIN and TARTOF (1973; Table 3, columns 4 plus 5). The ratios were obtained by dividing the number of breaks or grains in a region by the average of all 23 regions considered. The subdivision in each division is arbitrarily made according to Table 3 of RUDKIN and TARTOF. For instance, division 1 is subdivided into 1A-B and 1C-F. Therefore, the abscissa shown does not reflect quantitatively either the number of bands or the amount of DNA, but it merely indicates the locations in the Bridge's map of the X chromosome. A total of 803 breaks and 3,337 grain counts were used in this plot.

high frequency of these nonmutant breakpoints is found in the regions, 1B, 3C, 7A, 8C, and 12E. These regions appear as prominent peaks in Figure 1, except for 7A as mentioned above. The implication that follows is that the repetitious DNA involved in the recombinatory joining is not informational with regard to its genetic function. Further evidence is found in the frequency of X;Y translocations (NICOLETTI and LINDSLEY 1960; STEWART and MERRIAM 1973). The highest frequency is found in the regions 3C-F, 11A-F and 12C-F, and the least or none in 5A-C, 8A-B, 9A-B, 10D-F and 12A-B, which are again revealed as prominent peaks and dips in Figure 1. A further examination as to which type of

repetitious DNA is involved—highly repetitious or intermediately repetitious—will be of interest. It has been shown in *Lilium* that a very small amount of DNA synthesized during the pachytene stage of meiosis is intermediately repetitious according to DNA reassociation experiments (SMYTH and STERN 1973). This pachytene DNA synthesis is presumed to be due to the repair during crossing over.

I thank DR. BURKE JUDD for many helpful discussions and critical reading of this manuscript. This work was supported by National Science Foundation Grant (GB-37253).

LITERATURE CITED

- BOSTOCK, C., 1971 Repetitious DNA. *Advan. Cell Biol.* **2**: 153–223.
- BOTCHAN, M., R. KRAM, C. W. SCHMID and J. E. HEARST, 1971 Isolation and chromosomal localization of highly repeated DNA sequences in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **68**: 1125–1129.
- FLAMM, W. G., 1972 Highly repetitive sequences of DNA in chromosomes. *Intern. Rev. Cytol.* **32**: 1–51.
- GALL, J. G., E. H. COHEN and M. L. POLAN, 1971 Repetitive DNA sequences in *Drosophila*. *Chromosoma* **33**: 319–344.
- JONES, K. W. and F. W. ROBERTSON, 1970 Localization of reiterated nucleotide sequences in *Drosophila* and mouse by *in situ* hybridization of complementary RNA. *Chromosoma* **31**: 331–345.
- KAUFMANN, B. P., 1939 Distribution of induced breaks along the X-chromosome of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **25**: 571–577. —, 1946 Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. *J. Exptl. Zool.* **102**: 293–320.
- LEFEVRE, G., JR., 1973 The one band-one gene hypothesis: Evidence from a cytogenetic analysis of mutant and nonmutant rearrangement breakpoints in *Drosophila melanogaster*. *Cold Spring Harbor Symp. Quant. Biol.* **38**: 591–599.
- NICOLETTI, B. and D. L. LINDSLEY, 1960 Cytogenetic analysis of T(X;Y)'s. *Drosophila Inform. Serv.* **34**: 95–97.
- RAE, P. M. M., 1970 Chromosomal distribution of rapidly reannealing DNA in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **67**: 1018–1025.
- RUDKIN, G. T. and K. D. TARTOP, 1973 Repetitive DNA in polytene chromosomes of *Drosophila melanogaster*. *Cold Spring Harbor Symp. Quant. Biol.* **38**: 397–403.
- SMYTH, D. R. and H. STERN, 1973 Repeated DNA synthesized during pachytene in *Lilium henryi*. *Nature New Biol.* **245**: 94–96.
- STEWART, B. and J. R. MERRIAM, 1973 Segmental aneuploidy of the X-chromosome. *Drosophila Inform. Serv.* **50**: 167–170.
- WALKER, P. M. B., 1972 "Repetitive" DNA in higher organisms. *Progr. Biophys. Mol. Biol.* **23**: 145–190.

Corresponding editor: G. LEFEVRE