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THE BEHAVIOUR OF X AND Y CHROMATIN IN MITOMYCIN-C INDUCED MITOTIC ECOMBINATION IN BOTH MALES AND FEMALES OF *DROSOPHILA MELANOGASTER*

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Abstract: This investigation studied mitotic recombination in X-chromosome eu- and heterochromatin as well as in Y-chromosome heterochromatin in both males and females of *Drosophila melanogaster* by Y-chromosome participation in mitotic recombination events. The potent chemical mutagen mitomycin-C was applied and mitotic recombination induced in males was studied between X and Y and between XY and Y chromosome. In females, the recombination was studied between the two $X^{'S}$ as well as between the two $XY^{'S}$ chromosomes. The results showed: that:(1) Y-heterochromatin was not engaged in the induction of mitotic recombination by mitomycin-C. (2) The heterochromatin of X and Y might react differently with respect to mitomycin-C induced mitotic recombination. (3) Mitotic recombination took place in eu- as well as in heterochromatin of X–chromosome. The results suggested that, at the molecular level, X and Y heterochromatin are highly different and behave differently with respect to one or more of induced recombination mechanisms suggested by Haendle (1971 a, b and 1974).

Introduction:

Mitotic recombination in *Drosophila melanogaster* is a powerful tool in the study of developmental biology and mutagenecity (Graf, 1984). It provides a method for labeling single cells genetically and for analyzing the fate of descendants whenever they are detected as mosaic spots. The nature of X-ray induced mitotic recombination has been thoroughly investigated by various investigators. Such studies showed that the frequency of X-ray induced mitotic recombination is positively correlated with the amount of heterochromatin present in the chromosome and most of the exchange events take place in the proximal heterochromatin near the centromere (Becker,1969, Walen, 1964). In addition, the X-chromosome euchromatin and heterochromatin behave differently with respect to the induction of recombination and much of the induced recombination takes place in the X-heterochromatin (Haendle,

1979). On the other hand, mitotic recombination between X and Y is confined to the heterochromatic region of both chromosomes, since the Y-chromosome is almost completely heterochromatic while the X-chromosome contains about 40% of its length heterochromatin located in the region of the centromere (Cooper, 1959; Pimpinelli et al, 1983).

However, in earlier studies mitotic recombination between X- and Y-chromosomes proved to be infrequent (Von Glasenapp, 1975; El-Sayed, 1989). In such investigations, X-ray induced mitotic recombination was studied in both males and females of *Drosophila melanogaster* by Y-chromosome participation in mitotic recombination events. The results showed that Y-hetrochromatin does not recombine neither with X nor with Y, neither in males nor in females. In the present work the potent chemical mutagen, mitomycin-C was applied instead of X-rays that had been used in earlier works and could not overcome the refractoriness of the Y-heterochromatin. Mitomycin C was suggested to be very effective in the induction of mitotic recombination in the somatic cells of *Drosophila melanogaster* (Lehman et al., 2000 and Santos et al., 1999) Moreover, mitotic recombination was studied in males between X and Y and between XY and Y chromosomes. In addition, the recombination between the two X's as well as between the two XY's chromosomes was studied in females.

Material and methods:

Drosophila stocks:

Zeste mutant strain (Z):

The zeste is a sex-linked recessive mutant gene located at locus 1.0 on the X-chromosome. zeste females have lemon-colored eyes, while the males always show dark red-colored eyes. The description and genetic analysis of the zeste allele has first given by Gans (1953). The expression of the zeste allele depends upon the number of w^+ loci; females have two w^+ loci, whereas, the males have only one w^+ locus. The zeste females which have only one copy of w^+ are wild type, while the males containing a w^+ duplication have yellow eyes.

White- Apricot; suppressor of forked strain:

The flies of this stock carry X-chromosomes marked with white-apricot (w^a) and with suppressor of forked (su-f). The (su-f) gene enhances the eye coloured mutant (w^a) so that w^a su-f / w^a su-f flies are almost white eyed (Lindsley and Grill, 1968). Nearly all the heterochromatin lies between the su-f (Locus 65.9) and the centromere, and nearly all the euchromatin lies between w^a and su-f.

Attached X-Y chromosome stocks:

All the compound X-Y chromosome stocks are given in Table (1)

Females of the first six (1-6) stocks have all the same type of reversed metacentric compound X-chromosome [C (1) RM] consisting of two X-chromosomes joined in equal orientation to the centrally located centromere. These females are homozygous for the sex-linked recessive markers y, v, bb and have an extra Y-chromosome. Females of stocks 7

Table (1): Genetics structure of attached X.Y chromosome stocks.

Stock No.	Female genotype	Male genotypes
1	C (1) RM, y, v, bb	$XY^{L}.Y^{S}$, z
2	C (1) RM, y, v, bb	$XY^{S}.Y^{L}$, z
3	C (1) RM, y, v, bb	$XY^{L}.Y^{S}$, w
4	C (1) RM, y, v, bb	$XY^{S}.Y^{L}$, w
5	C (1) RM, y, v, bb	$XY^{L}.Y^{S}$, w^{a} su (f)
6	C (1) RM, y, v, bb	$XY^{S}.Y^{L}$, w^{a} su (f)
7	$XY^{L}.Y^{S}/XY^{L}.Y^{S}$, z	$XY^{L}.Y^{S}$, z
8	$XY^{S}.Y^{L}/XY^{S}.Y^{L}$, z	$XY^{S}.Y^{L}$, z

and 8 have attached XY chromosomes in homozygous condition marked with z marker

In strains 1,3,5 and 7 the $XY^{L}.Y^{S}$ is indicative of the attached XY chromosome in which the Y-chromosome is appended with its long arm (Y^{L}) to the X-euchromatin. In strains 2,4,6 and 8 $XY^{S}.Y^{L}$ symbolizes the attached XY chromosome in which the Y-chromosome is appended with its short arm (Y^{S}) to the X-euchromatin.

The genetic and cytological structures of these stocks were described in detail by Lindesley and Grill (1968).

Determination of mitotic recombination in males:

The criteria for mitotic recombination in males are zeste single spots in adult flies. In zeste males, which have normal eyes, a mitotic recombination event should yield, when induced in larvae, a zeste single spot, since the twin partner most probably does not survive. Figure (1a) presents an example for the mitotic recombination induced in X/Y and XY/Y males. In the present study, mitotic recombination was studied

between normal Y and three different types of chromosomes, normal X, XY^L. Y^S and XY^S. Y^L. For this purpose three different types of males: X/Y, $XY^L.Y^s$ and $XY^S.Y^L$ were mated to the attached – X/Y females and the resulting males were treated in their larval stage with the chemical agent.

Determination of mitotic recombination in females:

Two different series of experiments were made: one to determine the frequency of recombination over the entire length of the x-chromosome and the second to compare recombination frequencies in the eu- and heterochromatin, in the first series, heterozygous zw^+/z^+w females were used, in which mitotic recombination leads to yellow/white twin spots. Figure (1b) indicates the mitotic recombination in zw^+/z^+w females between two normal X chromosomes and between the two XY chromosomes. Three different types of females resulting from the following crosses were used:

X/Y, w x X/X, z

XY^L. Y^S, w x XY^L. Y^S/ $XY^L.Y^S$, z

$XY^S.Y^L$, w x $XY^S.Y^L$/ $XY^S.Y^L$, z

In the second series of experiments, the method of Haendle (1979) with a slight modification was used.. Mitotic recombination in the euchromatin of Heterozygous (zw^+ su-f / z^+w su-f) females results in white - apricot/yellow twins, while in the heterochromatin they result in white/yellow twins [Figure (1c)]. In this series of experiments, three different types of females resulting from the following crosses were used:

X/Y, z x XX, w^a su-f

$XY^L.Y^S$ w^a su-f x $XY^L.Y^S/XY^L.Y^S$, z

$XY^S.Y^L$ w^a su-f x $XY^S.Y^L/XY^S.Y^L$, z

Chemical treatments and data analysis:

Mitomycin-C was obtained from Kyowa Hakko Kaygo Ltd, Tokyo (Japan). For treatments of *Drosophila* larvae , an acute feeding treatment method was used as described by El-Sayed and Hashad (1997). The drug concentration (1.4 mM mitomycin-c) was relatively higher than that used in other somatic assays. Such dose was selected according to a survival pre-test made previously, giving about 30% survival. The eyes of the adult flies developed from the treated larvae were observed and statistical analysis was done as described by El-Sayed (1989). Mutation frequencies were calculated as percentage of mosaic spots (number of mosaic spots/100 eyes). All strains were maintained on a sugar-cornmeal medium at room temperature and experiments were conducted at 25°C.

Results and Discussion

The results of mitomycin-C induced mitotic recombination in males are shown in Table (2). The

rate of mitotic recombination was extremely low not only between the X and Y, but also between Y and X's with various Y-heterochromatin. The rates were 0.48%, 0.28% and 0.25% in the case of X/Y, $XY^L . Y^s$ /Y and $XY^s . Y^L$/Y males, respectively. In X/Y males mitotic recombination should take place between X and Y, whether this type of recombination occurs between X-heterochromatin and either the Y^L or the Y^s arm of the Y-chromosome remains an open question. In XY/Y males mitotic recombination most likely took place between two homologous arms of Y, between two Y^L's (in case of $XY^L . Y^s$/Y) or between two Y^s's (in case of $XY^s . Y^L$/Y). Similar results were obtained by von Glasenapp (1975) and El-Sayed (1989) after irradiation of males with X-ray.

Table (2): Frequency of mitomycin-C induced single spots in males.

Genotypes	Number of eyes scored	Single spots	
		No.	%
X / Y	2290	11	0.48
$XY^L.Y^s$ / Y	2150	6	0.28
$XY^s.Y^L$ / Y	2410	6	0.25

Table (3) presents the results of induced mitotic recombination in females, in which the recombination was determined over the entire length of the X-chromosome. The results showed that the rate of mitotic recombination was 29.82% in X/X females. In XY/XY female the rates of mitotic recombination between the two XY's were reduced to about one half of that observed between the two X's. The rate was 13.12% in $XY^L.Y^s/XY^L.Y^s$ and 13.89% in the $XY^s.Y^L$ / $XY^s.Y^L$ females. In addition, when the rate of induced recombination was determined in the eu- and the heterochromatin separately (Table 4), the results showed that in the X/X females recombination took place in both eu- and the heterochromatin 11.70% in the eu and 13.2% in the heterochromatin). These results are in agreement with those of Haendle (1979) and El-Sayed (1989), who found that in X/X female treated with X-rays mitotic recombination took place in heterochromatin as well as in the euchromatin of X-chromosome. In XY/XY females, mitotic recombination was induced only in heterochromatin [10.24% in $XY^L.Y^s/XY^L.Y^s$ and10.13% in the $XY^s.Y^L$ / $XY^s.Y^L$ females.] . Similar results were observed by El-Sayed

(1989) after irradiation of XY/XY females with X-rays and by El-Sayed and Hashad (1997) after chemical treatments with anticancer drugs.

Table (3): Mitomycin-C induced mitotic recombination frequencies over the entire sex-chromosome of three different genotypes of females.

Genotypes	Number of eyes scored	Twin spots	
		No.	%
X / X	3122	931	29.82
$XY^L.Y^s / XY^L.Y^s$	2876	376	13.12
$XY^s.Y^L / XY^s.Y^L$	2231	310	13.89

Table(4): The frequency of mitomycin-C induced mitotic recombination in twin spots/100 eyes of both eu- and hetero-chromatin of three different genotypes of females.

Genotypes	Number of eyes scored	Recombination in euchromatin		Recombination in heterochromatin		Total	
		No.	%	No.	%	No	%
X / X	4010	469	11.70	530	13.2	999	24.91
$XY^L.Y^s / XY^L.Y^s$	3230	331	10.24	-	-	331	10.24
$XY^s.Y^L / XY^s.Y^L$	3760	381	10.13	2	0.53	383	10.19

The above results had led to the conclusion that : (1) Y-heterochromatin did not engage in the induction of mitotic recombination by mitomycin-C neither in males nor in females, (2) Y-heterochromatin did not mitotically recombine neither with homologous Y nor with X-heterochromatin, and (3) X and Y heterochromatin might react differently with respect to mitomycin-C induced mitotic recombination.

In discussing these results the following should be considered : It is assumed that mitotic recombination is the result of multiple hit events (Haendle, 1971a&b) and there are two types of breaks involved in the induction of mitotic recombination, one type is fast and the other is more slowly repairing. However, mitotic recombination events occur only after pairing of chromosomes (Haendle, 1974). Chromosome pairing does not seem to be unspecific lumping together of chromosomes. Therefore, a certain degree of homology is obviously a necessary prerequisite for pairing. The scarcity of homologous regions between X and Y chromosomes

should reflect the rarity of pairing between them and, subsequently, the induced mitotic recombination events. This may explain the low frequency of mitomycin-C induced mitotic recombination between the X and The Y in the present work.

However, on the bases of this assumption one would assume that increasing of regions of homology of the two recombined chromosomes would result in more pairing and hence more recombination. In case of XY/Y males although recombination most likely took place between the two homologous arms of Y, the recombination rate did not go up. Therefore one can assume that the reduced rate of mitotic recombination between XY and Y chromosomes can be attributed to : (1) that the Y material did not pair (or paired very rarely), (2) in the absence of effective pairing restitution rather than rejoining is favored. In addition, the results of induced mitotic recombination in X/X showed that mitotic recombination took place in both eu- and hetrochromatin of X-chromosome. On the other hand, in XY/XY females (in which X-heterochromatin was replaced by Y-heterochromatin) mitotic recombination was induced only in the X-euochromatin. Therefore, we have to consider that the X and the Y heterchromatin in females as well as in males should react differently with respect to any one or more of the induced recombination mechanism suggested by Haendle (1971a, b and 1974).

Moreover, from these indications, it seems that, at the molecular level, X and Y-heterochromatin are highly different. The molecular difference between X and Y could be attributed to the number and the distribution of certain repetitive sequences as shown by the blotting experiments of Livak (1984) who found that, in Oregon R wild type strain of *Drosophila melanogaster*, there are an estimated 200 copies of the λ DM2L sequence on the X-chromosome and at least 80 copies on the Y-chromosome.

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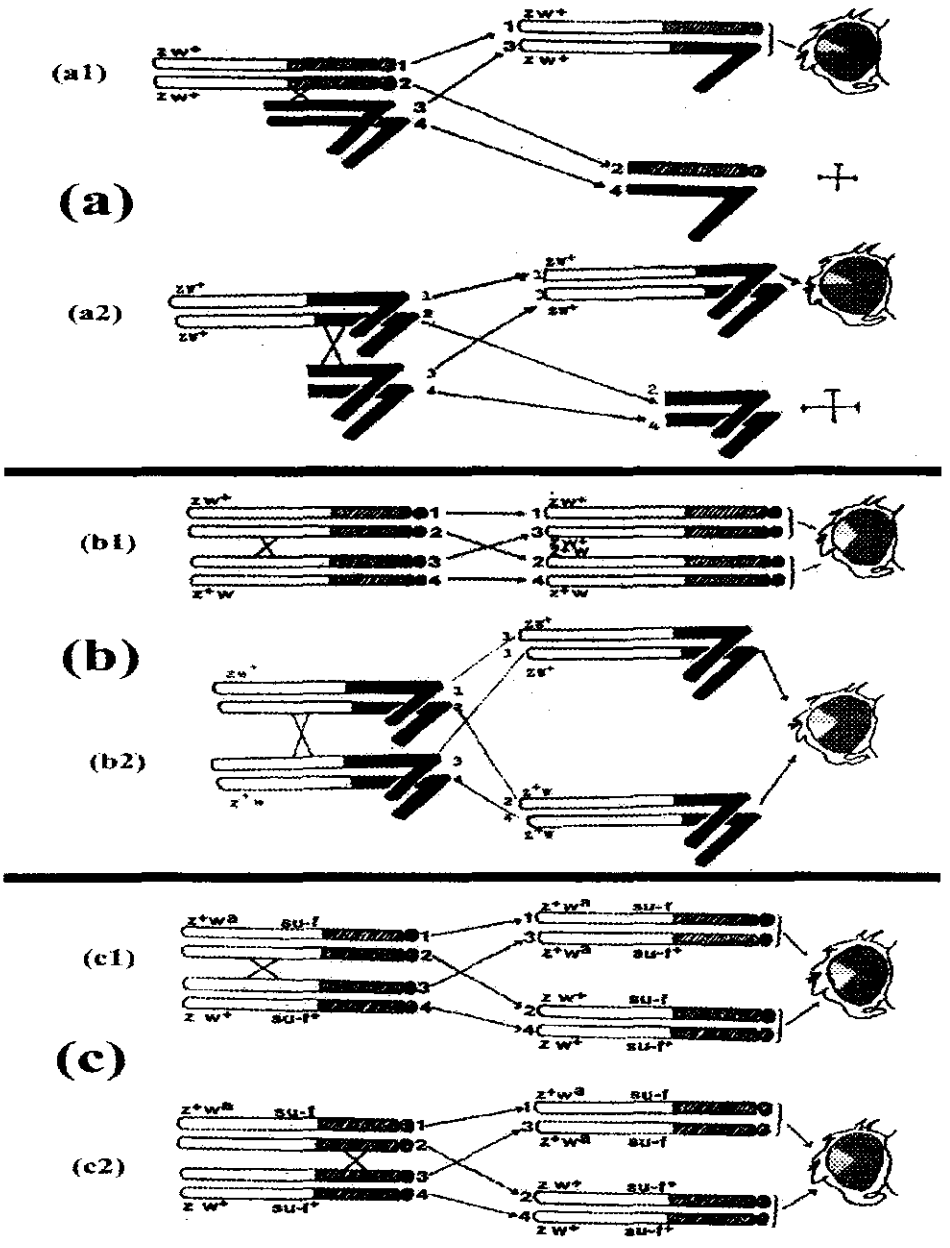

Figure (1): The mitotic recombination induced in X/Y and XY/Y males (1a) in XX and XY/XY females (1b) and in eu- and heterochromatin of females X separately (1c).

سلوك كرموسومي X , Y في عملية العبور الميتوزي المستحدث بواسطة مادة الميتوميسن سي في كل من إناث وذكور الدروسوفيلا ميلانوجاستر

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صمم هذا البحث لدراسة العبور الميتوزي في اليوكروماتين والهتروكروماتين الخاصين بكرموسوم X وكذا الهتروكروماتين الخاص بكرموسوم Y في كل من إناث وذكور الدروسوفيلا ميلانوجاستر عن طريق إدخال كرموسوم Y في عملية العبور الميتوزي . وعن طريق استخدام مادة الميتوميسن سي عالية الطفور تم دراسة العبور الميتوزي بين كرموسومي X , Y وبين كرموسومي XY , Y في الذكور. بينما في الإناث تم دراسة العبور المتيوزي بين كرموسومي X وكذا بين كرموسومي XY المتصلين.

أوضحت النتائج أن :-

١-هتروكروماتين كرموسوم Y لا يدخل في عملية العبور المتيوزي المستحدث بالميتوميسن سي

٢-إن كل من هتركروماتين كرموسوم Y وكذا هتروكروماتين كرموسوم قد يختلفان في استجابتهما لاستحداث العبور المتيوزي بواسطة الميتوميسن سي .

٣-أن العبور المتيوزي يتم حدوثه في كل من هتروكروماتين ويوكروماتين كرموسوم X.

تقترح هذه الدراسة أن هتروكروماتين Y , X قد يختلفان علي المستوي الجزيئي كما أنهما يختلفان عن بعضهما بالنسبة لسلوكهم وتفاعلهم مع الميكانيكيات المختلفة الخاصة بالعبور المتيوزي التي تم اقتراحها بواسطة Haendle 1971,a,b,1974.