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The cytogenetic boundaries of the rDNA region within heterochromatin of the *X* chromosome of *Drosophila melanogaster* and their relation to male meiotic pairing sites

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SUMMARY

The proximal breakpoints of the inversion chromosomes $In(1)w^{m4}$ and $In(1)^{m51b}$ were shown, by *in situ* hybridization, to define the boundaries of the ribosomal DNA region located within the *X* chromosome heterochromatin (*Xh*). We estimate that *at least* 95% of the rDNA is located between the $In(1)w^{m4}$ and $In(1)w^{m51b}$ proximal breakpoints. In contrast only 60–70% of the Type I intervening sequences located in *Xh* are located between these breakpoints. The Type I intervening sequences in the rDNA region occur as inserts in the 28S rRNA sequences while the remainder of the sequences are distal to the $In(1)w^{m4}$ breakpoint and not associated with rRNA genes.

The regions of Xh which contain rDNA and Type I intervening sequences were related to regions shown by Cooper (1964) to contribute to meiotic pairing between the *X* and *Y* chromosomes in male Drosophila. We demonstrate that the rRNA coding region contributes to *X*/*Y* pairing. However, no single region of Xh is required for fidelity of male meiotic pairing of the sex chromosomes.

1. INTRODUCTION

In *Drosophila melanogaster* the pairing sites responsible for the regular segregation of the *X* chromosome from the heterochromatic *Y* chromosome are located in the *X* chromosome heterochromatin (*Xh*) (Muller & Painter, 1932; Gershenson, 1940; Sandler & Braver, 1954; Cooper, 1964; Peacock, 1965). The pairing sites are not evenly distributed throughout *Xh*. Genetic and cytological studies (Gershenson, 1940; Cooper, 1964; Yamamoto & Miklos, 1977), show them to be absent from the most proximal segment of *Xh* and the short heterochromatic right arm (*XR*). In this report we map the cytogenetic boundaries of the rDNA region in Xh and find they *include* regions of conjunctive homology between *Xh* and *Y* as determined cytologically by Cooper (1964). We provide direct evidence that the rDNA region of *Xh* contributes to the male meiotic pairing of the *X* and *Y* chromosomes and document that no *single* site within *Xh* is essential for successful sex chromosome meiotic pairing during spermatogenesis.

2. MATERIALS AND METHODS

Sex Chromosome Rearrangements. $In(1)w^{m4}$, $In(1)w^{m51b}$, $In(1)w^{m4L}w^{m51bR}$,$y\,f\,m$, $In(1)w^{m51bL}w^{m4R}$, and Y^{bb-} were obtained from Dr J. Spofford; B^sY and the $C(1)\,y\,v\,f/O$ stock from Dr W. J. Peacock, and $Df(1)mal^{12}$, sc^8B, $Df(1)y^{x2}$, sc^8f, y^+Ymal^+ and $Ymal^+$ from Drs A. Schalet and K. Tartof. Further information on these chromosomal rearrangements may be found in the Results section, Lindsley & Grell (1968) and Schalet (1969). The chromosomes $Df(1)C1$ and $Df(1)C4$ are radiation-induced $su(f)^-$ deletions of $In(1)w^{m51b}$.

(i) *Assay of chromosome pairing at meiosis*

One assay for chromosome pairing at meiosis is the segregational behaviour of the respective chromosome as determined from scoring progeny. Males heterozygous for an X chromosome rearrangement and a marked Y chromosome (y^+Ymal^+, $Ymal^+$ or B^sY) were crossed singly in vials to either $C(1)RM$, $y\,v\,f/0$ or $In(1)49$, $y\,v\,f\,mal\,su(f)$ (homozygous) females and brooded for 4 days at 25 °C after which parents were cleared. Cultures remained at 25 °C until all progeny were scored.

The genetic assay is an indirect estimate of chromosome pairing but the meiotic behaviour of the X and Y chromosomes, as determined genetically, is consistent with their cytology (Cooper, 1964). Furthermore, Peacock (1965) found that the frequency of synapsis failure between $In(1)sc^{4L}sc^{8R}$ and the Y chromosome, as determined cytologically, was in good agreement with the frequency of X/Y genetic nondisjunction. This close correlation exists despite the dispartate zygotic recovery of reciprocal classes of nondisjunctional gametes due to sperm dysfunction (reviewed in Peacock & Miklos, 1973). Since the advantage of genetically assaying male meiotic pairing is that many more meioses can be sampled and low frequencies of chromosomal nondisjunction detected we have employed both cytological and genetic assays.

(ii) *Cytological analysis*

Polytene chromosomes from larval salivary glands and mitotic chromosomes from larval ganglia were prepared by fixation, staining and squashing in aceto-lacto-orcein (2% orcein). Salivary glands were dissected in 45% acetic acid, transferred to stain and immediately squashed. Ganglia were dissected in Ringers (0·75% NaCl) and pretreated with 1% sodium citrate for 1 min before transfer to stain and, after 5 min, squashed. Testes from newly emerged males were treated in the same manner as larval ganglia. Alternatively testes were fixed, stained and squashed in aceto-orcein (2% orcein dissolved in 60% acetic acid).

(iii) In situ *hybridization*

In situ hybridization of [^{3}H]cRNA to *Drosophila* mitotic chromosomes was performed as described in Steffensen, Appels and Peacock (1981). The more extensive washing procedure described by Appels, Steffensen & Craig (1979) was

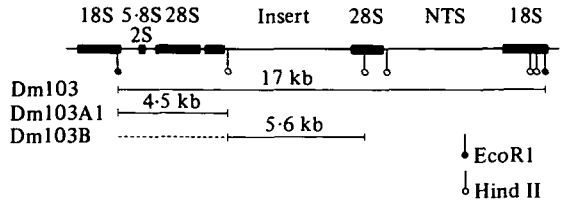

Fig. 1. Structure of a cloned DNA segment containing the *Drosophila melanogaster* ribosomal DNA repeat unit (Dm103) and two derivative subclones (Dm103A1 and Dm103B). The Dm103 plasmid carries the rDNA repeating unit associated with the Type 1 intervening sequences. The symbols ● and ○ locate the cleavage sites for the EcoR1 and Hind 111 restriction enzymes respectively within the rDNA repeating unit. NTS refers to the nontranscribed spacer. The details of Dm103 and its derivative clones were taken from Peacock *et al.* (1980).

used. The [^{3}H]cRNA probes were synthesized from the subclones Dm103A1 and Dm103B derived from Dm103 (Fig. 1). The plasmids were kindly provided by Dr D. M. Glover, Imperial College, London.

3. RESULTS AND DISCUSSION

(i) *The cytogenetic boundaries of rDNA sequences in Xh*

The *in situ* hybridization of [^{3}H]cRNAs homologous to rDNA and Type I intervening sequences to somatic chromosomes demonstrate that the proximal breakpoint of $In(1)w^{m4}$ is at the distal border of the region spanned by rDNA (Fig. 2). Grain counts indicate that 1·8% (10/547 grains, 17 chromosomes) of the rDNA is distal to the $In(1)w^{m4}$ breakpoint; whereas, 34% (41/120 grains, 10 chromosomes) of the Type I intervening sequences are distal to this break. The fraction of rDNA proximal to the $In(1)w^{m4}$ breakpoint is a minimum estimate since it is likely that we underestimated the grains located there due to grain coincidence; to detect the distal sequences, the autoradiograms required long exposures which resulted in near saturation of grains in the major, proximal, site of rRNA genes. The Type I intervening sequences located distal to the $In(1)w^{m4}$ breakpoint represent sequences which are not inserted into 28S rRNA genes and have been detected earlier by biochemical techniques (Dawid & Botchan, 1977; Peacock *et al.* 1981).

If we examine the *in situ* hybridization of the above probes to $In(1)w^{m4L}w^{m51bR}$ (see Fig. 3 for the synthesis of this chromosome) the $In(1)w^{m4}$ result is confirmed and the position of the right breakpoint of $In(1)w^{m51b}$ with respect to the rDNA is established. For the $In(1)w^{m4L}w^{m51bR}$ chromosome, a small but reproducible number of grains are observed on both sides of the chromosome (Fig. 4) and thus the right breakpoints of $In(1)w^{m4}$ and $In(1)w^{m51b}$ define the distal and proximal boundaries, respectively, of the rRNA gene cluster in Xh. At most 3–4 repeat units of rRNA genes being located outside each boundary (using the grains located distal to the $In(1)w^{m4}$ breakpoint as an internal standard). The intervening sequences

are found only on one side of the $In(1)w^{m4L}w^{m51bR}$ chromosome, indicating that the Type I intervening sequences not inserted into the 28S rRNA genes are located largely in the distal quarter of Xh. Fig. 4 also shows that the chromosome $In(1)w^{m51bL}w^{m4R}$, which is the reciprocal recombinant of $In(1)w^{m4L}w^{m51bR}$, has the distribution of sequences expected if this chromosome is duplicated for the region deleted in $In(1)w^{m4L}w^{m51bR}$. Thus the deletion $In(1)w^{m4L}w^{m51bR}$, which removes approximately 50% of *Xh* (Fig. 3), largely defines the extent of *Xh* encompassed by the rRNA genes.

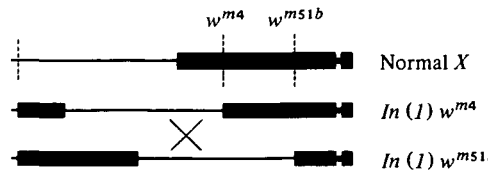

Fig. 3. Derivation of the $In(1)w^{m4L}w^{m51bR}$ Xh deletion and the $In(1)w^{m51bL}w^{m4R}$ Xh duplication is by meiotic recombination. Xh is represented by the thickest line and the centromere by the short line of intermediate thickness on the right. Cytological analysis of $In(1)w^{m4}$ and $In(1)w^{m51b}$ mitotic chromosomes allowed us to determine that the approximate *Xh* breakpoints are as indicated in the figure.

(ii) *The meiotic segregation of the* X *and* Y *chromosomes in male Drosophila*

Cooper (1964) determined that cytological conjunction between Xh and *Y* occurred proximally and distally to the nucleolus organizer in Xh. Since $In(1)w^{m4L}w^{m51bR}$ deletes 30–50% of Xh it very likely includes all or part of the pairing regions in blocks B and C defined by Cooper (1964). It was therefore surprising that the $In(1)w^{m4L}w^{m51bR}$ deletion segregates normally from the *Y* chromosome during male meiosis (Table I and Fig. 5). We examined this situation further by examining the segregation of $Df(1)y^{x2}$ and $Df(1)mal^{12}$ which delete Cooper's blocks B and C, and, D and C, respectively (see Fig. 6*a*) for a diagrammatic representation of these chromosomes). These two overlapping deletions thus encompass, between them, the entire set of *Xh* male meiotic pairing sites. However, neither $Df(1)mal^{12}$ nor $Df(1)y^{x2}$ is associated with significant *X*/*Y* non-disjunction (Table 1).

Clearly, removing a subset of the male meiotic pairing sites within *Xh* does not necessarily result in an elevated level of *X*/*Y* meiotic non-disjunction. This is consistent with earlier observations that certain free *X* duplications, consisting of the telomeric region of the *X* chromosome and only a portion of the region of *Xh* possessing pairing affinity with the *Y*, will segregate from an attached *X*. *Y* chromosome in *X*. *YDp*(*1*;*f*) males (e.g. Lindsley & Sandler, 1968; Parry & Sandler, 1973). Our data further confirm that no single region of pairing affinity between *Xh* and *Y* is required for correct segregation of the sex chromosomes.

Table 1. *Gametic frequencies of paternal sex chromosomes recovered from F1 progeny*

Paternal *X* chromosome	Gametic types *X*	*Y*	Nullo	*X/Y*	*N*
In(1)w^{m4}*	0·484	0·514	0·001	0·000	1524
In(1)w^{m51b}*	0·500§	0·500	0·000	0·000	1612
In(1)w^{51bL}*w*^{m4R}*	0·472	0·472	0·001	0·001	1533
In(1)w^{m4L}*w*^{m51bR}*	0·498‖	0·498	0·002	0·001	1523
Df(1)y^{x2}†	0·498¶	0·498	0·002	0·001	2282
Df(1)mal^{12}†	0·498¶	0·498	0·003	0·000	957
Df(1)C4‡	0·591	0·380	0·027	0·001**	876
Df(1)C1‡	0·434	0·566	0·000	0·000	1207

* Determined from *In(1)-B*^{s}*Y* males crossed to C(1)*RM, y v f/O* females.

† Determined from *Df(1)y*^{x2}*/y*$^{+}$*Ymal*$^{+}$ and of *Df(1)mal*^{12}*/y*$^{+}$*Ymal*$^{+}$ males mated to *C*(1)*RM, y v f/O* females.

‡ Determined from *Df(1)C4Ymal*$^{+}$, and of *Df(1)C1/Ymal*$^{+}$, males mated to *In(1)dl-49, y ac v mal f su(f)/In(1)dl-49, y ac v mal f su(f)* females.

§ Estimated from the *Y* bearing F1 as *In(1)w*^{m51b}*/0* is of reduced viability.

‖ Estimated from the *Y* bearing F1 as *In(1)w*^{m4L}*w*^{m51bR}*/0* is lethal.

¶ Estimated from *Y* bearing F1, as *Df(1)y*^{x2}*/0* and *Df(1)mal*^{12}*/0* are lethal.

** The low recovery of the *X/Y* gamete has also been observed in studies of the *In(1)sc*^{4L}*sc*^{8R} and *Df(1)bb*^{l481} chromosomes (see Peacock & Miklos, 1973).

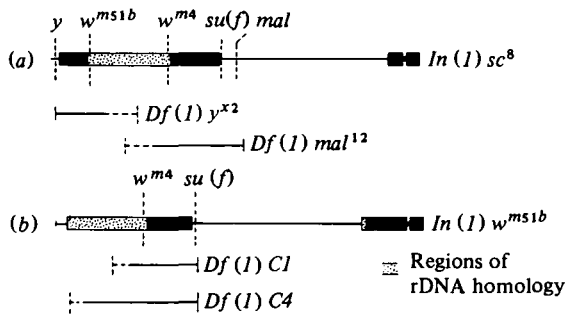

Fig. 6(*a*). The structure of the Xh deletions *Df(1)y*^{x2} and *Df(1)mal*^{12}, synthesized from *In(1)sc*^{8} (Schalet, 1969). For references, the sites in Xh associated with the corresponding breakpoints in *In(1)w*^{m4} and *In(1)w*^{m51b} are shown. (*b*) The structure of the Xh deletions *Df(1)C1* and *Df(1)C4* synthesised from *In(1)w*^{m51b}. The euchromatic boundary of *Df(1)C1* is in 19E2-3 while that of *Df(1)C4* is proximal to 20A; the euchromatic extents of the deletions argue against euchromatic pairing sites contributing to the meiotic segregation discussed in the text (Hilliker and Appels, manuscript in preparation).

(iii) *Assay for male meiotic pairing sites within the rDNA region of Xh*

Since Cooper (1964) determined that conjunction between the *X* and *Y* chromosomes did occur in blocks B and C of *Xh* we sought alternative means of assaying the region deleted by *In(1)w*^{m4L}*w*^{m51bR} for male meiotic pairing sites. In preliminary experiments, we have found that *In(1)w*^{m4L}*w*^{m51bR} competes less effectively with

a free duplication of Xh for pairing with the *Y* chromosome than does either $In(1)w^{m4}$ or $In(1)w^{m51b}$ (Hilliker, unpublished), consistent with the conclusion that the region deleted by $In(1)w^{m4L}w^{m51bR}$ contains male meiotic pairing sites.

The derivatives of $In(1)w^{m51b}$ recently isolated by Hilliker & Appels (manuscript in preparation) provided useful chromosomes for the assay of male meiotic pairing sites in the rDNA region. Two derivatives, *Df(1)C1* and *Df(1)C4*, were examined. The *Df(1)C1* deletion removes *su(f)*, the distal (non-rDNA) heterochromatin and approximately half of the rDNA from $In(1)w^{m51b}$; while *Df(1)C4* is an even larger deletion removing 90% or more of the rDNA (see Fig. 6*b*). Thus the two chromosomes are essentially identical except for a difference in rDNA content.

Cytological examination of testis preparations from $Df(1)C1/Ymal^{+}$ and $Df(1)C4/Ymal^{+}$ males indicated that *Df(1)C1* always paired with $Ymal^{+}$ (30 metaphase I cells examined) while *Df(1)C4* occasionally did not pair with $Ymal^{+}$ (lack of pairing seen in 2/22 metaphase I cells examined) (see Fig. 7). Progeny analysis demonstrated that the *Df(1)C4* chromosome had significant non-disjunction (Table 1) while *Df(1)C1* did not. The level of non-disjunction of *Df(1)C4* is low, but it is clearly higher than that of *Df(1)C1*, as would be predicted if the rDNA contributed to male meiotic pairing; the low level of non-disjunction observed for *Df(1)C4* reflects the fact that the pairing sites in the proximal heterochromatin left undisturbed in $In(1)w^{m51b}$ (and its derivatives) are sufficient to provide a high level of pairing between the *X* and *Y* chromosomes.

4. CONCLUSIONS

Our analysis of the cytological extent of the rRNA genes in *Xh*, the one well-defined site of genetic homology between *Xh* and *Y*, demonstrates that the rDNA region, which lies between the proximal breakpoints of $In(1)w^{m4}$ and $In(1)w^{m51b}$, must be considered a site of meiotic pairing between *Xh* and *Y*. This region however is not solely determinative in sex chromosome segregation (as an *X* chromosome bearing a deletion of the region will segregate regularly from the *Y*), as other DNA sequences in *Xh* and *Y* must also be of importance in this segregation. These conclusions are consistent with those of Cooper (1964); our data assign rDNA and Type I intervening sequences to block C (and possibly block D) and rDNA to block B.

DNA sequence homology *per se* is not sufficient for male meiotic pairing, as pointed out by Yamamoto & Miklos (1977). This applies to both heterochromatin and euchromatin since several free *X* duplications consisting of the most proximal portion of *Xh* and the euchromatin near the tip of the *X* chromosome, (which do not disrupt *X*/*Y* disjunction), fail to segregate from one another during meiosis in $X/YDp(1;f)/Dp(1;f)$ males (Yamamoto & Miklos, 1977). The assignment of specific DNA sequences to regions involved in pairing activity should provide useful starting points for further analyses of pairing sites at a molecular level.

We wish to thank Dr J. Spofford for specifically encouraging the analysis of the $In(1)w^{m_4}$ and $In(1)w^{m_{51b}}$ chromosomes and her continued interest in the *in situ* analysis. We thank Mrs Lyndall Moran and Miss Kathryn Ferguson for technical assistance and we acknowledge Drs B. John and W. J. Peacock for stimulating criticism during the course of this study.

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EXPLANATION OF PLATES

PLATE 1

Fig. 2. *In situ* localization of rDNA and Type I intervening sequences on $In(1)w^{m4}$. The *in situ* localization of [^{3}H]-cRNA synthesized from Dm103A1 (to assay rDNA) and Dm103B (to assay Type 1 intervening sequences) was carried out at 45 °C in 3XSSC, 50 % formamide for 5 h. These hybridization conditions are stringent as discussed in detail by Peacock *et al.* (1980). The complete metaphase preparation shown in the bottom portion of the figure illustrates a result consistently obtained, namely the absence of rDNA on the Y^{bb-} chromosome associated with the $In(1)w^{m4}$ stock even in the long autoradiographic exposures used. The absence of rDNA from Y^{bb-} is consistent with the observation of Spear (1974) using filter hybridizations.

Fig. 4. *In situ* localization of [^{3}H]cRNA complementary to rDNA (Dm103A1) and the Type I intervening sequence (Dm103B) on $In(1)w^{m4L}w^{m51bR}$ and $In(1)w^{m51bL}w^{m4R}$. Exposure times of autoradiographs was 3·5 months.

Fig. 5. A metaphase I cell from the testis of a $w^{m4L}w^{m51bR}/Y$ male. Normal pairing of the sex chromosomes was observed in 18 metaphase I cells.

PLATE 2

Fig. 7. Metaphase I cells from the testis of (*a*) $Df(1)C1/Ymal^+$ males and (*b*, *c*) $Df(1)C4/Ymal^+$ males. In (*c*) the *X* and *Y* chromosomes appear to be unpaired.

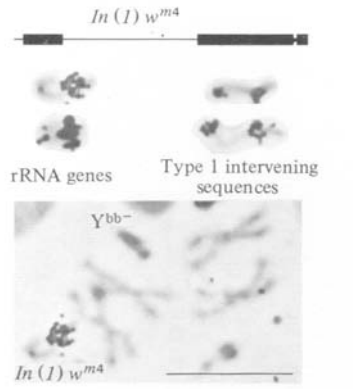

Fig. 2

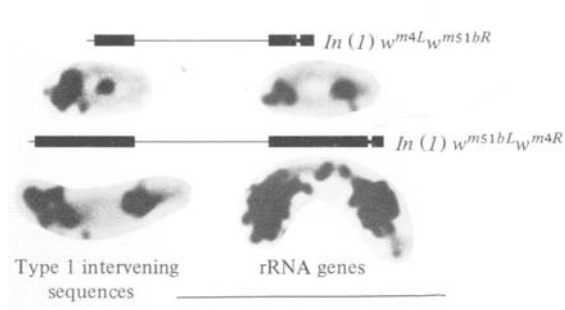

Fig. 4

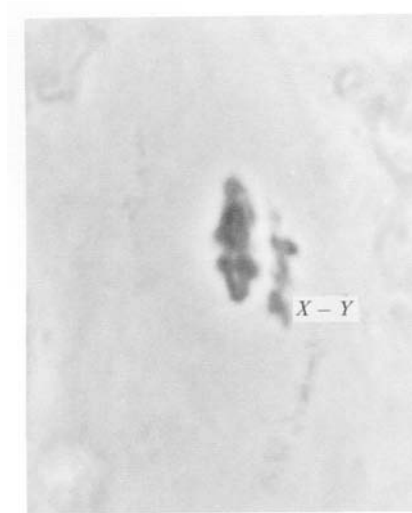

Fig. 5

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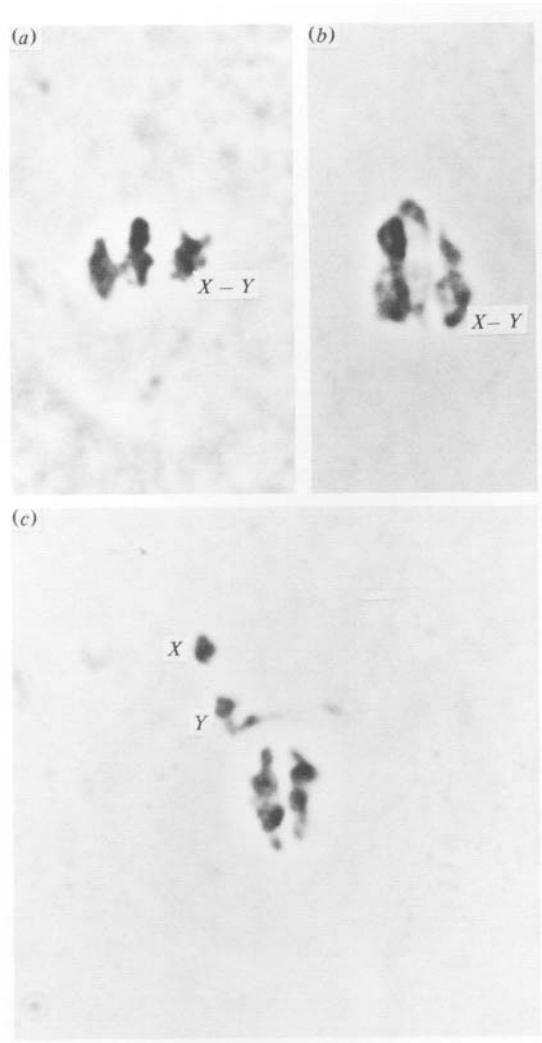

Fig. 7

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