8-2000

The effects of the promotor region of the 240bp repeats of the rRNA genes on x-y chromosome disjunction in Drosophila melanogaster males

Yunsang Lee

To the Graduate Council:

I am submitting herewith a thesis written by Yunsang Lee entitled "The effects of the promotor region of the 240bp repeats of the rRNA genes on x-y chromosome disjunction in Drosophila melanogaster males." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biochemistry and Cellular and Molecular Biology.

Bruce D. McKee, Major Professor

We have read this thesis and recommend its acceptance:

Ranjan Ganguly, Mary Ann Handel

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council.

I am submitting herewith a thesis written by Yunsang Lee entitled "The Effects of the Promoter Region of The 240bp Repeats of The rRNA Genes on X-Y Chromosome Disjunction in *Drosophila melanogaster* Males " I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biochemistry and Cellular and Molecular Biology.

Bruce D. McKee

Bruce D McKee, Major Professor

We have read this thesis

and recommend its acceptance·

Ranjan Ranganathan

Mary Ann Handel

Accepted for the Council·

Associate Vice Chancellor and
Dean of The Graduate School

THE EFFECTS OF THE PROMOTER REGION OF THE 240bp REPEATS OF THE rRNA GENES ON X-Y CHROMOSOME DISJUNCTION IN *Drosophila melanogaster* MALES.

A thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Yunsang Lee

August 2000

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Bruce McKee for his guidance and support during my research Additionally, I would like to thank my other committee, Dr. Mary Ann Handel, and Dr. Ranjan Ganguly for their time and valuable comments.

Also, I am indebted thanks to Dr. McKee's lab members, Amy Creekmore, C - S. Hong, Morvarid Bejnood, Suchita Das, Siuk Yoo and Dr. Wendy Stapleton for their help and friendship.

Finally, I would like to thank my family members who have given me support, encouragement and love

ABSTRACT

Pairing between homologous chromosomes is essential for successful meiosis In *Drosophila melanogaster* males, sex chromosome pairing during meiosis I is mediated by rDNA, located in heterochromatin. Several analyses of rDNA fragments showed that 240bp repeats in the intergenic spacer (IGS) have the ability to stimulate X-Y chromosome pairing and disjunction In addition, point mutations within the promoter of the 240bp repeats failed to mediate X-Y chromosome pairing and disjunction. These previous studies imply that promoter activity of the 240bp repeats is involved in X-Y chromosome pairing in *Drosophila* males In this study, I made a construct comprised of 16 copies of the 72bp fragment within the 240bp repeat, which has promoter activity and obtained transformant lines with the construct. The construct was transferred to Df(1)X-1, an rDNA deficient X chromosome, by recombination. The effect of the transgene on the frequency of X-Y disjunction were analyzed both by cytological and genetic experiments. The transgene in Df(1)X-1 chromosome induced increased X-Y chromosome disjunction frequency The result indicates that promoter activity of the 240bp repeats may be responsible for X-Y chromosome pairing in *Drosophila* males.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

TABLE **PAGE**

CHAPTER 1

INTRODUCTION

General characteristics of meiosis

Meiosis is a special type of cell division by which gametes containing only half the amount of genetic material are produced. The meiotic process contains two divisions, meiosis I and meiosis II, and one round of DNA replication. After the meiosis I, gametes become haploid, and meiosis II is very similar to mitosis Each of these divisions has four stages prophase, metaphase, anaphase, and telophase.

Among these stages, many important events occur at prophase I that lead to production of gametes containing only half the number of chromosomes Prophase I is traditionally divided into five stages. leptotene, zytotene, pachytene, diplotene and diakinesis. At zygotene, a synaptonemal complex (SC), a tripartite ladder-like structure, typically begins to develop between two homologous chromosomes. This major morphological marker for pairing is completely assembled by pachytene. The SC is present between the paired chromosomal axes during these stages and is thought to have important functions in meiotic recombination (von Wettstein et al , 1984). The important role of SC in meiotic recombination is indicated by the appearance of recombination nodules at intervals on the synaptonemal complex before and after synapsis is complete Recombination nodules are small, dense proteineous structures thought to contain localized meiotic recombination enzymes (Carpenter, 1975a, b, 1988) which mediate chromosomal exchange and

recombination (Rasmusen and Holn, 1978). After the disassembly of the SC at the end of pachytene, the two homologous chromosomes pull away from each other to a certain extent and are stabilized by chiasmata. The chiasmata, discrete attachment sites arising from crossovers, are first seen at diplotene stage and hold bivalents together until anaphase I to ensure chromosome segregation in most organisms (Jones, 1987; Hawley, 1988) Therefore, mutations that disrupt meiotic recombination and inhibit chiasma formation cause high frequencies of meiosis I nondisjunction (Roeder, 1990· Hawley, 1988, Baker et al , 1976)

Spermatogenesis in *Drosophila melanogaster*

Recombination is not always necessary for pairing and segregation of homologs. In *Drosophila melanogaster* males, homologous chromosomes pair stably until anaphase and segregate without either the SC or recombination (Cooper, 1950; Meyer, 1960) This achiasmatic meiotic system has apparently evolved many times from chiasmatic meiosis (White, 1973), implying that the two meiotic systems may be very similar. Easy determination of gene loci, and easy cytological studies, many mutants, and small number of chromosome numbers (only 4 pairs) (Fig 1A), facilitate the to study of meiosis in *Drosophila melanogaster* Therefore, the study of chromosome pairing in *Drosophila melanogaster* males, which is rather straightforward because of the absence of SC, recombination and chiasmata, may provide insights to the spermatogenesis of higher organisms.

Figure 1. Schematic diagram of chromosomes

A. Normal karyotype of Drosophila male

B. Df(1)X-1; X chromosome having a deficiency of large part of the heterochromatin containing the rDNA region

C. $B^{s}Yy+$; Y chromosome which has a duplicated X tip containing y+ and proximal X containing Bs

D. $B^{s}Y$, Y chromosome containing a duplicated X tip

Rectangles = heterochromatin, lines = euchromatin, circles = centromere

crosshatched region = rDNA

A. Normal karyotype of male *Drosophlia*

X

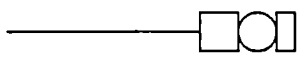
Y

2

3

4

B Df(1)X-1



C B^sYy^+

D B^sY

Spermatogenesis of *Drosophila* occurs within the coiled testis. Two types of stem cells, which are located in an apical position in the tip of the testis start spermatogenesis. Spermatogonia, generated from stem cells, undergo four mitotic divisions, resulting in cysts of 16 primary spermatocytes. These spermatocytes enter a growth phase during which their volume is increased 25-fold After two meiotic divisions of the primary spermatocytes, 64 haploid secondary spermatocytes are produced (Fig 2). At late prophase I three clumps appear near the nuclear membrane. One clump is the X-Y bivalent, and the other two are major autosomal bivalents; however the tiny fourth chromosome bivalent is not visible (Fig. 3A). During metaphase I the bivalents congress toward the metaphase plate, and the fourth chromosome is often visible at this stage (Fig 3B). The paired bivalents start to segregate toward opposite poles at anaphase I (Fig. 3C). At metaphase II three chromosomes appear (Fig. 3D) and are segregated at anaphase II (Fig 3E).

How homologous chromosomes are held together in achiasmatic *Drosophila melanogaster* males has been an important question that remains largely unsolved. Identification of pairing sites may provide the clues to the nature of interhomolog interaction which happens during meiotic pairing. The X-Y pairing site in *Drosophila melanogaster* males is the most studied. Therefore, detailed studies of X-Y pairing of *Drosophila* males may help to elucidate the mechanism of homolog pairing

Figure 2. Illustration of spermatogenesis in *Drosophila melanogaster*

Spermatogenesis start at the tip of the testes. Four mitotic divisions of a spermatogonium makes 16 primary spermatocytes After meiosis I and II of the 16 primary spermatocytes, 64 haploid secondary spermatocytes are produced Open circles = nuclei, gray regions = germ cell cytoplasm, white cells = somatic cell, black spots in (a-d) = nucleoli and big black spots in (e) = protein body Illustration is obtained from Maines and Wasserman (1998)

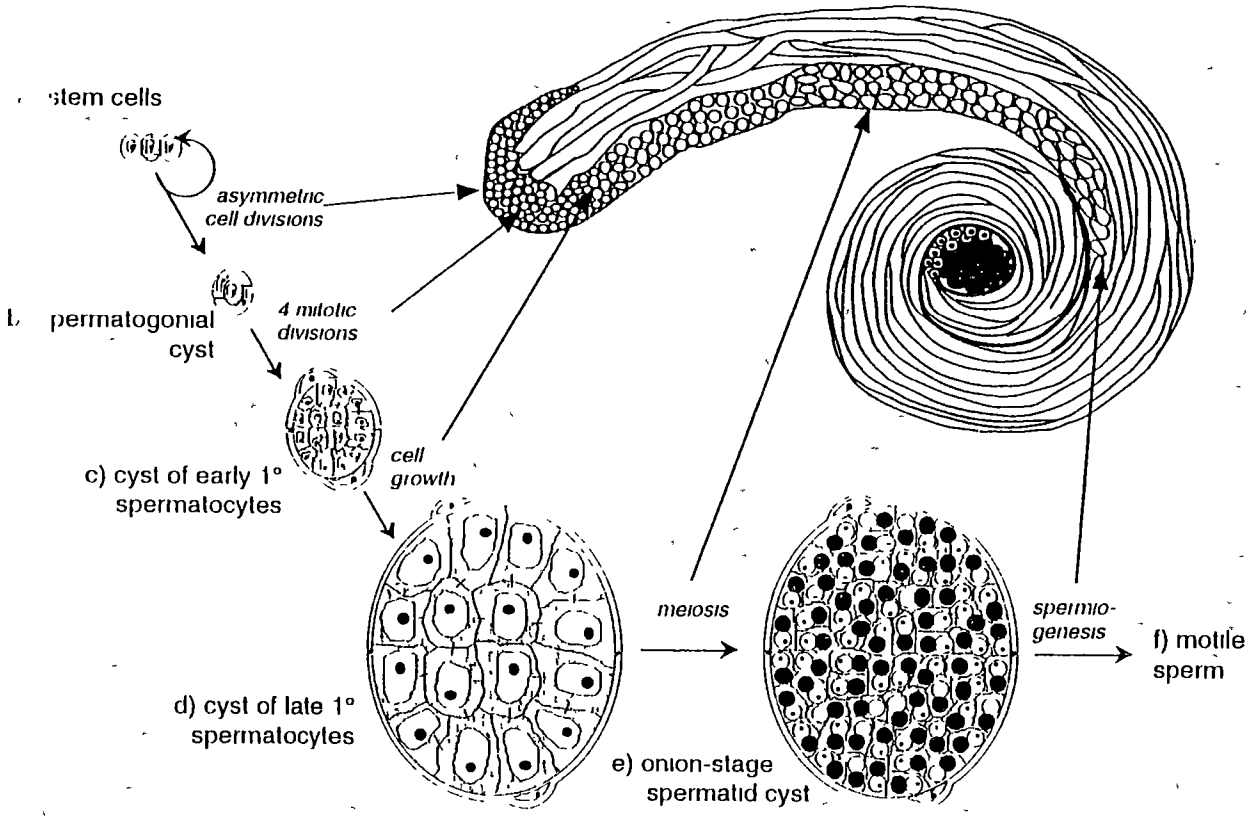

stem cells
asymmetric cell divisions
4 mitotic divisions
b) spermatogonial cyst
c) cyst of early 1° spermatocytes
cell growth
d) cyst of late 1° spermatocytes
meiosis
e) onion-stage spermatid cyst
spermio-genesis
f) motile sperm

Fig 3 Orcein-stained Spermatocytes in *Drosophila* males

A. Prophase I· Three clumps appear. Arrow indicates typical prophase I cell

B Metaphase I: Three bivalents congress toward metaphase plate.

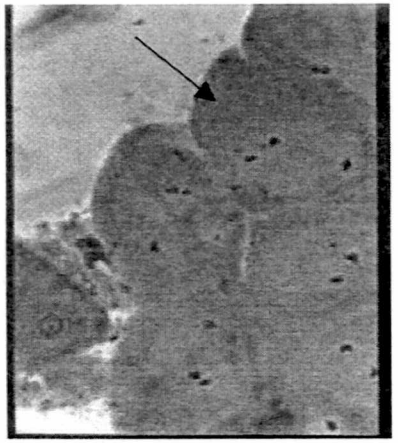
Arrow indicates chromosomes at metaphase I.

C. Anaphase I: Homologous chromosomes are segregated to opposite poles.

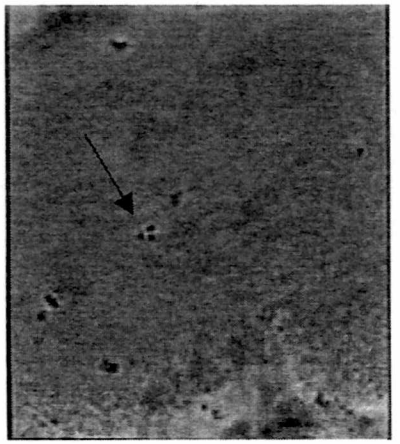
D. Metaphase II. Three chromosomes appear at metaphase plate

E. Anaphase II Sister chromatids are segregated to opposite poles

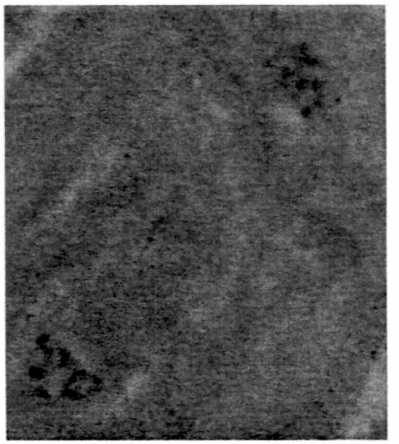
Arrow indicates cell at anaphase II.



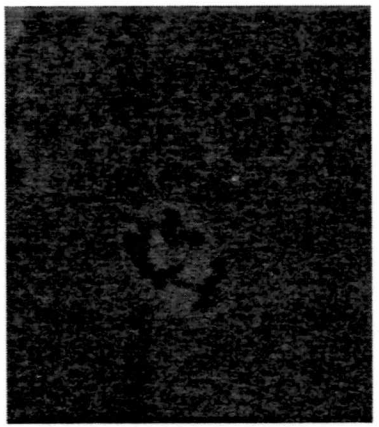
(A) Prophase I



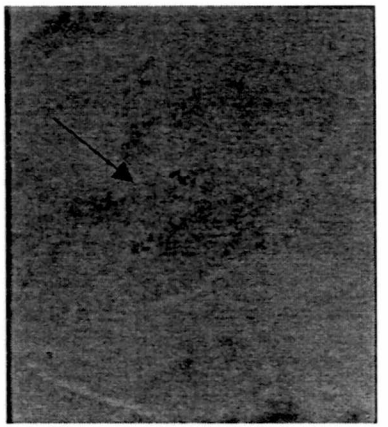
(B) Metaphase I



(C) Anaphase I



(D) Metaphase II



(E) Anaphase II

X chromosome pairing site of *Drosophila melanogaster*

Cytological studies showed that the X-Y pairing region in *Drosophila* male is confined to heterochromatin (Cooper, 1964, Appels and Hilliker 1982) The X pairing site can be moved by inversions to the distal end of the X chromosome, causing the X chromosome to pair at its distal end (Cooper, 1964) Additional evidence for a heterochromatic X pairing site comes from deletion experiments. *Drosophila melanogaster* males carrying X chromosome from which most heterochromatin is deleted showed high frequencies of X-Y nondisjunction (Sandler and Braver 1954, Peacock, 1965; McKee and Lindsley 1987). Df (1) sc4L-sc8R and Df(1) X-1 have a deletion of heterochromatin of X chromosome. Df(1) sc4L-sc8R males show X-Y nondisjunction, depending on genetic background and temperature (Gershenson, 1933; Sandler and Braver, 1954 Cooper, 1964 Peacock, 1965, Peacock et. al., 1975). Df(1)X-1 males exhibit very high levels of X-Y nondisjunction, generating X, Y, XY, and nullo-XY secondary spermatocytes in equal numbers(McKee and Lindsley, 1987; McKee and Karpen 1990). In contrast, Dp(1;f)AR111, which is an X chromosome deficient for most of the euchromatin, but which has a small part of heterochromatin containing rDNA, retains the ability to pair with and disjoin from their partner. The Dp(1;f)AR111 disjoins regularly from an attached X-Y in XY/DpAR111 males (Park and Yamamoto,1995) while Dp(1;f)1187, which lacks most of the heterochromatin, segregates randomly from an attached-XY (Lindsley and Sandler, 1958). Fig.4 shows summarized results that map the X-

chromosome pairing site. From these data, it is concluded that central heterochromatin is sufficient to mediate X-Y pairing.

rDNA and its role in X-Y pairing in *Drosophila melanogaster* males

Several lines of evidence imply that rRNA genes are responsible for X-Y pairing First, cytological studies suggested that sex chromosome pairing in *Drosophila* males might be limited to the region around and including the nucleolus organizers (NOs) (Cooper, 1964) The NOs contain 200-250 copies of the rRNA genes in tandem repetition (Ritossa, 1976; Long and David, 1980). Second, all X chromosomes containing rDNA have the ability to disjoin regularly from Y chromosomes, while all X chromosomes with rDNA deletions show X-Y nondisjunction. Most importantly, decisive evidence for pairing ability of rDNA came from an analysis of transgenic rDNA insertions using a P-element mediated transformation experiments McKee and Karpen (1990) inserted a single copy of rDNA onto the tip of the X chromosome [rib7 (1A1-4)] When [rib7(1A1-4)] was recombined onto a pairing deficient X chromosome, Df(1) X-1, rib7(1A1-4) significantly restored the X chromosome's ability to pair with Y chromosomes, B^sYy^+ and B^sY, and caused increased levels of X-Y disjunction. Interestingly, when more copies of rDNA were inserted onto the Df(1) X-1 chromosome, its pairing capacity increased and the magnitude of restoration was proportional to the number of inserted rDNA repeats. The capacity of rDNA repeats to mediate pairing does not depend on an intact rDNA transcription unit. One complete rDNA copy consists

Figure 4. Mapping the X chromosome pairing site

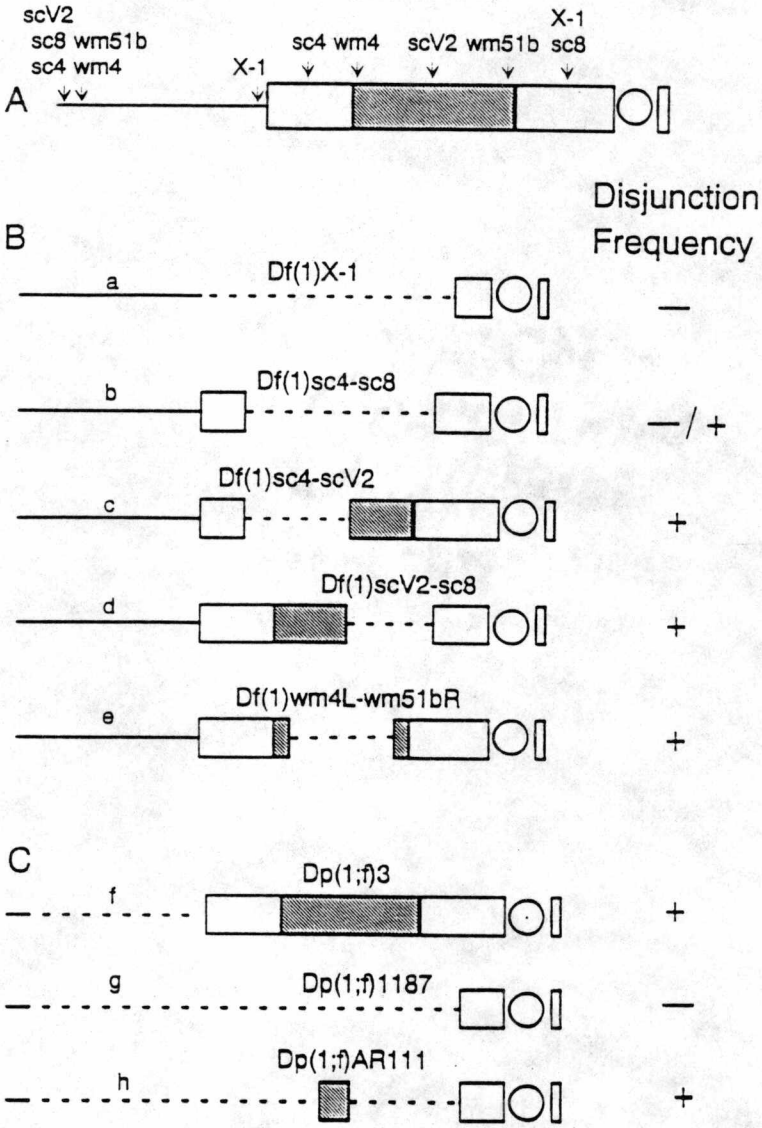
A. Breakpoints of some inversions and deficiencies used to map the pairing site

B and C. Deficiencies (B) and free X duplications (C) used to map X chromosome pairing site

Boxes = heterochromatin, crosshatched boxes = rDNA, lines =euchromatin and dotted lines = deleted region

- = nondisjunction, + = disjunction, -/+ = nondisjunction depending on genetic background and temperature

This Diagram is obtained from McKee (1998)


scV2
sc8 wm51b
sc4 wm4
X-1
sc4 wm4
scV2 wm51b
X-1
sc8
A
Disjunction
Frequency
B
a
Df(1)X-1
—
b
Df(1)sc4-sc8
—/+
c
Df(1)sc4-scV2
+
d
Df(1)scV2-sc8
+
e
Df(1)wm4L-wm51bR
+
C
f
Dp(1;f)3
+
g
Dp(1;f)1187
—
h
Dp(1;f)AR111
+

on an 8kb transcription unit (TU) and a 3-4kb intergenic spacer (IGS). A TU contains the18S, 5 8S, and 28S rRNAs as well as an external transcribed spacer (ETS) and internal transcribed spacer (ITS) (Fig. 5A) Merrill et al (1992) generated deletions of part or all of the transcription unit and obtained X chromosome insertions using a P-element Three different constructs containing IGS regions and varying amount of the 5'end of the transcription unit mediated pairing in a dose-dependent manner McKee et al. (1992) also generated a series of X-linked rDNA deletions which retained most or all of the IGS region but lacked part or all of the rDNA transcription unit More precisely, 240bp sequences of the IGS region showed pairing capacity The 240 bp sequences are present in 6-12 tandem repeats upstream of the rDNA promoter, and at least 6 copies of the sequences are required for X-Y pairing (Fig 5B)

Ren et al (1997) showed that the transcriptional unit alone does not stimulate X-Y pairing , despite containing up to six times as much rDNA as some of the IGS-only insertions (Fig 5Bk). This result indicates that the absence of the transcription unit had no effect on pairing capacity, suggesting that the 240bp repeats alone suffice to mediate X-Y pairing Gene constructs used to map the rDNA pairing site are summarized in Fig 5B

Additional evidence for the key role of 240 repeats in X-Y pairing comes from cytological study of *Drosophila simulans*, which has a very similar chromosome structure to *Drosophila melanogaster* except that *D simulans* does not have a nucleolus organizer (NO) in Y chromosome, which contains rDNA However, the Y chromosome of *D simulans* contains a very large (3000kb)

Figure 5 Constructs of rDNA and used to map rDNA pairing site.

A. a copy of a complete rDNA

   IGS, Intergenic spacer

   ETS; External transcribed spacer

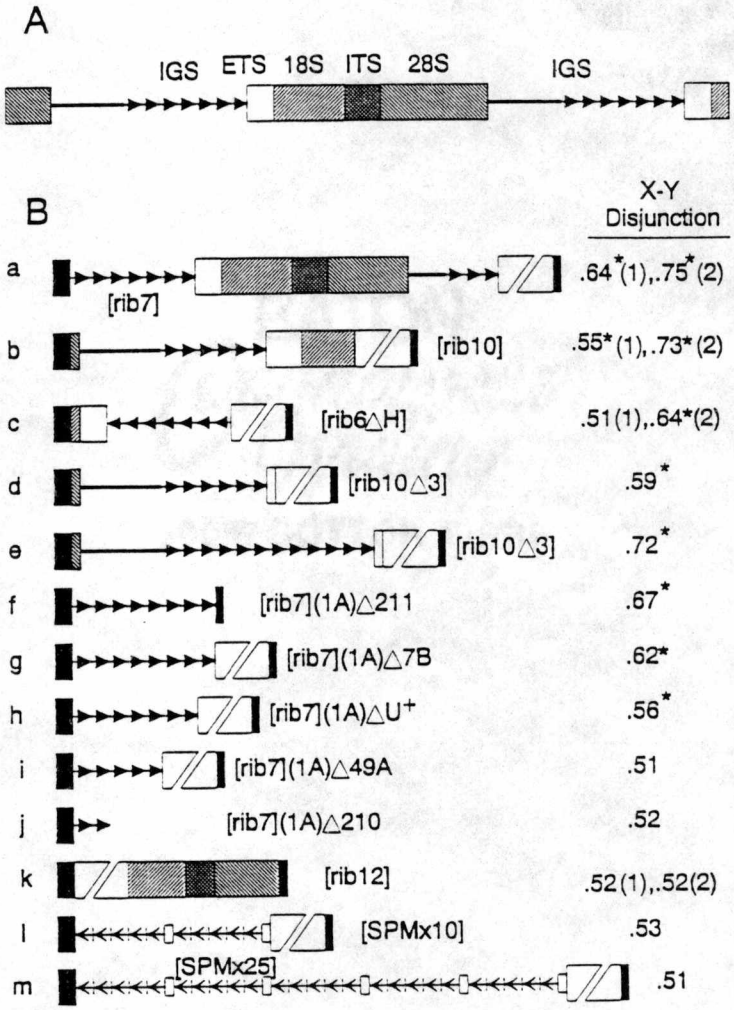
   ITS, Internal transcribed spacer, which contains 5.8S

B. Gene constructs used to map rDNA pairing site

   At least 6 copies of 240bp repeats are required to stimulate X-Y disjunction

   Crossed rectangles = P-element sequences, open rectangles = rosy gene for eye color marker and crossed triangles = 240bp repeats.

   Number in parentheses is copy number of the insertion Numbers indicate X-Y disjunction frequencies Asterisk indicates a result significantly different from random disjunction The diagram is obtained from McKee (1998)


A
IGS
ETS
18S
ITS
28S
IGS
B
X-Y Disjunction
a
[rib7]
.64*(1),.75*(2)
b
[rib10]
.55*(1),.73*(2)
c
[rib6△H]
.51(1),.64*(2)
d
[rib10△3]
.59*
e
[rib10△3]
.72*
f
[rib7](1A)△211
.67*
g
[rib7](1A)△7B
.62*
h
[rib7](1A)△U+
.56*
i
[rib7](1A)△49A
.51
j
[rib7](1A)△210
.52
k
[rib12]
.52(1),.52(2)
l
[SPMx10]
.53
m
[SPMx25]
.51

block of 240bp IGS repeats (Lohe and Roberts 1990), and it was proven that this region of the Y chromosome of *D simulans* pairs to the X chromosome (Ault and Rieder,1994). Therefore, it seems that this block of 240bp repeats provides pairing function in the absence of a NO

Transcription and Pairing

A role for transcription in chromosome pairing is supported by some evidence First, a promoter-containing fragment of yeast ribosomal DNA stimulates mitotic recombination of an adjacent gene 25 to 100 fold (Voelkel-Meiman et al , 1987). Second, some meiotic recombination hot spots have been mapped to promoter and/or enhancer regions in yeast and mice(Nicolas et al., 1989; Sun et al., 1989; Shenkar et al., 1991). Finally, in *Drosophila melanogaster* both transcription and pairing are positively correlated with the number of 240bp repeats

Each 240bp IGS repeat from the rDNA has nearly perfect copy of a 52bp fragment and a functional RNA polymerase I promoter located at the beginning of the transcription unit (Coen and Dover, 1982, Kohorn and Rae, 1982, Miller et al , 1983; Simeone et al., 1985) These "spacer promoters" initiate transcription *in vivo* (Murtif and Rae, 1985) and *in vitro* (Kohorn and Rae 1982) In addition, the spacer promoters function as directional enhancers of transcription from the rDNA promoter (Grimaldi and Di Norera, 1988, Grimaldi et. al , 1990).

To determine whether transcription is related to the pairing capacity of 240bp repeats, Sun et al made four single base pair mutated 240bp repeats by site-directed mutagenesis, and introduced the constructs into X chromosomes, which is deficient of X heterochromatin. The mutation sites include -16, -13, -1 and +1 positions with the respect to the transcription initiation site. These arrays did not stimulate X-Y pairing and disjunction despite containing 25 copies of the mutant repeat (Fig.5Bm) (unpublished data). This result suggests a link between transcription and pairing because 240bp IGS repeats have promoter activity and the 4 sites mutated are thought to be important for transcription.

Now, it is necessary to determine whether promoter region of 240bp repeats itself promotes X-Y pairing in *Drosophila* males, or whether there are other sites which are required for pairing with the 240bp IGS repeat Grimaldi et al. (1990) tested promoter activity of several fragments derived from 240bp repeat They showed that some of the fragments have promoter activity, and that the 72bp fragment extending from –62 to +10 is the smallest segment having promoter activity (Fig. 6) On the basis of this result, the pC72X16 plasmid containing 16 copies of the 72bp fragment was constructed and microinjected into embryos to test the above question X-linked insertions were tested for X-Y pairing and disjunction frequency

Mini-chromosome, Df (1;f)1187

A free X duplication, Dp(1,f)1187 or Dp1187, contains only 1000kb of centromere heterochromatin and 300kb of distal DNA (Fig 4). This Dp1187 has

Figure 6 Sequences of the 240bp repeat and the 72bp fragment.

The 72bp fragment sequence is from –62 to +10 with the respect to the transcription initiation site. The four mutated sites belong to the 72bp fragment. Bold sequences = the 72bp fragment sequences, underlined sequences = primer sequences for subcloning of the 72bp fragment, and arrowhead = direction of primers

Sequences of the 240bp repeats and the 72bp fragment

CTAAG TATTA TAGAG AAAAG CCATT T**TAGT GAATG GATAT**

AGTAG TGTAA GCTAG CTGTT TTACG ACAGA GGGTTCAAAA

ACTAC TATAG GTAGG CAGTG GTTGC CGACC TCTCA TATTG

-1 +1

TTCAA AACGT ATGTG TTCAT ATGAT TTTGG CAATT ATATG

AGTAA ATTAA ATCAT ATACA TATGA AAATT AATAT TTATT

ATATG TATAT GGAAA AATGT TGAAA TATTC CCATT TTCTC

TAAG

been characterized regarding both structure and transmission properties (Karpen and Spradling, 1992; Karpen et. al., 1996). The Dp1187 is transmitted efficiently in both sexes, and two Dp1187 disjoin regularly in *Drosophila* female meiosis. However, two copies of Dp1187 randomly segregated from each other and from sex chromosome in males (Karpen et al., 1996), indicating that Dp1187 does not contain male-meiotic pairing sites This property provides the possibility to test whether any sequence has pairing activity using the mini-chromosome, Dp1187, in *Drosophila* males. Although Dp1187 is very small, transgenes can be inserted onto the Dp1187 using a P-element insertion because it has hot spots. Therefore, P-element insertions onto the mini-chromosome can be obtained by remobilization.

To determine whether 16 copies of 72bp fragment onto Dp1187 stimulate disjunction, pC72x16 onto X-chromosome was remobilized onto Dp1187 by crossing the flies containing transposase source, and disjunction frequency between Dp1187[72x16] and $B^{s}Y$ chromosome was tested.

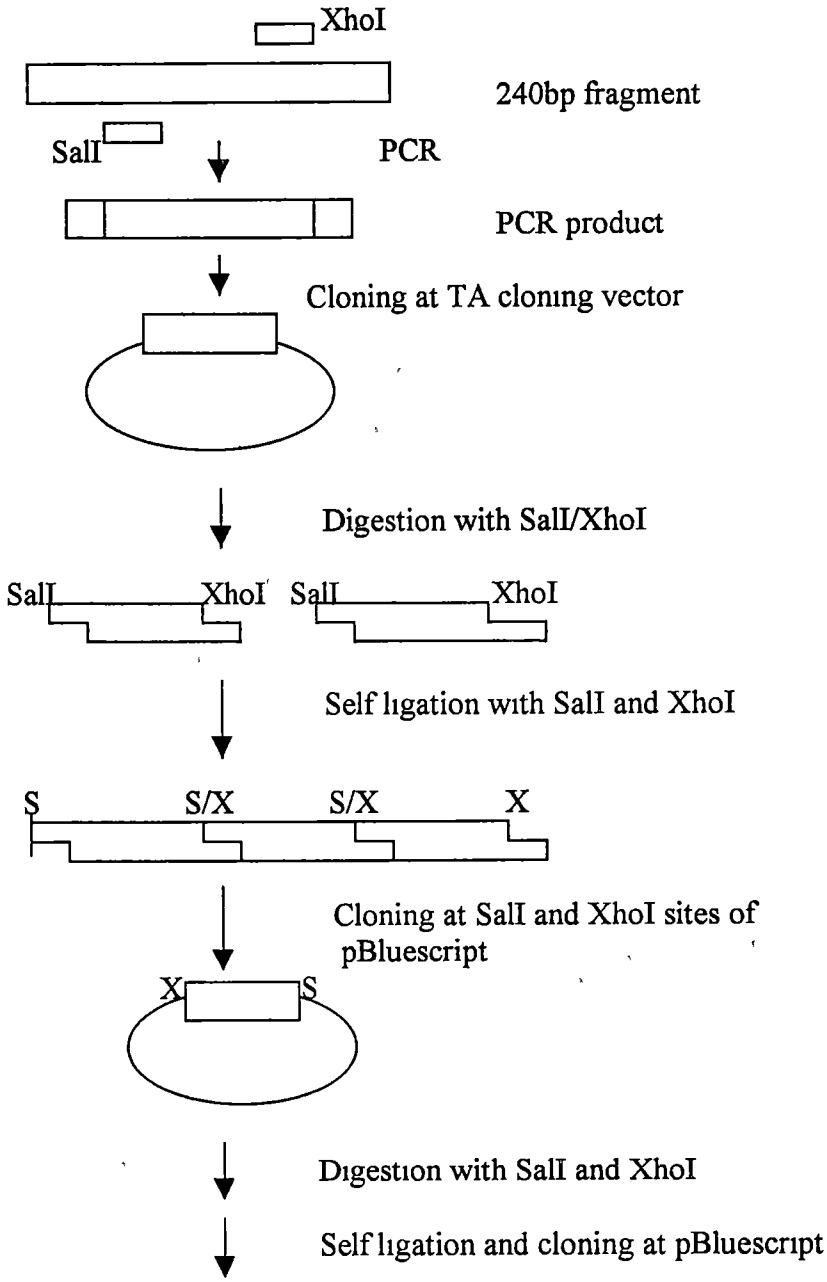
CHAPTER 2

MATERIALS AND METHODS

Plasmid construction

The plasmid pC72x16 was constructed to produce transformants using microinjection The plasmid pC72x16 contains 16 copies of the 72bp fragment which are from -62 to +10 in the 240bp repeat of an rRNA gene. In order to make the array fragment, 240bp repeat DNA was digested with DdeI and the DdeI fragment was used as a template To obtain the 72bp fragment, forward primer (Sal 72F) and reverse primer (Xho 72R), which contain SalI and XhoI enzyme sequence at the 5' end respectively, were used (Sal 72F; GCCGTCGACTAGTGAATGGATATAGTA, Xho 72R, GCGCTCGAGCTGCCTACCTATAGTAGTT) The 72bp PCR products were subcloned into TA cloning vectors (Stratagene). Correct sequence was confirmed by sequencing. After digestion with SalI and XhoI, the linerized 72bp fragments were head to tail self-ligated together After transformation, a four tandemly repeated fragment was obtained. The 16 repeated array was accomplished after one more self-ligation of four repeated 72bp fragment The cloned fragment was inserted at SalI site of Carnegie 20 vector Fig. 7 diagrams how pC72x16 was obtained. The direction and sequence were determined by sequencing and enzyme digestion Fig. 8 shows schematic diagram of pC72x16 construct.

Figure 7. Flow chart showing procedure for making the pC72x16 construct PCR was used to obtain the 72bp fragment, with forward primer (Sal 72F), reverse primer (Xho 72R) and 240bp repeats as a template 72bp PCR products were subcloned into TA cloning vectors The linearized 72bp fragments were head to tail self-ligation together. After transformation, a four tandemly repeated fragment was obtained. The 16 copies of 72bp fragment array was made after another round of self-ligation. The cloned fragment was inserted at SalI site of the Carnegie 20 vector



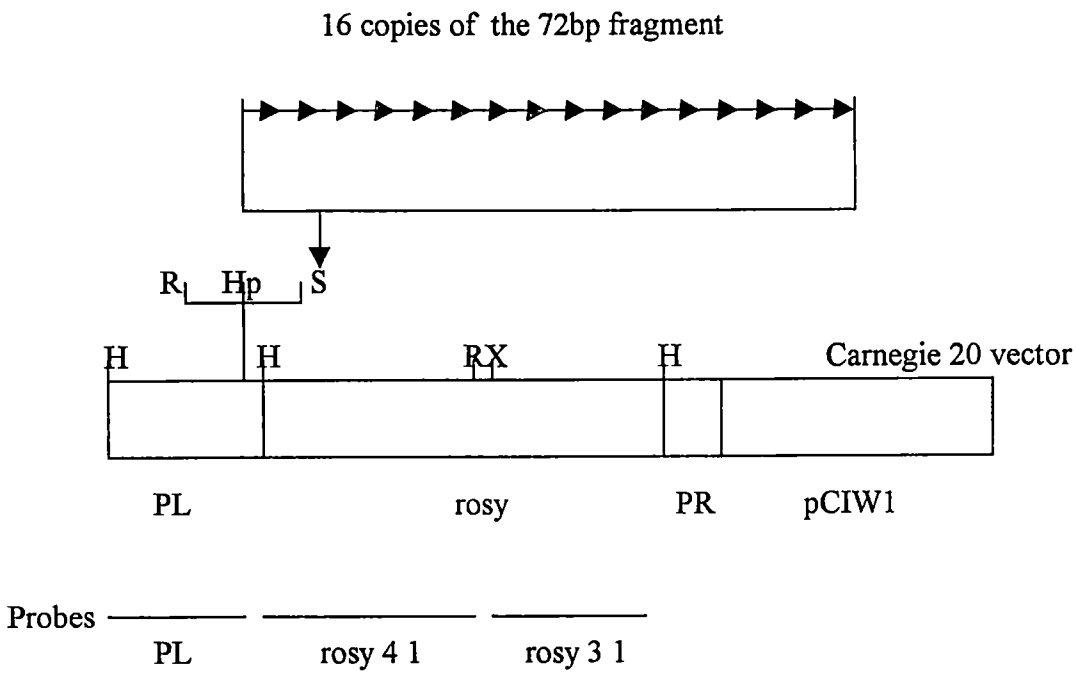
Insertion of 16 copies of the 72bp fragments
at SalI site of Carnegie 20 vector

Figure 8 Schematic diagram of pC72x16 construct.

Sixteen repeated 72bp fragment was inserted at SalI site of the Carnegie 20 vector P-left, rosy 4 1 and rosy 3 1 probes were used for Southern blot analysis

PL = P- element left end , PR = P- element right end, and rosy = eye marker for selection of transformants

R = EcoRI, Hp = Hpa I, S = SalI, X = XhoI, and H = HindIII


16 copies of the 72bp fragment
R
Hp
S
H
H
RX
H
Carnegie 20 vector
PL
rosy
PR
pCIW1
Probes
PL
rosy 4 1
rosy 3 1

***Drosophila* strains and culture**

Flies were cultured at 25^0C on standard cornmeal, molasses, and agar medium supplemented with yeast. The following stocks were used in this study and the brief descriptions are given.

TMS (Sb) A third chromosome balancer with dominant stubble (Sb) marker, and Δ2,3(99B) ry^+ Δ2,3 is a P-element transposase source

ry· A spontaneous mutation of wild type rosy gene, with dark red eyes

FM 6 An X chromosome balancer containing multiple inversions which effectively eliminates crossovers in FM 6/+ heterozygote. This chromosome has w^a and B marker w^a· white-apricot eyes, B: eyes restricted to narrow vertical bar.

B^sYy^+. Y chromosome that has duplicated X tip containing y^+ and three bands from the proximal X containing B^s marker B^s, X-ray induced derivative of B, produces narrower eye than B (Fig 1)

Df (1) X-1· X chromosome containing a large deletion of heterochromatin including the NO and a portion of proximal X euchromatin. Males carrying Df (1) X-1 and a normal Y are inviable because of the proximal euchromatic deletion Thus, B^sYy^+ or B^sY chromosome is required for the male carrying Df (1) X-1(Fig.1)

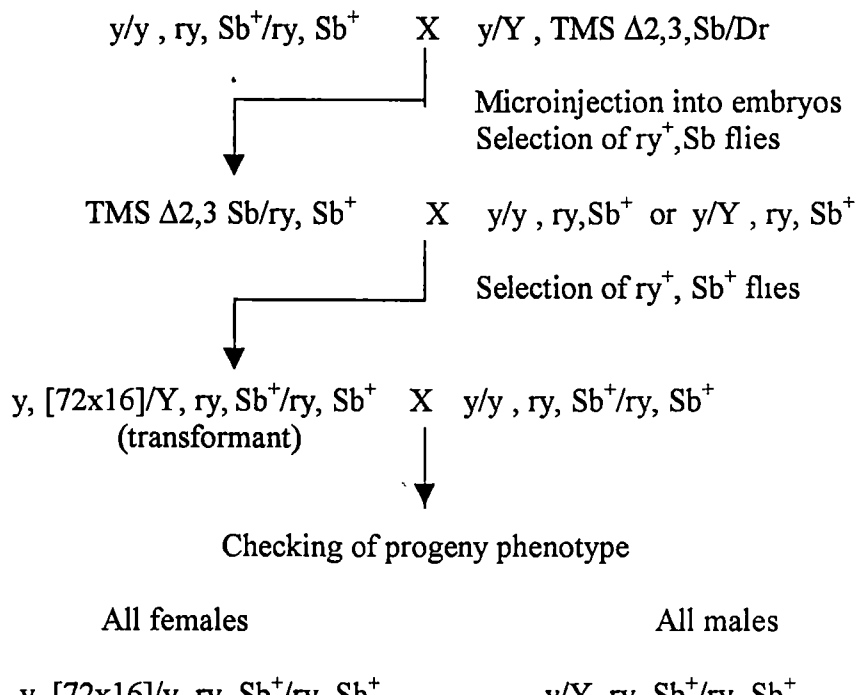
B^sY: Y chromosome which contains duplicated proximal X having B^s marker (Fig 1)

y,w· Two recessive genes on X chromosome which determine body color (yellow) and eye color (white).

***Drosophila* transformation**

The pC72x16 containing 16 repeats of the 72bp fragment were microinjected into the *Drosophila* germ line cell using the method described by Spradling and Rubin (1982, 1986). Two strains were used to produce embryos for this purpose One was TMS, Δ2,3/Dr, which carries a stable source of transposase on the third chromosome, and the other was y, ry The cross TMS, Δ2,3/Dr X y; ry was carried out at room temperature. Fresh embryos were collected, dechorionated, desiccated, and microinjected with the pC72x16 plasmid at a concentration of 300-600ug/μl in injection buffer (5mM KCl, 0 1mM NaH2PO4) ry^+, Sb progeny were selected and crossed individually with opposite sex of y, ry flies. G1 flies having ry^+ Sb phenotype were discarded to get rid of the Δ2,3 transposase source and the ry^+ gene which is linked with Sb gene Thus, ry^+ Sb^+ flies carry ry^+ gene that is from the injected plasmid Male flies with ry^+ Sb^+ were crossed singly with y; ry virgins to determine whether or not the plasmid was inserted into the X chromosome (Fig 9)

Figure 9 Cross scheme for microinjection and identification of transformants y, ry virgin flies were crossed to flies carrying Δ2,3 transposase, and pC72 x16 was microinjected into fresh embryos from this cross. ry^+ Sb flies were selected and crossed to y, ry to select transformants. Transformants show ry^+,Sb^+ phenotype The transformants were crossed to y, ry flies to determine if the insertion is X-linked X-linked transformants give ry^+ females and ry males.



X-linked transformants show all female progeny are ry^+ eyes and all male progeny are ry eyes

Remobilization of transgene

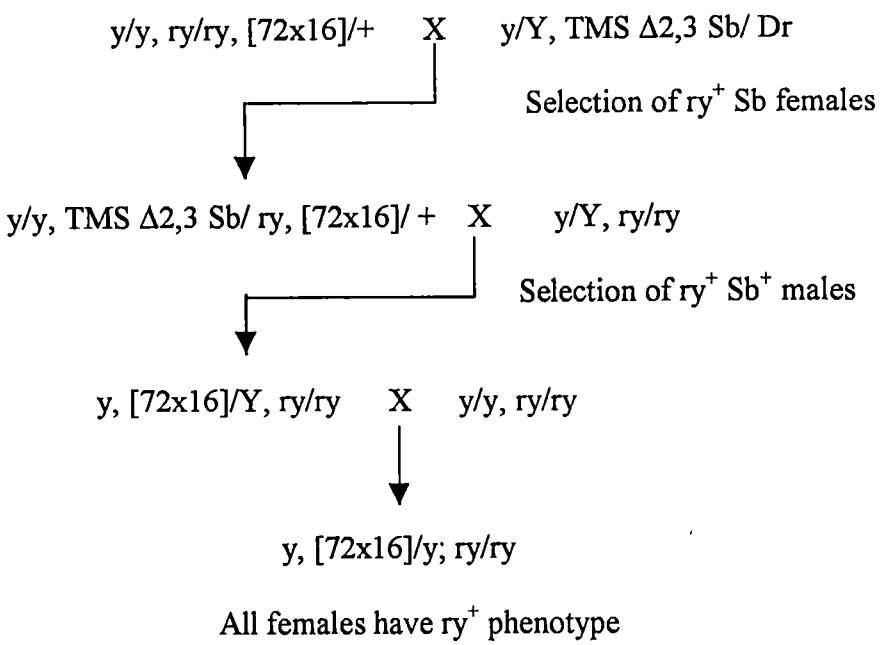
No X-linked insertion line was obtained, so remobilization of the insertion was carried out. The Autosome-linked insertion line was crossed to TMS, Δ2,3/Dr at room temperature Sb, ry^+ progeny were selected, and crossed with opposite sex y; ry flies Males with ry^+, Sb^+ phenotypes were selected and crossed with y, ry virgins to check whether the transgene was remobilized onto X-chromosome If transgene is remobilized onto X-chromosome, all female would be ry^+, and all male would be ry phenotype (Fig 10) From this remobilization, 11 X-linked transformants were made

Southern blot analysis

Genomic DNA was prepared from 10-15 adult flies as described by Bender et al (1983). The DNA was digested overnight by appropriate restriction enzymes and separated on a 0.8-1% agarose gel by electrophoresis in Tris-Borate-EDTA (TBE) buffer (Sambrook et al , 1989). The DNA was nicked by soaking the gel for 15min in 0.25M HCl, then denatured by incubation for 30 minutes in 0 4M NaOH/ 0.6M NaCl and neutralized by incubation for 30 minutes in 0.5M Tris-HCl (pH7 5)/1 5M NaCl DNA was transferred to Gene Screen Plus hybridization transfer membrane (Dupont/NEN) by the Southern blot method (Southern, 1975) The membrane was baked at 100°C for 2hrs under vacuum. After baking, the membrane was prehybridized for 2-4hr at 42°C in 50% formamide, 1% SDS, and 5X SSPE

Figure 10. Cross scheme for remobilization of autosome linked pC72x16 to a X chromosome

Transformants carrying pC72x16 on an autosome were mated to flies containing transposase. Progeny with ry^+, Sb were selected and crossed to y, ry opposite sex flies. At the next generation, ry^+, Sb^+ males were selected and crossed to y, ry females individually to check whether an insertion was remobilized to an X chromosome. Flies containing pC72x16 on a X chromosome give all female progeny ry^+ phenotype


y/y, ry/ry, [72x16]/+ X y/Y, TMS Δ2,3 Sb/ Dr
Selection of ry+ Sb females
y/y, TMS Δ2,3 Sb/ ry, [72x16]/ + X y/Y, ry/ry
Selection of ry+ Sb+ males
y, [72x16]/Y, ry/ry X y/y, ry/ry
y, [72x16]/y; ry/ry
All females have ry+ phenotype

(0 75M NaCl, 0.05M MaH2PO4 H20, 5mM EDTA), and salmon sperm DNA (Sambrook et al., 1989). The DNA probes were labeled by using a random primers DNA labeling system (BRL). Hybridization was performed at 42^{0}C overnight. Membranes were washed with shaking in 2X SSC for 5 minutes at room temperature, 2X SSC/ 1% SDS for 30 minutes at 65^{0}C twice, 0.1X SSC for 30 minutes at room temperature, and then exposed for various times at -80°C using Kodak XAR5 films

***In situ* hybridization**

In situ hybridization to salivary gland chromosomes using biotin-labeled DNA probes was carried out as described in Engels et al. (1991). Briefly, Salivary glands were dissected from wandering third instar larvae, fixed in 45% acetic acid on subbed slides, squashed under siliconized cover slips and kept at 4°C overnight. The cover slips were removed after freezing the slides in liquid nitrogen and chromosomes were fixed in 3:1 mixture of methanol and acetic acid. After dehydrating in ethanol, the slides were air dried. The slides were then washed in 2X SSC at room temperature for 2 minutes The slides were immersed in 2 liters of 1000mM TEA, and 10ml of acetic anhydride was added while stirring the solution vigorously. Once the acetic anhydride was dissolved, stirring was stopped, and the slides were left to sit in the solution for 10 minutes. The slides were washed in 2X SSC twice for 5 minutes each and dehydrated in 70% ethanol twice and in 90% ethanol twice. After denaturing in

70mM NaOH for 1 5 minutes at room temperature, the slides were dehydrated, air dried, and hybridized.

The DNA probes for hybridization were prepared using the BioNick labeling system (Gibco-BRL) and purified using column chromatography The probes were denatured by boiling the probe for 5 minutes 10µl of the probe was placed on the chromosomes The chromosomes were covered with siliconized cover slips, sealed with rubber cement, and incubated at 58°C in a moisturized chamber overnight Next day, the rubber cement was peeled off and the cover slips were removed by soaking the slide in 2X SSC at 53°C The slides were washed with three changes in 2X SSC at 53°C for 20 minutes each and two changes in PBS solution at room temperature for 10 minutes each The slides were then incubated in 2% BSA in TMN1, Triton X-100 to prevent non-specific binding. Fifty µl of SA/AP (2µl streptavidin/ alkaline phosphatase conjugate in 1ml TMN1, Triton X-100) was pipeted onto each slides and left to sit for 5 minutes Another 50µl of SA/AP was added to each slides and incubated for 5 minutes Each slide was rinsed with 3-4ml of TMN1, Triton X-100 by holding the slide over a beaker The slides were washed twice in TMN1, Triton X-100 and twice in TMN2. 30µl of NBT/BICP solution was pipeted onto the chromosomes. The slides were then placed on a flat tray and incubated in the dark for 2 hours. The slides were then rinsed in distilled water and air dried To examine the slides, a drop of sterile water was pipeted on the chromosomes and a siliconized cover slip was placed on it To photograph, the cover slip was sealed with rubber cement to prevent oil from going under the cover slip

Testes squashes

Male meiotic chromosomes were prepared by the method of Lifschytz and Hareven (1977). Briefly, 0-2 day adult males were dissected in 7% NaCl. (Ashburner, 1989) and the testes from the dissected flies were fixed in 45% acetic acid for 30 seconds, and stained in 3% orcein-60% acetic acid for 5 minutes. The testes were transferred to a drop of 60% acetic acid, torn at their apical end and gently squashed with a cover slip containing a drop of 2% lactic-acetic-orcein. The well-spread chromosomes were examined under phase-contrast optics using a Zeiss Universal Axioplan photomicroscope and photographed using Kodak T-MAX 100 film.

Data analysis

The X-Y disjunction frequency (P) is defined as the fraction of secondary spermatocytes that include an X or Y but not both ((X+Y)/ (X+Y+XY+O)). P values were estimated from testis squash data using the formula P=(2a+d)/(2a+2b+c+d+e) (where a=X and Y going to different poles, b=X and Y going to the same pole from Meiosis I, and c= a cell has no sex chromosomes at all, d= a cell contains either X or Y, e= a cell contains both X and Y from Meiosis II.) The variance is V=P(1-P)/N

P values were also estimated from progeny class frequencies by the method of McKee (1984; also see McKee and Lindsley, 1987). The formulae are

$P=1/[1+(o\,(XY)o(0)/\,o(X)o(Y)^{1/2}]$

$Rx = [\,(o(XY)o(X)/(o(Y)o(0))\,]^{1/2}$

$Ry = [\ (o(XY)o(Y)/o(X)o(0)\ ]^{1/2}$

The variances are. $V_J = 1/[\Sigma I\ (\ (1/e(i)\ )\ (\ e(1)/J\ )^2\ )\ N\ ]$,

where I represents the progeny classes. X, Y, XY, and 0. Rx and Ry are chromosome viability parameters defined as the viability of sperm that carry the relevant chromosome divided by the viability of otherwise identical sperm that lack it J represents the parameters. Rx and Ry (Kempthorne, 1969). Parameter comparisons involving different lines were made by the Z-test which uses the following equation: $Z= (J_1-J_2) / (V_{J1}+ V_{J2}\ )^{1/2}$

CHAPTER 3

RESULTS

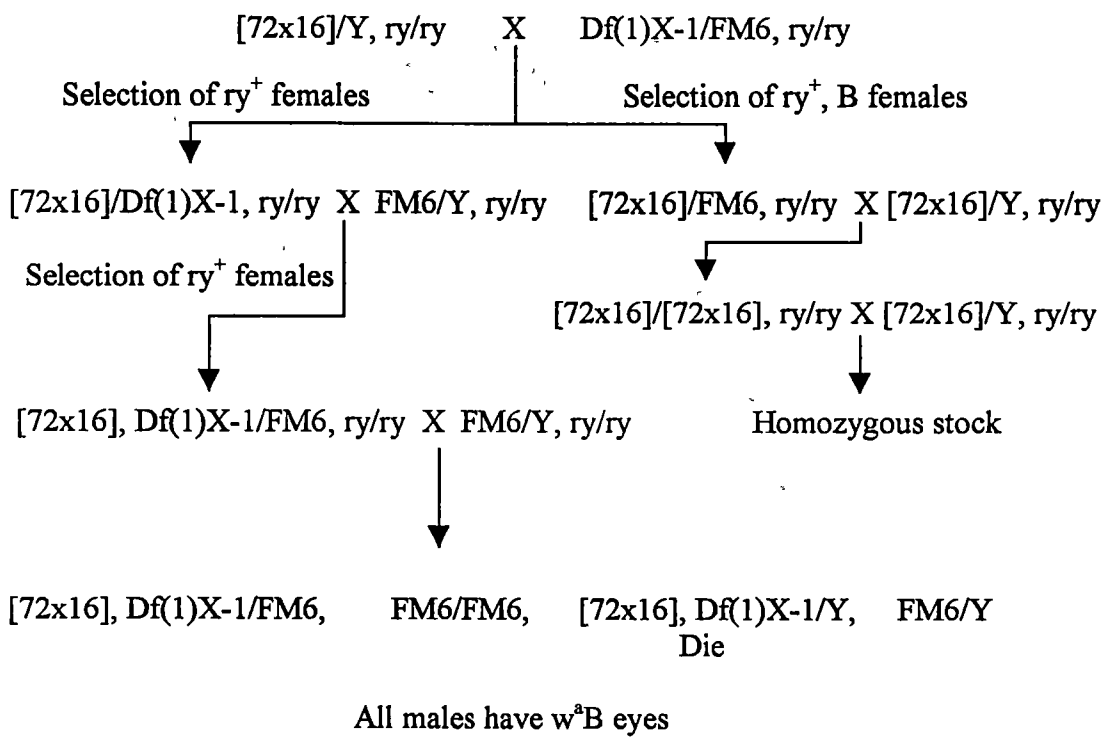
Transformants

Plasmid pC72X16 includes 16 copies of 72bp fragment, covering from –62 to +10 of the 240bp repeat sequence. The 72bp fragment contains RNA polymerase I promoter activity The 16 copies of the 72bp fragment were inserted in a Carnegie-20 transformation vector, which contains a wild type *rosy* gene as an eye-color marker (Rubin and Spradling, 1983) The plasmid pC72X16 was transformed into the germline of y, TMS, Δ2,3/ry embryos by microinjection The Δ2,3 is a P-element insertion on chromosome 3 that is a source of transposase, which facilitates P-element insertion into genomic DNA. Thus, co-injection of helper DNA is not needed in this experiment (Fig. 9)

One independent transformant was identified, and the transformant was not X-linked. In order to make an X-linked transformant, the insertion was remobilized, and several X-linked transformants were identified The cross scheme for the remobilization of insertion is showed in Figure 10 Four of the X-linked transformants (T1, T2, T3 and T4) were selected randomly and recombined onto a heterochromatically deficient X-chromosome, Df(1) X-1 by crosses (Fig 11) Insertions of all four transformants were transferred onto the Df(1) X-1 chromosome.

Figure 11. Cross scheme for making homozygous stocks and [72x16] –Df(1)X-1 recombination lines.

X-linked transformant males were crossed to Df(1)X-1/FM6 female flies ry^+ female flies were selected and crossed to FM6/Y, ry/ry male flies At the next generation, ry^+ females were crossed to FM6/Y; ry/ry males singly to detect recombinants between the X chromosome containing insertion and the Df(1)X-1 chromosome. When recombination occurs, male progeny will have w^aB eyes



All males have w^aB eyes

Southern blot analysis

Inserted DNA was analyzed by Southern blot hybridization using probes derived from P-left and rosy sequences (Fig. 12). Genomic DNAs of four transformants and ry flies which were digested with EcoRI were run on agarose gel and transferred to nylon membrane followed by hybridization with a P^{32} labeled ry3.1 fragment, which is 3.1kb fragment of 5'end of rosy gene As expected, 2 bands were observed on the blot. One is from the native *rosy* gene because all of the transformants carry the *rosy* gene on their third chromosome and the other one is from the transgene. The size of one band was same among the transformants and ry flies The size of the other band was different among the transformants indicating that all inserts are at different locations on the X chromosome, and ry flies which did not have insertion did not show this band. Hybridization with P-left probe yielded the same result.

In order to determine whether transformants contained 16 copies of the 72bp fragment, genomic DNA was digested with EcoRI, and then blotted and hybridized with the ry4 1 probe, which is 4 1kb of 3'end of rosy gene. Genomic DNAs of ry flies were also used as control. Fig 12 shows the two bands from the transformants One is from endogenous ry gene because the size of the bands are same as that of ry flies. The other is from the transgene The size of this band was about 5.3kb (1.2kb from transgene + 4.1kb from the ry gene) indicating all transformants have correct copy number of the 72bp fragment.

Figure 12. Autoradiograms of Southern blot analysis.

A. Enzyme, EcoRI, Probe, P-left, 600bp of left end of P-element

All transformants show one different size band, implying different insertion sites.

B Enzyme; EcoRI, Probe; rosy 3.1, 3.1kb of 5'end of rosy gene

All transformants show one different size band from transgene and one same size band from endogenous rosy gene

C. Enzyme, EcoRI, Probe, rosy 4.1, 4 1kb of 3'end of rosy gene

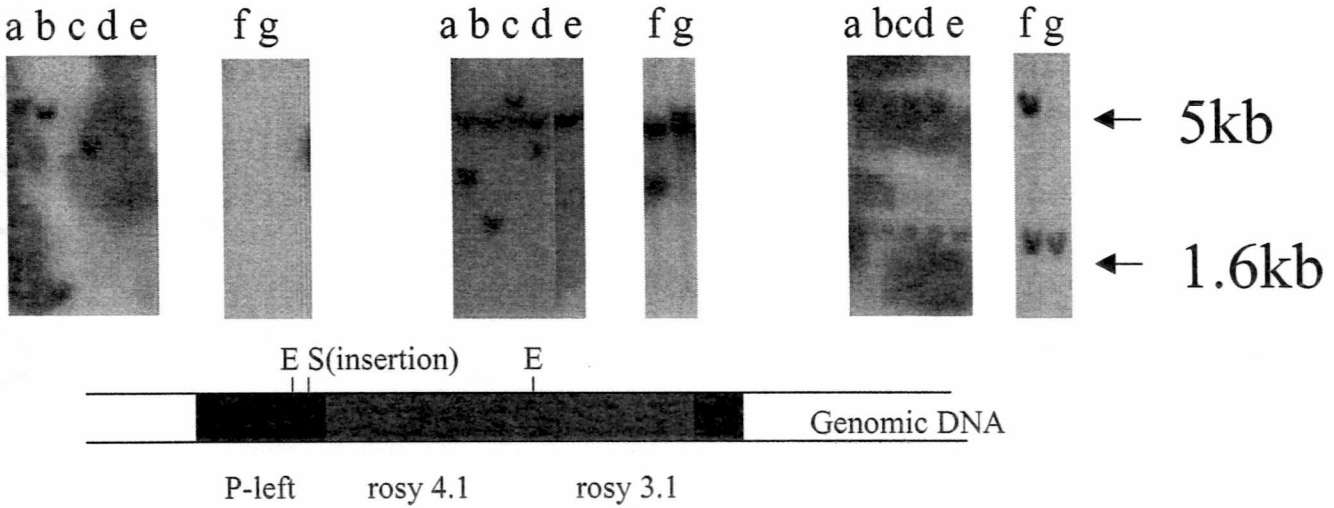
All transformants show above 5kb bands, indicating the insertion is intact

Southern blot analysis

EcoRI P-left

EcoRI rosy 3.1

EcoRI rosy 4.1



a: T1, b: T2, c: T3, d: T4, e: ry

ertre

f: ry,Dp1187[72x16], g: ry,Dp1187

***In situ* hybridization**

Polytene chromosomes in salivary glands replicate 9-10 times without cell divisions, and they have characteristic banding pattern Thus, the precise location of transgene can be determined by *in situ* hybridization to the polytene chromosomes.

Salivary glands of third instar larvae of T1, T2, T3 and T4 were squashed and polytene chromosome were probed with biotin-labeled 3 1kb fragment of the rosy gene. From the *in situ* hybridization experiment, it was determined that inserts of T2 and T3 transformants were located in 11E and 15A respectively (Fig. 13). The locations of the insertions of the T1 and T4 transformants were not determined.

X-Y pairing and disjunction analysis

Disjunction requires proper homologous chromosome pairing and segregation, thus failure of chromosome pairing leads to nondisjunction. To determine if the transformants carrying 16 copies of the 72bp fragment have the ability to stimulate X-Y disjunction, X-linked transformants were recombined onto Df(1)X-1. The Df(1)X-1 is an X chromosome deficient for most of the heterochromatin containing the native pairing site and disjoins at random from the Y chromosome (McKee and Lindsley, 1987). These recombinant X chromosomes were made heterozygous with the Y chromosome B^sYy^+ because the B^s duplication which is present in B^sYy^+ compensates for the missing function of the proximal euchromatic deletion of Df(1)X-1.

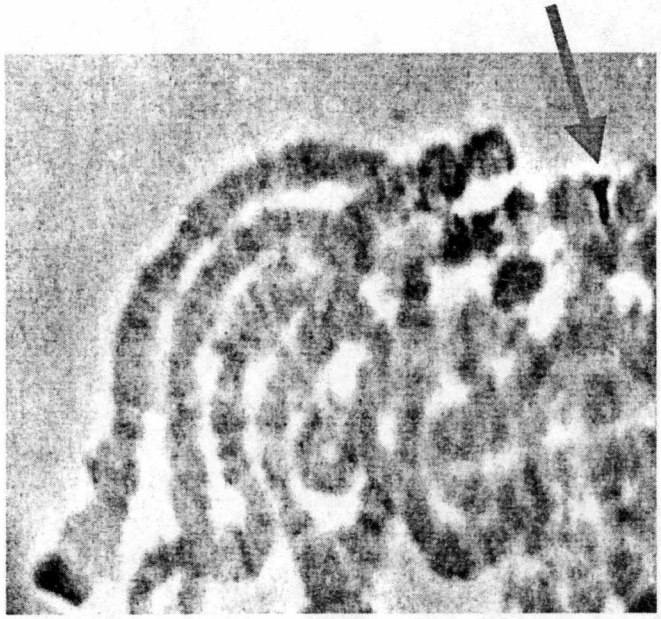
Figure 13 *In situ* hybridization of rosy 3.1 to polytene chromosomes.

Arrows indicate the locations of the insertions

A. T2 transformant ; 11E

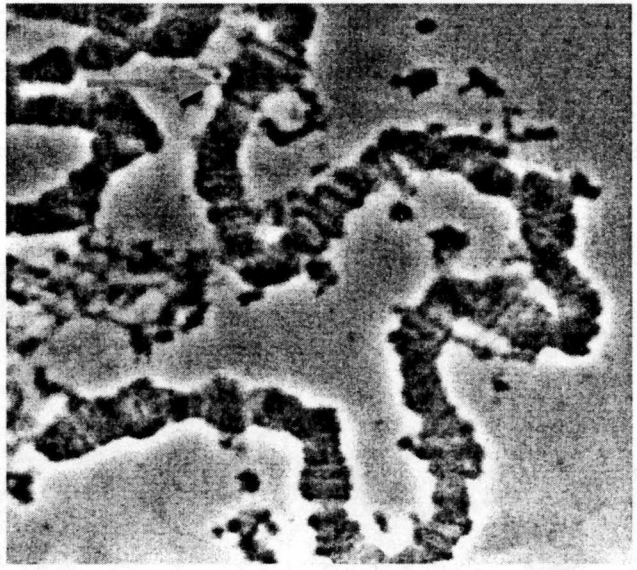
B. T3 transformant ; 15A

In situ hybridization



T2

Insertion site: 11 E



T3

Insertion site: 15 A

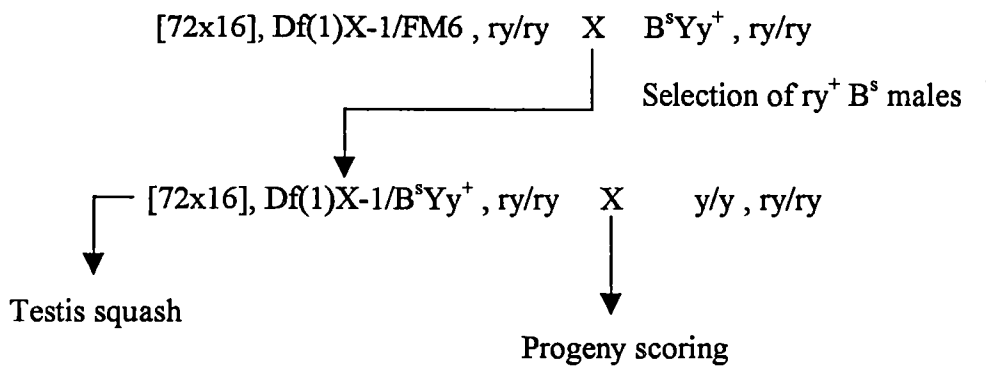
Pairing and disjunction were assayed cytologically in orcein-stained squash preparations of 0-2day young male testes. Late prophase I and metaphase I chromosomes were analyzed for meiotic pairing pattern. Unlike Df(1) X-1, where the X and Y chromosome are unpaired (0-5% pairing), all four transformants showed increased X-Y chromosome pairing

Disjunction was measured cytologically for all four transformants by scoring anaphase I, prophase II, metaphase II, and anaphase II spermatocytes for the presence of the X and Y chromosomes. Disjunction frequency is defined as the fraction of secondary spermatocytes with an X or Y but not both (X+Y)/(X+Y+XY+O). Df(1) X-1 chromosome shows about 50% disjunction frequency because unpaired Df(1) X-1 chromosomes disjoin randomly The disjunction frequencies for the four transformants were higher than that of Df(1) X-1 flies. T1, T2, T3, and T4 transformants showed 63%, 61%, 61%, and 63% disjunction frequencies respectively. This result implies that 16 copies of 72bp fragment partially rescue X-Y pairing and disjunction (Table 1.)

Disjunction was also measured genetically by crossing the [pC72x16]-Df(1) X-1/$B^{s}Yy^{+}$ males to ry females and scoring the recovery of the 4 sperm classes (X, Y, XY, and nullo-X, nullo-Y) in their progeny (Fig 14) This genetic method allows analysis of many more meioses than does cytological analysis, thus giving greater reliability To estimate the disjunction frequency from progeny count data, an adjusted formula (see Materials and Methods) was used to compensate for skewed progeny ratios resulting from sperm mortality associated with genotypes with irregular X-Y pairing. The results of the genetic

Figure 14 Cross scheme for testis squash and progeny scoring

Flies with [72x16]-Df(1)x-1 were crossed to $B^{s}Yy^{+}$ males. Males carrying Df(1)X-1 chromosome require a duplication of proximal X to compensate the missing function of the proximal euchromatic deletion of Df(1)X-1 The [72X16]-Df(1)X-1/ $B^{s}Yy^{+}$ males were used for testis squashes. For progeny scoring the male flies were crossed to ry female flies. Sperm classes were determined by the phenotypes of the progeny



Phenotype	Sperm class	
B^+y^+ females	X	Disjunction
B^sy^+ male	Y	Disjunction
B^sy^+ females	X Y	Nondisjunction
B^+y male	0	Nondisjunction

Table 1. Cytological and genetic analysis data.

A. Cytological analysis

a = X and Y go to different poles

b = X and Y go to same poles

c = No sex chromosomes

d = X or Y chromosome

e = X and Y chromosomes

$P = (2a + d)/ (2a + 2b + c + d + e)$

P = frequency of disjunction C.I = 95% confidence interval

B Genetic analysis

$P = 1/[1 + ((XY) (0)/ (X) (Y))^{1/2}]$

P = frequency of disjunction C I = 95% confidence interval

A Testes squash data

Flies	Meiosis I		Meiosis II			P± C I
	X-Y (a)	X-Y (b)	0 (c)	X or Y (d)	XY (e)	
Df(1)X-1	25	23	22	108	87	0 5 ± 0 06
T 1	51	22	40	111	43	0 63± 0 06
T 2	21	14	33	104	33	0 61± 0 07
T 3	45	22	24	74	35	0 61± 0 07
T 4	41	22	41	123	38	0 63± 0 06

B Progeny count data

Flies	Sperm class				P± C I
	X	Y	XY	0	
Df(1)X-1	339	99	28	1335	0 51± 0 03
T 1	855	275	49	1812	0 62± 0 02
T 2	537	154	26	1072	0 64± 0 02
T 3	1584	536	137	3234	0 58± 0 01
T 4	769	218	43	1728	0 60± 0 02

analysis are coincident with those of the cytological analysis. All of the insertions showed increased X-Y disjunction frequency· T1 showed 62%, T2 showed 64%, T3 showed 58% and T4 transformant showed 60% (Table 1) These results indicate that promoter region of 240bp repeat can stimulate X-Y pairing and disjunction

Mini-chromosome assay

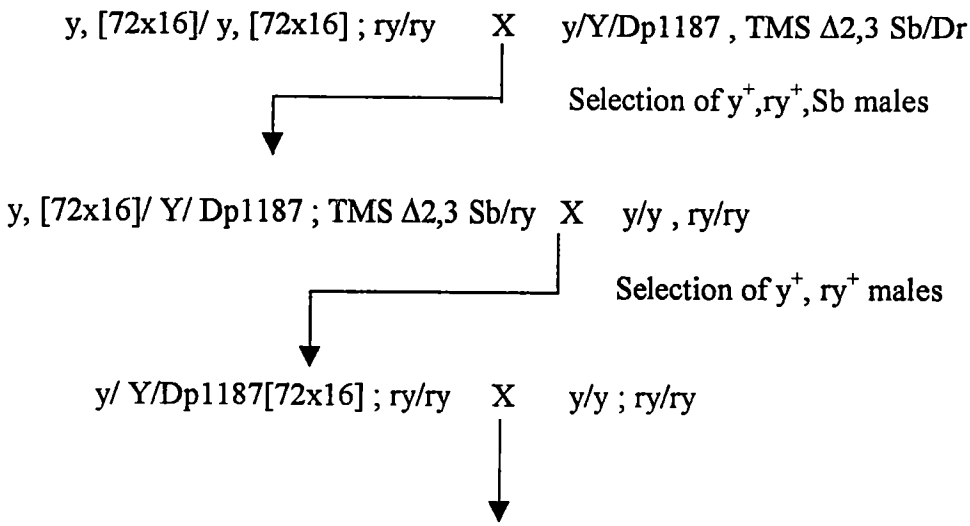
The pC72x16 was used to determine whether the mini-chromosome assay can be used to test pairing site 16 copies of the 72bp fragment, which is X-linked, was remobilized onto Dp(1,f) 1187, a very small X chromosome containing no pairing site, by exposure to a transposase source (Fig 15), and one transgenic line was made Southern blot analysis was carried out with genomic DNA of the transformant to determine whether it has transgene Hybridization with rosy 3.1 probe showed two bands One is from endogenous rosy gene, and the other one is from transgene Hybridization with rosy 4 1probe showed a band around 5.3kb indicating correct copies numbers of 72bp fragment Thus pC72x16 appear to be transferred to Df(1)X-1 successfully. However no band was shown when the genomic DNA was hybridized with P-left probe, suggesting that some P-left sequences were lost during remobilization

Df(1) X-1/B^sY/Dp1187 [72x16] flies were tested to determine whether Dp1187 [72x16] disjoins from B^sY Disjunction frequency was measured genetically by crossing Df(1) X-1/B^sY/Dp1187[72x16] males to y, w females and scoring the recovery of the sperm classes (Y, Dp1187[72X16], B^sY,Dp1187

[72x16], nullo-B^{s}Y; nullo-Dp1187[72x16]) (Fig 16). The result of the analysis showed below 50% disjunction frequency, indicating random separation between B^{s}Y and Dp1187[72x16](Table 2).

Figure 15 Cross scheme for remobilization of pC72x16 to Dp1187

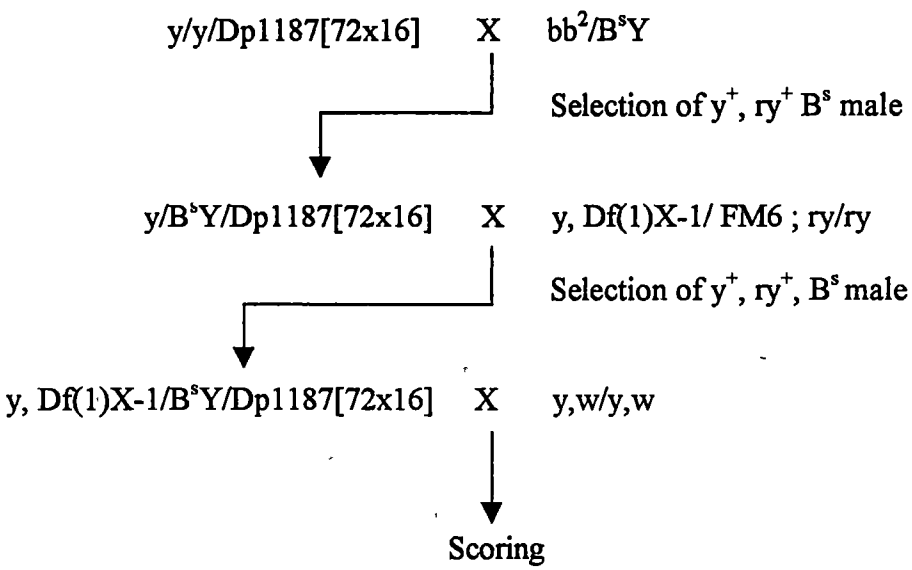
Autosome-linked transformants were mated with flies carrying transposase Progeny with y^+, Sb males, which carry transposase and Dp1187 were selected and crossed to y, ry opposite sex flies. At the next generation, Sb progeny were discarded to get rid of transposase, and y^+, Sb^+, ry^+ males were selected and crossed to y, ry female singly. Flies containing Dp1187[72x16] show that y^+ and ry^+ phenotypes go together



Progeny showing y^+ always goes with ry^+ contain Dp1187[72x16]

Figure 16. Cross scheme for mini-chromosome assay

Dp1187[72x16] female flies were crossed to B^sY male Flies containing B^sY and Dp1187[72x16] were selected and crossed to y, Df(1)X-1/FM6, ry/ry flies. y, Df(1)X-1/B^sY/Dp1187[72x16] males were chosen and crossed to y, w females, and progeny were scored



Phenotype	Sperm class	B^sY and Dp1187[72x16]
y female	X	Nondisjunction
y^+ female	X, Dp[72x16]	Disjunction
yB^s female	X, Y	Disjunction
y^+B female	X, Y, Dp[72x16]	Nondisjunction
y, w male	0	Nondisjunction
y^+, w male	Dp[72x16]	Disjunction
y, w, B^s male	Y	Disjunction
y^+, w, B^s male	Y, Dp[72x16]	Nondisjunction

Table 2. Mini-chromosome assay data.

P = frequency of disjunction

DJ = Disjunction , NDJ = Nondisjunction

P = DJ spermatocytes / NDJ + DJ spermatocytes

Mini-chromosome assay data

Sex	Female				Male				P
Phenotype	(a) y	(b) y^+	(c) yB	(d) y^+B	(e) y,w	(f) y^+,w	(g) y,w,B	(h) y^+,w,B	
Sperm class	X	X, Dp	X,Y	X,Y,Dp	0	Dp	Y	Y, Dp	
DJ or NDJ	NDJ	DJ	DJ	NDJ	NDJ	DJ	DJ	NDJ	
Df(1)X-1/ B^sY/Dp1187	149	113	4	1	736	655	32	25	0 469
Df(1)X-1/ BsY/Dp[rib7]	72	95	5	3	325	423	14	12	0 566
Df(1)X-1/ B^sY/Dp[72x16]	108	83	0	2	592	460	10	20	0 433

$$P = (b + c + f + g) / (a + b + c + d + e + f + g + h)$$

CHAPTER 4

DISCUSSION

In *Drosophila melanogaster* males, 240bp repeats, clustered in tandem arrays within the rDNA intergenic spacer (IGS), are essential for X-Y pairing and disjunction. The 240bp repeats contain nearly the same 52 base pair sequence as a RNA polymerase I promoter and have promoter activity Grimaldi et al , (1990) analyzed the transcriptional activity of several recombinant constructs in which distinct segments were derived from a 240bp repeat The result indicated that 72bp extending from –62 to +10 is the smallest region having promoter activity Interestingly, it was found that the mutations at –16, -13, -1, and +1 which are within 72bp fragment and thought to be important for transcription, in 240bp repeat eliminate the ability of 240bp repeats to stimulate X-Y disjunction This finding implies that X-Y pairing and disjunction are connected to transcription of the 240bp repeats.

From this result, one question has been raised. Can the 72bp fragment itself stimulate X-Y disjunction? In order to solve this question, the plasmid pC72x16, 16 repeats of the 72bp fragment, was constructed and microinjected to obtain transformants. The effect of the X-linked insertion of pC72X16 on X-Y disjunction was determined by recombining the insertion onto a Df(1) X-1, which contains no X-Y pairing site and measuring the X-Y disjunction frequency both cytologically and genetically Although four transformants showed different insertion location, all of them stimulated X-Y disjunction All

transformants showed about 60% X-Y disjunction frequency while the negative control showed about 50% disjunction (random separation)

The present result can be compared to those of previous experiment (McKee and Karpen, 1990; McKee et al , 1992, Merrill et al , 1992). Previous studies showed that 240bp repeats of the intergenic spacer (IGS) is critical for X-Y pairing and disjunction, and the copy number of the 240bp repeat is also important factor. At least six copies of the 240bp repeat are required for increased X-Y disjunction, and pairing capacity is proportional to the copy number of the 240bp repeats. Twelve copies of the 240bp repeats showed 72% disjunction frequency while six copies of the 240bp repeats showed 64% disjunction frequency. However, 16 copies of 72bp fragments showed about 60% disjunction frequency We don't know why 72bp fragment showed reduced stimulation of X-Y disjunction compared to whole 240bp repeats One possible answer to the question is that sequences outside of the 72bp fragment may also be involved in X-Y disjunction Therefore, it is needed to see whether the rest of the 240bp repeat sequence (240bp minus 72bp) has the ability to stimulate X-Y disjunction Another possible answer may be different promoter activity Although the 72bp fragment has promoter function, the activity could be lower than that of whole 240bp repeats, and therefore, lower disjunction frequency

RT-PCR was carried out to detect transcripts which is transcribed from 72bp fragment, but no transcripts were detected. This finding could result from

transcription activity being too low to detect transcripts *in vivo* although it was proven that 6 copies of 72bp fragment contain promoter activity.

How might the promoter region and pairing be related? The answer to this question is currently unknown. However, there are some possible models to explain the relationship between promoter region and pairing The first one is that transcription generates transiently single stranded DNA, permitting access by a pairing enzyme In this model, both rDNA on X and Y chromosome must be activated by transcription because mutated rDNA on X chromosome does not stimulate X-Y disjunction. A second possibility is that nascent transcripts could base pair with complementary region of a homologous template strand, thus could form a RNA-DNA heteroduplex RNA bridge. The RNA bridge model is attractive for two reasons. One is that this model does not require DNA breaks for linking homologous chromosome. The other is that a linkage of this type could be dissolved by removing the RNA without DNA crossovers. Finally, there is also the possibility of an indirect connection between pairing and transcription The four single base pair mutations in 240bp repeat could have altered the promoter recognition sequence preventing the transcription factors from binding to the promoter region. Thus, protein-protein interactions between transcription factor, or other proteins which are recruited by transcription factors, could mediate X-Y disjunction, and the lack of transcription factors may induce abnormal X-Y pairing and nondisjunction.

Molecular mapping of the pairing site was determined using transgenic fly experiment, however, this technique has a limitation If pairing sites are

dispersed throughout a chromosome, transgene assay can not be used. In order to overcome this limitation, a mini-chromosome assay was designed.

A mini-chromosome assay was carried out using a free X duplication Dp(1;f)1187 (Dp1187) that consists of 1300kb of X-derived sequence excluding rDNA region. This Dp1187 is transmitted well through both mitotic and meiotic division in both sexes (Karpen and Spradling, 1992; Karpen et al., 1996) In addition, the Dp1187 segregates randomly from sex chromosomes in males, and has hot spots near the telomere. Thus, insertion of any pairing candidate sequence whether it is from sex chromosome or even autosomes onto the Dp1187 may show disjunction frequency.

The results of the mini-chromosome assay did not coincide with previous results. While 16 copies of 72bp fragment on Df(1) X-1 stimulate X-Y disjunction (around 60%), the same transgene on Dp1187 did not induce X-Y disjunction. The [rib 7] transgene also showed reduced X-Y disjunction frequency in Dp1187 from 64% to 56%. The reason why Dp1187[72x16] shows less disjunction frequency than Df(1) X-1[72x16] is not known .

REFERENCES

Appels, R. and Hilliker, A. J (1982). The cytogenetic boundaries of the rDNA region within heterochromatin of the X chromosome of *Drosophila melanogaster* and their relation to male meiotic pairing sites. Genet Res Camb 39, 149-156

Ashburner, M. (1989) *Drosophila* · A Laboratory Manual. Cold Spring Harbor Press, New York

Ault, J. G. and Rieder, C. L. (1994) Meiosis in Drosophila males. I. The question of separate conjunctive mechanisms for the XY and autosomal bivalents. Chromosoma 103, 352-356

Baker, B. S , Carpenter, A T. C., Esposito, M S and Sandler, L (1976) The genetic control of meiosis Annu. Rev. Genet 10, 53-134

Bender, W , Spierer, P. and Hogness, D S (1983). Chromosome walking and jumping to isolate DNA from Ace and rosy loci and the bithorax complex in *Drosophila melanogaster*. J. Mol. Biol. 168, 17-33

Carpenter, A T. C. (1975a) Electron microscopy of meiosis in *Drosophila melanogaster* females I. Structure, arrangement, and temporal change of the synapotnemal complex in wild-type Chromosoma 51, 157-182

Carpenter, A. T. C. (1975b). Electron microscopy of meiosis in *Drosophila melanogaster* females. II The recombination nodules – a recombination-associated structure at pachytene? Proc Natl. Acad. Sci. USA 72, 3186-3189

Carpenter, A T C (1988) Thoughts on recombination nodules, meiotic recombination, and chiasmata In Genetic Recombination. American Society of Microbiology. Washington, D.C pp 529-548

Coen, E S , and Dover, G. A (1982). Multiple polI initiation sequences in rDNA spacers of *Drosophila melanogaster* Nucleic Acids Res. 10, 7017-7026.

Cooper, K. W (1950) Normal spermatogenesis in *Drosophila*. In "Biology of Drosophila" (M. Demerec, Ed.), pp 1-61. Wiley, New York.

Cooper, K.W. (1964) Meiotic conjunctive elements not involving chiasmata Proc Natl. Acad. Sci. USA 52, 1248-1255.

Engels, W. R., Preston, C. R., Thompson, P., and Eggleston, W B (1991) In situ hybridization to Drosophila salivary chromosomes with biotinylated DNA probes and alkaline phosphatase. Life Technologies (BRL).

Gershenoson, S (1933) Studies on the genetically insert region of the X-chromosome of Drosophila. I Behavior of an X chromosome deficient for a part of the insert region. J Genet. 28, 297-312

Grimaldi, G. and Di Nocera, P P (1988) Multiple repeated units in *Drosophila melanogaster* ribosomal DNA spacer stimulate rRNA precursor transcription. Proc Natl. Acad Sci. USA 85, 5502-5506

Grimaldi, G., Fiorentino, P. and Di Nocera , P P. (1990) Spacer promoters are orientation-dependent activators of pre-RNA transcription in *Drosophila melanogaster* Mol. Cell Biol 10, 4667-4677.

Hawley, R. S (1988) Exchange and chromosomal segregation in eucaryotes In Genetic Recombination American Society of Microbiology Washington, D C. pp 497-527

Jones, G H (1987) Chiasmata in meiosis. (Moens, P B. Ed) Orlando. Academic Press 213-244

Karpen, G. H , and Spradling, A C (1992). Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single p element insertion mutagenesis Genetics 132, 737-753

Karpen, G.H , Le, M-H , and Le, H (1996) Centric heterochromatin and the efficiency of achiasmatic disjunction in *Drosophila* female meiosis Science 273, 118-122

Kempthone, O (1969) An Introduction to Genetic Statistics (Ames, Iowa The State University Press).

Kohorn, B. D. and Rae, P. M M. (9182) Non-transcribed spacer sequences promote in vitro transcription of *Drosophila* ribosomal RNA Nucleic Acids Res 10, 6879-6886

Lifschytz, E , and Harevan, A. (1977) Gene expression and control of spermatic morphogenesis in *Drosophila melanogaster*. Genetics 43, 547-563

Lindsley, D. L., and Sandler, L. (1958). The meiotic behavior of grossly deleted X chromosomes in *Drosophila melanogaster*. Genetics 43, 547-563

Lohe, A. R. and Roberts, P. A. (1990) An unusual Y chromosome of *Drosophila simulans* carrying amplified rDNA spacer without rRNA genes Genetics 125, 399-406

Long, E. O. and David, I. B. (1980). A possible role for the symaptonemal complex in chiasma maintenance. Exp. Cell Res 112, 297-308

Maines, J , and Wasserman, S. (1998). Regulation and execution of meiosis in *Drosophila* males. In Meiosis and gametogenesis, M. A Handel, ed (San Diego Academic Press), pp 301-326

McKee, B D. (1984). Sex chromosome meiotic drive in *Drosophila melanogaster* males. Genetics 106, 403-422

McKee, B D. (1998) Pairing sites and the role of chromosome pairing in Meiosis and spermatogenesis in male *Drosophila* In Meiosis and Gametogenesis M. A. Handel, ed (San Diego Academic Press), pp.77-115

McKee, B D , Habera, L and Vrana, J A (1992). Evidence that intergenic spacer repeats of *Drosophila melanogaster* rRNA genes function as X-Y pairing sites in male meiosis, and a general model for achiasmatic pairing. Genetics 132, 544-549

McKee, B D and Karpen, G. H. (1990) *Drosophila* ribosomal RNA genes function as an X-Y pairing site during male meiosis Cell 61, 61-72

McKee, B. D. and Lindsley, D L (1987). Inseparability of X heterochromatic functions responsible for X:Y pairing, meiotic drive, and male fertility in *Drosophila melanogaster* males. Genetics 116, 399-407

Merrill, C. J , Chakravarti, D., Habera, L., Das, S., Eisenhour, L. and McKee B. D (1992). Promoter-containing ribosomal DNA fragments function as X-Y meiotic pairing sites in *D. Melanogaster* males. Dev. Gen 13, 468-484

Meyer, G. F. (1960) The fine structure of spermatocyte nuclei of *Drosophila melanogaster*. In proceedings of the European Regional Conference on Electron Microscopy Delft: Die Nederlandse Verening voor Electron microscopic Delft. pp. 951-954

Miller, J. R., Haywark, D. C. and Glover, D. M. (1983). Transcription of the " non-transcribed" spacer of *Drosophila melanogaster* rDNA Nucleic Acids Res 11, 11-19

Murtif, V. L and Rae P. M. M. (1985) *In vivo* transcription of rDNA spacers in *Drosophila*. Nucleic Acids Res 13, 3221-3239

Nicolas, A., Treco, D , Schultes, N. P., and Szostak, J. W. (1989) An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae* Nature 338, 35-39

Park, H -S , and Yamamoto, M.-T. (1995) The centric region of the X chromosome rDNA function in male meiotic pairing in *Drosophila melanogaster* Chromosoma 103, 700-707

Peacock, W. J (1965) Nonrandom segregation of chromosomes in Drosophila males Genetics 51, 573-583

Peacock, W. J , Miklos, G L G., and Goodchild, D. J (1975). Sex chromosome meiotic derive systems in *Drosophila melanogaster*. I. Abnormal spermatid

development in males with a heterochromatin deficient X chromosome (sc4sc8). Genetics 79, 613-634

Rasmusen, S. W. and Holm, P B. (1978) Human meiosis II Chromosome pairing and recombination nodules in human spermatocytes. Carlsberg Res Commun. 43, 423-43.

Ren, X -J., Eisenhour, L , Hong, C -S , Lee, Y , and McKee, B. D (1997) Roles of rDNA spacer and transcription unit sequences in X-Y meiotic pairing in *Drosophila melanogaster* Chromosoma 106, 29-36

Roeder, G S. (1990) Chromosome synapsis and genetic recombination Their roles in meiotic chromosome segregation. Trends Genet. 6, 385-389

Ritossa, F (1976) The bobbed locus In the Genetics and Biology of Drosophila Vol 1B (Ashbumer, M., and Novitski, E. Ed) London: Academic Press pp 801-864.

Rubin, G M. and Spradling, A. C. (1983). Vectors for P-element mediated gene transfer in Drosophila. Nucl. Acids Res 11, 6341-6351

Sambrook, J , Fritsich, D F. and Maniatis, T (1989). Molecular Cloning. A Laboratory Manual Second edition Cold Spring Harbor Press, New York

Sandler, L. and Braver, G. (1954). The meiotic loss of unpaired chromosomes in *Drosophila melanogaster* Genetics 39, 51-63.

Shenkar, R , Shen, M. and Arnheim, N. (1991) DNase I hypersensitive sites and transcription factor-binding motifs within the mouse Eβ meiotic recombinational hotspot. Mol. Cell Biol. 11, 1831-1819.

Simeone, A., La Volpe, A., and Boncinelli, E (1985) Nucleotide sequence of a complete ribosomal spacer of *D melanogaster*. Nucleic Acids Res. 13, 1089-1101

Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol. 98, 503-517

Sun, H., Treco, D , Schultes, N. P. and Szostak, J W (1989) Double-strand breaks at an initiation site for meiotic gene conversion. Nature 338, 87-90.

Voelker-Meiman, K., Keil, R. L and Roeder, G S. (1987) Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequence regulation transcription by RNA polymerase I Cell 48, 1071-1079

von Wettstein, D., Schultes, N. P. and Holm, P. B. (1984) The synaptonemal complex in genetic segregation. Annu. Rev. Genet. 18, 331-414

White, M J D (1973). Animal cytology and evolution 3rd ed Cambridge Cambridge University Press

Yamamoto, M and Miklos, G. L G (1977) Genetic dissection of heterochromatin in *Drosophila*· The role of basal X heterochromatin in meiotic sex chromosome behavior. Chromosoma 60, 283-296.

VITA

Yunsang Lee was born in Seoul, Korea in May 1967 In 1986 he entered Inha University, Incheon, Korea and was awarded the Bachelor of Science Degree in Biology with honor in 1990 In March of that year, he entered Seoul National University, Seoul, Korea and received Master's Degree in Molecular Biology in 1992. After graduation, he joined military service for about 2 years In 1995 he began graduate studies at the University of Tennessee, Knoxville and received the Degree of Master of Science in Biochemistry and Cellular and Molecular Biology in August 2000.