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Test for Rex and Su(Rex) in
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Leonard D. Kaplan

Major professor

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TEST FOR *REX* AND *Su(REX)* IN NATURAL POPULATIONS OF *D. MELANOGASTER*

BY

Mekki Boussaha

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ABSTRACT

TEST FOR REX AND Su(REX)

IN NATURAL POPULATIONS OF D. MELANOGASTER.

By

Mekki Boussaha

Rex (Ribosomal exchange) is a genetic element of Drosophila melanogaster that induces mitotic recombination in the ribosomal RNA genes (rDNA, nucleolus organizer, bb locus). In the course of this study some features of Rex were characterized.

(1) Crosses and progeny counts were used to test some sixteen wild-caught X chromosomes from a world-wide sample of populations for Rex activity, as well as for suppressors of Rex (Su(Rex)). The results show that Rex was not detectable in natural populations whereas Su(Rex) was present in all samples. These observations leave open the question of whether Rex is present in wildtype Drosophila since the presence of Su(Rex) precludes knowing whether Rex was present.

(2) Rex-induced exchange can be used to construct rDNA maps quickly, but we need to measure rDNA copy number reliably for fairly large numbers of samples. Tecniques for rapidly determining rDNA copy number were, therefore, examined. A single-insect squash-blot technique while promising, shows too much variation. Quantitative dot-blots, however, are reliable.

To my parents

for their support and love

AND

to Leonard G. Robbins

for his support psychologically, academically and financially

ACKNOWLEDGMENTS

I am grateful for the superb technical assistance, precious advice and, encouragment of **Dr. Leonard G. Robbins.** I also thank **Dr. Thomas Friedman** and **Dr. Larry Snyder** for their critical reading of the manuscript. I am grateful to **Dr. Scott Williams.** His promptness in sending requested stocks greatly facilitated the research presented here. Special thanks are also due Susan Lootens, Robin Steinman, Rob Morell, Lorri Wallrath, Peter Crawley and Mark Thompson for their help and for being wonderful officemates. I also thank Ellen Swanson for her patient assistance.

Finally, I have dedicated this work to my family, and here add my special thanks to all my friends back home.

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INTRODUCTION

Genetic and physical maps of chromosomes have provided a framework for further studies in molecular biology. The heterochromatin, however, has been refractory to conventional mapping techniques. Even though, for example, 40% of the length of the Drosophila X chromosome and all of the Y chromosome are heterochromatic (Hilliker et al., 1980), the large-scale organization of heterochromatin remains mysterious for a variety of reasons: (1) there are very few phenotypic markers; (2) the recombination frequency is extremely low (approximately 0.02% for the X heterochromatin), and (3) repeated gene families within the heterochromatin are too large to be cloned using conventional vectors.

Rex-induced recombination in the ribosamal RNA genes (rDNA) of Drosophila melanogaster is at least two-orders of magnitude more frequent than spontaneous events. Moreover, the rDNA is located in the X heterochromatin and occupies approximately 40% of its physical length (Fig. 1) (Hilliker et al., 1980). The rDNA is, therefore, a model gene for establishing a methodology for mapping repeated genes in the heterochromatin (Williams et al., 1990).

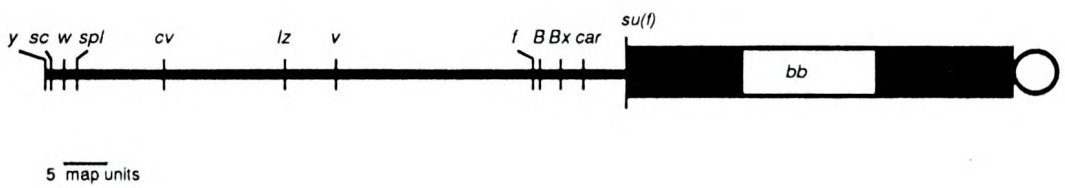


Figure 1 - Schematic representation of the X chromosome
The X chromosome is 66 map units long, and the map positions of many of the phenotypic markers are shown. The basal heterochromatin (black boxes) is approximately 40% of the physical length (Hilliker et al., 1980; Schalet & lefevre, 1976; Williams et al., 1989). The NO occupies about one-third of the basal heterochromatin and is shown as an open box.

AREAS OF INVESTIGATION

There are several questions about Rex, and, more importantly, about the rDNA that Rex can be used to answer. Two main questions have been addressed. The first forms the body of this thesis, the second is discussed in the appendix:

(1)- Is Rex present in natural populations? Rex-induced rDNA recombination is at least two orders of magnitude more frequent than spontaneous events. If Rex is present in natural populations, Rex-induced exchange would be a major factor in the concerted evolution of this gene family.

(2)- Rex-induced exchange events can be used to map the structure of the rDNA array. Improving the resolution of our rDNA maps, as well as being able to construct these maps quickly using Rex-induced exchange, depends on being able to determine rDNA copy number for fairly large numbers of samples. An attempt was made to devise a reliable and rapid technique for measuring rDNA copy number.

CHAPTER 1

LITERATURE REVIEW

I) - The rDNA of Drosophila melanogaster

In wild-type D. melanogaster there are two clusters of repeated rRNA genes (rDNA), each with approximately 250 copies (Long and Dawid, 1980; Tartof, 1973a). One of these is located in the proximal heterochromatin of the X chromosome and the other is located on the short arm of the Y chromosome. These two rDNA arrays correspond genetically to the bobbed (bb) loci. A single repeat unit contains coding regions for the 2S, 5.8S, 18S, and 28S rRNA subunits (Fig. 2) (Tautz et al., 1988). The series of alleles known as bobbed represent the range of subnormal numbers of these genes (Ritossa, Atwood and Spiegelman, 1966). Bobbed flies show delayed development and have a phenotype that includes small thoracic bristles, abdominal etching and, in severe cases, malformed genitalia (Lindsley and Zimm, 1985). The most extreme alleles are lethal.

Some of the rDNA repeats are interrupted in the 28S coding region by one of two types of nonhomologous insertion sequences. In two common laboratory wild-type stocks (Oregon-R and Canton-S), type 1 (T1) insertions are restricted to the X chromosome where they interrupt about 50% of the rDNA repeats. The DNA sequences at rDNA/T1 junctions and the location of

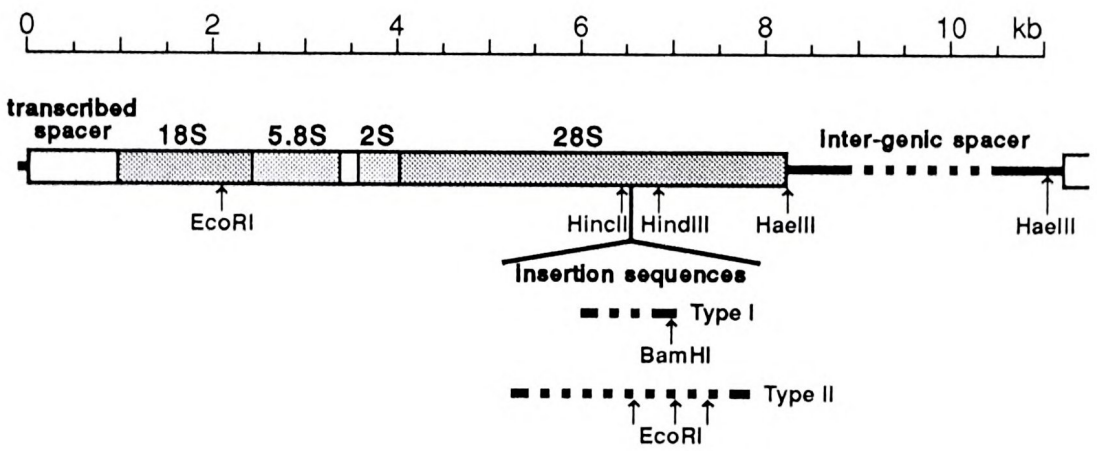


Figure 2 - Map of a single rDNA repeat unit.

The NO contains approximately 250 rDNA repeats or cistrons (Tartof,1973a). A single cistron is shown schematically, with the transcribed sequences in boxes. There are coding regions for four species of rDNA, and two transcribed spacers shown as open boxes, and a non-transcribed inter-genic spacer (IGS) (Tautz et al., 1988). Some cistrons in each NO are interrupted in the 28S coding region by insertion sequences which are classified as either type I or type II depending on sequence. Type I insertions appear to be unique to the X chromosome, while type II insertions are found both in X and Y NO's (Wellauer et al., 1978). Each insert class has a specific integration site in the 28S coding region, and the two sites are separated by fewer than 100 bp (Roiha et al., 1981). The dashed lines within the insertion sequences and within the IGS indicate the length of these regions.

sequences homologous to T1 insertions away from the nucleolus organizer suggest that these elements may be transposable (Kidd and Glover, 1981; Peacock et al., 1981; Roiha et al., 1981). Type 2 (T2) insertions interrupt about 15% of the rDNA repeats on both the X and Y chromosomes. These insertions are not homologous to T1 insertions and have a slightly different insertion point in the 28S rRNA coding region (Figure 2) (Roiha and Glover, 1981; Long and Dawid, 1980). Repeats containing T1 and T2 insertions do not appear to contribute significantly to the production of mature rRNA (Long and Dawid, 1979; Long et al., 1981)

Another source of heterogeneity within rDNA arrays is the non-transcribed or intergenic spacer (IGS) which separates the repeat units and is highly variable in length, ranging from 4kb to 20kb (Coen et al., 1982; Indik and Tartof, 1980; Terracol, 1986). The 5' portion of the IGS contains a series of 340bp sequences bounded by DdeI sites (Williams et al., 1987); whereas the central portion of the IGS contains a variable number of 240bp sequences bounded by Alu1 sites (Coen et al., 1982; Simeone et al., 1985)

II) - Concerted evolution

Evolution of repetitive gene families presents a serious problem. Independent mutations would be expected to yield divergence of members of a repeated array. However, homogeneity is most common. For Drosophila rDNA, although

there are diagnostic differences between the X and Y chromosomes, they too are quite similar. Moreover, the transcribed segments, within a chromosome, are nearly identical. Through genetic interactions among its members, the gene family may evolve together in concerted fashion, ie. as a unit (Arnheim, 1983). Mechanisms of molecular exchange, including unequal crossing over and gene conversion, are thought to account for concerted evolution (Dover, 1982; Arnheim, 1983; Smith, 1973; Arnheim et al., 1980; Tartof, 1988) because they are capable of rapidily homogenizing selectively neutral mutations. In the case of the Drosophila rDNA, however, analysis of spontaneous rDNA exchange indicates that reciprocal recombination and gene conversion are not sufficient to explain the observed patterns of homogeneity and difference found in natural populations (Williams et al., 1989).

Because Rex-induced rDNA recombination is at least two orders of magnitude more frequent than spontaneous events, if Rex is present in natural populations, Rex-induced exchange would be a major factor in the concerted evolution of this gene family.

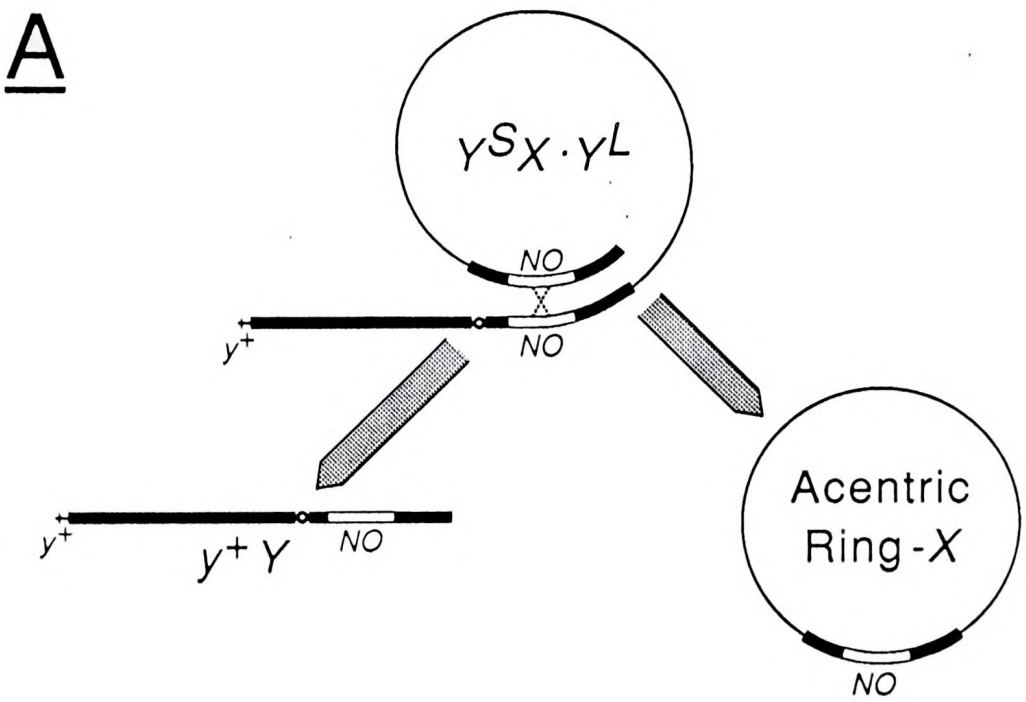
III) - Rex-INDUCED EVENTS

Rex is a genetic element of D. melanogaster that induces mitotic exchange in the ribosomal RNA (Robbins, 1981). Rex was not discovered as a new mutant. Rather, it was detected in the

Figure 3 - Detection of Rex activity in natural populations

A: The attached-XY target chromosome is schematically shown undergoing a Rex-induced exchange event. This exchange leads to loss of X euchromatin and detachment of a complete Y chromosome from the attached-XY chromosome. Heterochromatic regions are indicated by thick lines and the bb loci are indicated by open boxes.

B: The punnet square for a typical Rex mating is shown. The Rex-induced exchanges take place in XXY female zygotes, yielding XY sons or gynandromorphs. These are readily distinguished from regular sons because of the y^+ marker.



B $\dfrac{+\ Rex?}{y\ w} \times \dfrac{Y^SX \cdot Y^L,\ y\ v\ f\ B\ \cdot y^+}{0}$

	♀ \ ♂	0	$Y^SX \cdot Y^L$, y v f B·y^+
NCO	y w	y w ♂	B/+ ♀
	+	+ ♂	B/+ ♀
SCO	y^+w	y^+w ♂	B/+ ♀

Rex - induced mitotic events

$\dfrac{y\ w}{y^+Y}$ ♂

y^+♂ B/+♀ ♂ ♀

X chromosomes of a $Df(1)w^{rJ1}$ stock. This chromosome was first isolated as an unequal crossover in the w region. Aside from the exchange that generated the deficiency, the provenance of this chromosome is unrecorded. We, therefore, do not know the origin or molecular basis of Rex, but we can determine its properties and from that make inferences about both its origin and nature.

Rex is a maternal effect dominant locus (hence, probably encodes a product). It was detected because Rex mothers induced mitotic exchanges in their offspring between two ribosomal DNA arrays in an attached-XY chromosome. The result is the production of free Y chromosomes (Fig. 3) (Robbins, 1981). The exchange event is mitotic and takes place in the early zygote, either before S of the first cell cycle changing XXY daughters into XY sons, or after S of the first cell division or at the two cell stage producing gynandromorphs (Fig. 3) (Robbins, 1981).

The attached-XY chromosome in which the Rex-induced detachment was first detected is $Y^{s}X{\cdot}Y^{L}$ (Lindsley and Novitski, 1959). Fig. 4 shows the structure and origin of this chromosome. The short arm of the Y (Y^{S}) is attached to the distal X euchromatin, and the long arm (Y^{L}), marked with a small translocation of the X euchromatin carrying y^{+}, is attached to the centromere.

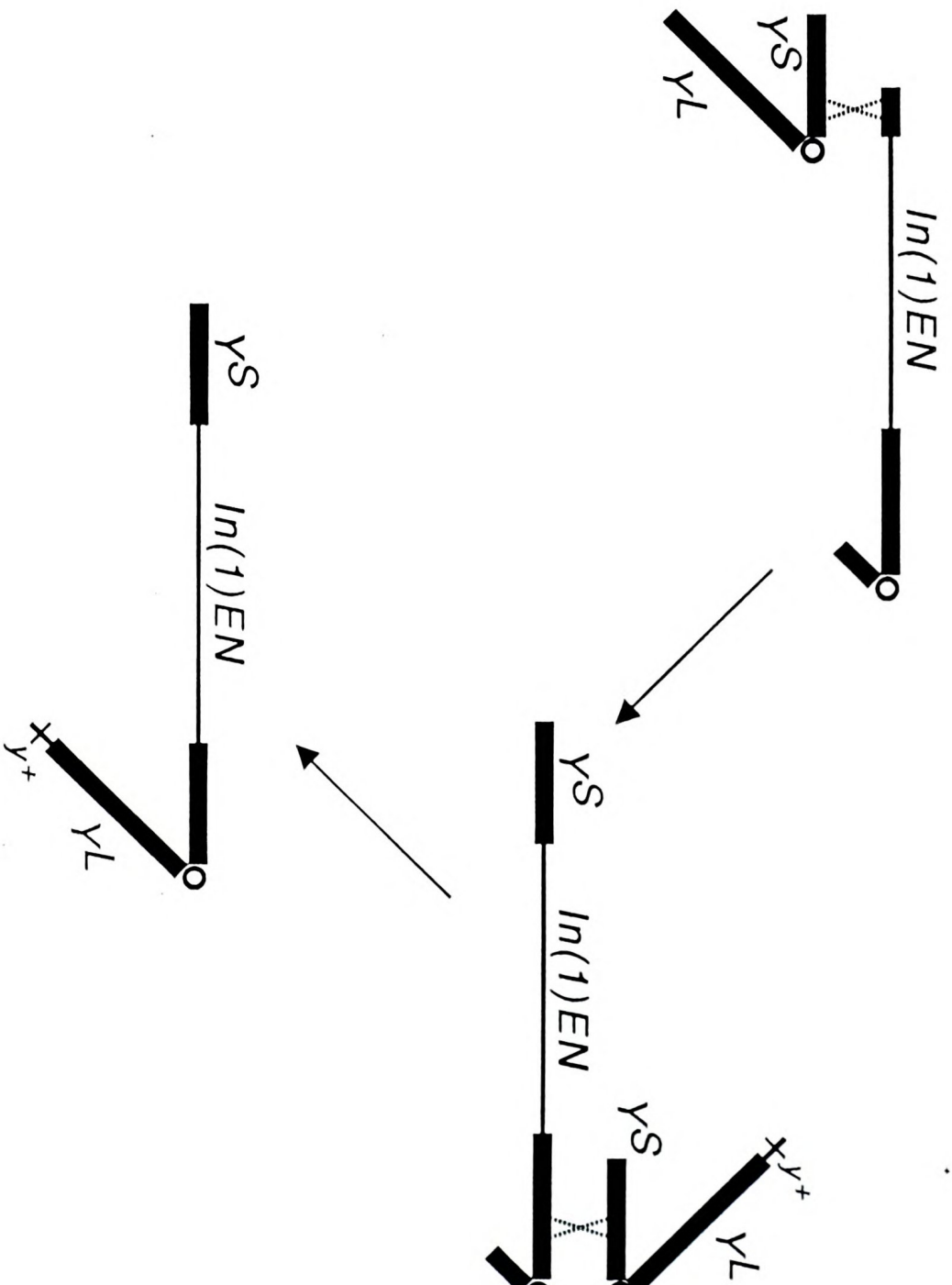


Figure 4 - Synthesis of $\underline{y^{s}xy^{L}}$.

1) - The responding site of Rex - Swanson (1984) demonstrated that Rex causes exchange between any two separated blocks of rDNA on a single chromosome. She tested various chromosomes with two rDNA blocks for ability to undergo detachment events. Even half a block of rDNA at each end of the chromosome can be a target. She has also shown that simple duplications of heterochromatin do not constitute a Rex target; In(1)w^{m4} which has type 1 insertion sequences at both ends of the chromosome (Hilliker and Appels, 1982) is not a target. These observations leave open the question of whether an exactly duplicated non-rDNA block of heterochromatin at both ends of a chromosome can act as a Rex target.

2) - The nature of Rex - Dosage studies indicate that, using the classification scheme proposed by Muller (1932), Rex is a neomorph or extreme hypermorph (Rasooly and Robbins, 1991). It therefore produces a novel function, or a normal product that is abnormally expressed.

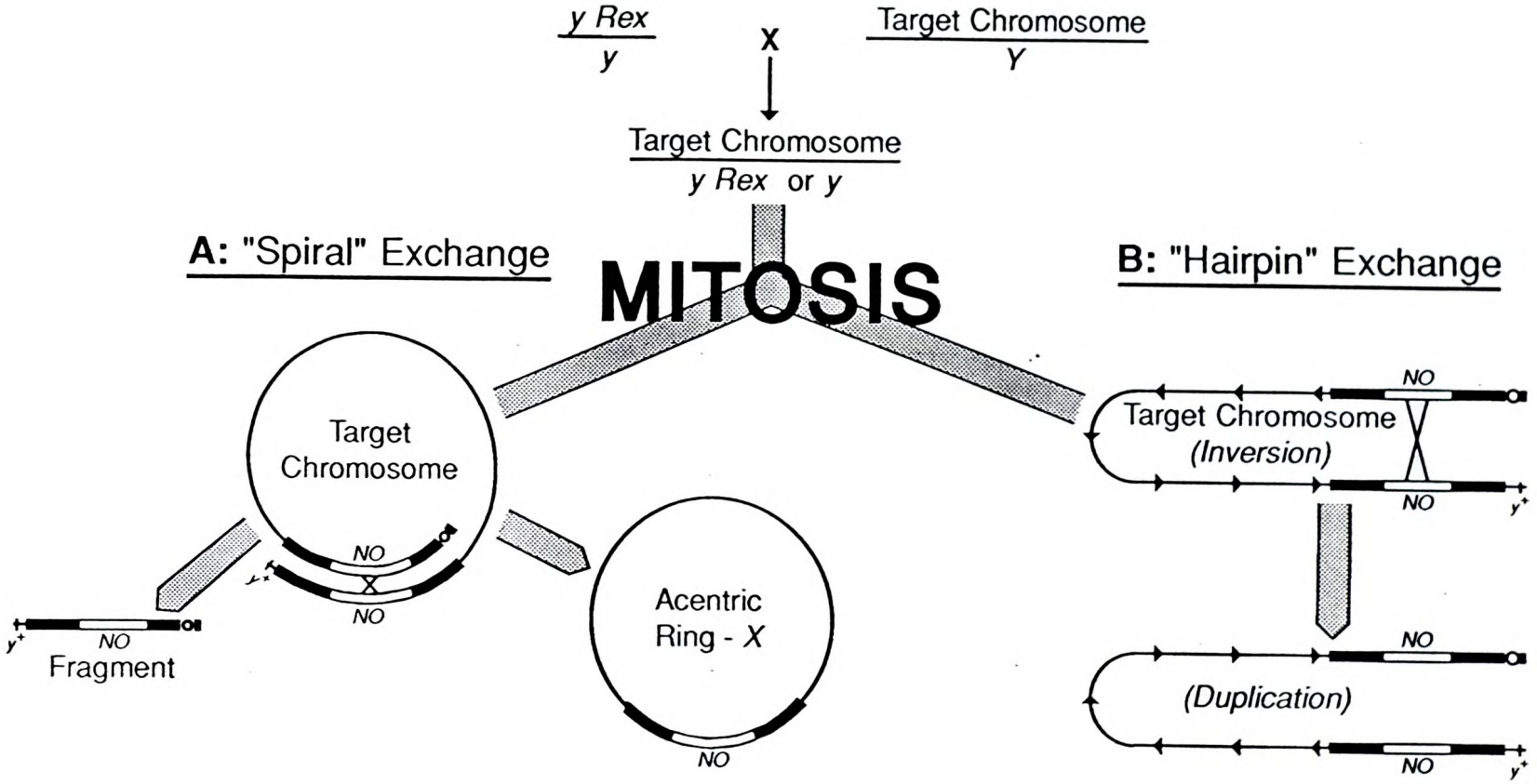
3) - Mapping of Rex and Su(Rex) - Rex maps to the heterochromatin of the X chromosome and its target is specifically the rDNA. Rex and a suppressor of Rex (Su(Rex)) not only map to the nucleolus organizer, but also map as repeated elements at discrete locations in the rDNA array (Rasooly, 1989; Rasooly and Robbins, 1991).

4) - Rex induces spiral and hairpin exchanges - Rex generates two kinds of exchanges, depending on whether the target chromosome pairs in a "spiral" or a "hairpin" configuration (Robbins and Swanson, 1988). When the target chromosome pairs as a spiral, the product is deleted for all of the material originally in between the two nucleolus organizers. If the deletion removes most of the X chromosome euchromatin, these products can, with the appropriate markers, be readily detected as phenotypically distinctive males or gynandromorphs. When the nucleolus organizers pair in the opposite orientation, the hairpin configuration, the exchange inverts the material between the nucleolus organizers rather than deleting it. A typical mating, the Rex-induced events, and their products are shown in Fig. 5.

5) - Deletions generated by Rex - Rex generates deletions at the exchange sites in the rDNA that can be identified both molecularly and genetically. These observations suggest that Rex may be an active version of one of the insertion sequences found in the rDNA. It could encode a site-specific endonuclease activity causing recombinogenic breaks in the target DNA. Resolution of these breaks would then lead to the exchanges we see, as well as to deletions at the exchange site.

Figure 5 - Two types of Rex-induced exchange

The target shown is an X chromosome duplicated for the NO and surrounding heterochromatin. Maternal Rex activity induces both types of exchange in the target chromosome in a single cross (Robbins & Swanson, 1988). The spiral exchange deletes the intervening X euchromatin, changing X/X daughters into sterile X/fragment males patroclinous for only y^+ or gynandromorphs with y^+ male tissue. The hairpin exchange simply inverts the material between the two NO's.


y Rex / y
X
Target Chromosome / Y
Target Chromosome
y Rex or y
A: "Spiral" Exchange
MITOSIS
B: "Hairpin" Exchange
Target Chromosome
NO
NO
Fragment
Acentric Ring - X
NO
Target Chromosome (Inversion)
NO
NO
(Duplication)
NO
NO

CHAPTER 2

MATERIALS AND METHODS

I) - FLY STOCKS AND REARING METHODS

All flies for stocks and crosses were reared on a standard Drosophila medium of cornmeal, molasses and brewer's yeast at 25^{0}C. Mass matings were done in polyethelene bottles, 15 males with 15 females. Matings for counting offspring and testing for fertility were done in glass shell vials, one female with three males and one male with three females, respectively. Unless otherwise noted, all phenotypic markers and standard chromosomes are described in Lindsley and Grell (1968) and Lindsley and Zimm (1985, 1987).

1) Wildtype stocks - Seventeen Drosophila stocks carrying wildtype X chromosomes were obtained from Scott Williams on July 9, 1990 (Table 1). These stocks come from a total of nine isofemale lines. The original isofemale lines were collected from Argentina (Arg4; Arg6), Australia (Aust.Bl-10; Aust.Bl-17); Central African Republic (Caf-15); Taiwan (Taiwan-20) and Vietnam (Viet. 13-I, Viet. 15-I). From each of these original lines, males were crossed individually to virgin females with compound X chromosomes and sons and daughters were mated (Williams, S., personal communication). Thus, all males in the stocks we received are isogenic for their X and Y chromosomes.

	ISOFEMALE LINES	OBSERVATIONS
ARGETINA	Arg-4 I Arg-4 II Arg-4 IV Arg-6 I Arg-6 II	Stock died
AUSTRALIA	Aust BL-10 I Aust BL-10 II Aust BL-17 IV Aust BL-17 V	
CENTRAL AFRICAN REPUBLIC	CAL-15 I CAL-15 II	
TAIWAN	Taiwan-20 I Taiwan-20 II	
VIETNAM	Viet 13-1 I Viet 13-1 II Viet 15-1 I Viet 15-1 II	

Table 1 - Origin of wildtype Drosophila.

The Roman numeral for each stock (e.g., Taiwan 20-II) signifies that it was from the second male. All the isofemale lines have more than one representative.

II) - CROSSING SCHEMES

1) Tests for Rex activity in natural populations - The mating scheme used to test for Rex-activity in natural populations is shown in Fig. 6. Homozygous y w spl virgin females are crossed to wiltype males and the F1 +/y w spl female offspring are crossed to males carrying the target chromosome, $Y^{S}X{\cdot}Y^{L}$, In(1)EN, y v f B· y^{+}/0. Regular males are X/0 and, therefore, sterile. Non-disjunctional males are v f B and are distinctive. y^{+} w males are generated by Rex-induced mitotic events. A crossover between y and w, however, also produces y^{+} w males. These are X/0 and sterile while the y^{+} w products of Rex activity would be X/y^{+}Y and fertile. All y^{+} w males recovered are, therefore, tested for fertility. Recovery of fertile males would reveal the presence of Rex in the corresponding wildtype stock.

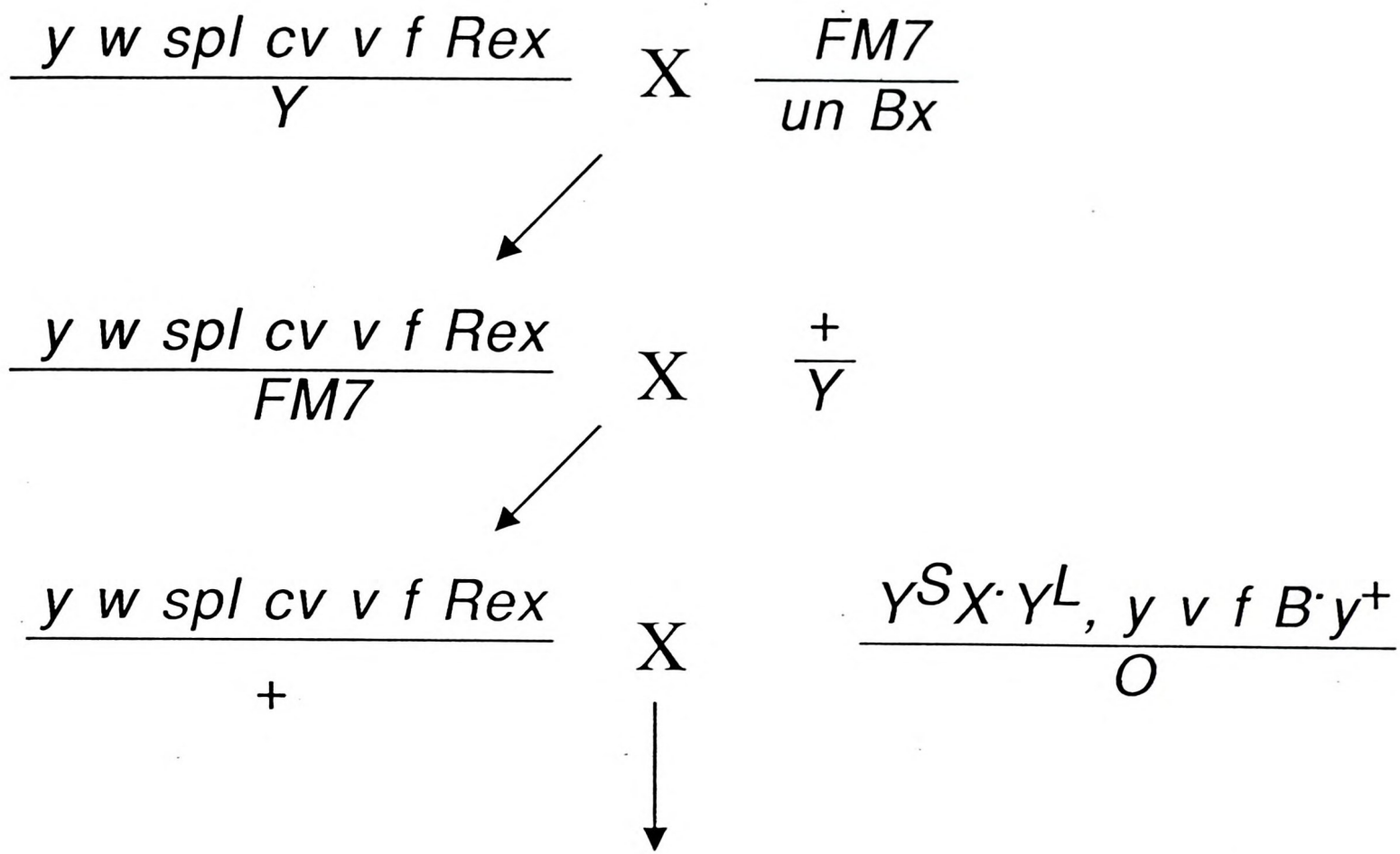
2) Test for Su(Rex) in natural populations - Fig. 7 shows the mating scheme used to test for Su(Rex) in sixteen of the wild-caught X chromosomes. Rex is kept in stocks as Rex/Y males x C(1)DX/Y females. FM7/un Bx females were crossed to y w spl cv v f Rex/Y males. The F1 y w spl cv v f Rex/FM7 virgin females were then crossed to wildtype males and the wildtype female

Figure 6 - Test for Rex activity in natural populations. Homozygous y w spl virgin females were crossed to wildtype males and the F1 +/y w spl female offspring were crossed to $Y^{S}X{\cdot}Y^{L}$, IN(1)EN, y v f B y^{+}/0 males.

y w spl
y w spl
X
y w spl
+
X
y
+
O
yS X.Y L, y v f B.y+
X
Score for production of y+ w males

offspring were then crossed to $\underline{Y^{S}X \cdot Y^{L}}$, $\underline{In(1)EN}$, $\underline{y}$ $\underline{v}$ $\underline{f}$ $\underline{B}.\underline{y^{+}}/\underline{0}$ males. $\underline{y^{+}}$ $\underline{w}$ males, that are either $\underline{y^{+}}/\underline{0}$ crossovers between $\underline{y}$ and $\underline{w}$, or $\underline{Rex}$-induced $\underline{X}/\underline{y^{+}Y}$, were scored and then tested for fertility. The $\underline{y^{+}}$ $\underline{w}$ males produced by Rex-induced mitotic events would be fertile. If $\underline{Su(Rex)}$ was present in a particular stock, no fertile $\underline{y^{+}}$ males would be detected.

Figure 7 - test for Su(Rex) in natural populations. y w spl cv v f Rex/Y males were crossed to FM7/un Bx females. The F1 y w spl cv v f Rex/FM7 virgin females were crossed to wildtype males and the wildtype female offspring were then crossed to $Y^{s}X \cdot Y^{L}$, IN(1)EN, y v f B y^{+}/0 males.


y w spl cv v f Rex
Y
X
FM7
un Bx
y w spl cv v f Rex
FM7
X
+
Y
y w spl cv v f Rex
+
X
Y^S X· Y^L, y v f B· y+
O
Score for production of y+ w males

CHAPTER 3

RESULTS AND DISCUSSION

I - RESULTS

1) Tests for Rex activity in natural populations - Table 2 shows the total progeny counted for each isofemale line of Drosophila melanogaster. Of a total of 358 y^+ w male progeny (Table 4), however, all were sterile. These observations suggest that Rex was not detectable or was not present in natural populations.

2) Tests for Su(Rex) in wildtype Drosophila - Total progeny counted for each isofemale line are shown in Table 3. Of a total of 401 y^+ w male offspring scored (Table 4), none has proven to be fertile. These observations imply that Su(Rex) is wide-spread in natural populations.

II - DISCUSSION

Two possibilities can be considered to explain the absence of Rex activity in the X chromosomes derived from these populations:

(1) - Rex is not present in natural populations. Rather, it is limited to some laboratory stocks, hence, Rex can not be considered as a major factor in concerted evolution of the rDNA gene family.

Wildtype Lines	B/+ Females	y w spl Males	W.T. Males	W.T. Females	v f B Males	y^+ w spl Males	♂ ♀	y w^+ males
Arg-4 III	3160	1891	2108	2	4	21	0	17
Arg-4 IV	3465	1943	1843	5	7	27	0	21
Arg-6 I	3334	1857	2049	5	3	17	0	21
Arg-6 II	2895	1441	1966	4	6	21	0	19
Aust BL-10 I	2972	1447	1905	1	4	21	0	15
Aust BL-17 II	2862	1600	1787	3	6	26	0	17
Aust Bl-17 IV	2822	1458	2008	2	5	23	0	19
Aust BL-17 V	2857	1444	1631	3	6	23	0	17
CAL-15 I	3178	2059	2204	2	6	24	0	18
Cal-15 II	2802	1751	1828	3	2	16	0	10
Taiwan-20 I	3670	1974	2059	2	6	21	0	16
Taiwan-20 II	3212	1558	2466	4	5	23	0	19
Viet-13-1 I	3200	1731	2100	5	6	24	0	19
Viet-13-1 II	3285	1847	2134	4	4	17	0	17
Viet-15-1 I	3746	1814	2229	3	12	33	0	21
Viet-15-1 II	3192	1864	2001	4	4	21	0	16

Table 2 - test for Rex activity in natural populations of D. melanogaster.

(2) - Rex might be present in wildtype Drosophila, but it is heavily suppressed by Su(Rex). The presence of Su(Rex) in natural population does not, however, necessarily imply the presence of Rex since a suppressor may have other functions as well as suppressing Rex.

Su(Rex) was, indeed, present in all of the samples. These observations leave open the question of whether Rex is present in wildtype Drosophila since the presence of the suppressor precludes knowing whether Rex is there as well. One way to resolve this question would be to define the function(s) of Su(Rex). If Su(Rex) has only one function, to suppress Rex activity, then the presence of the suppressor would imply that Rex is also wide-spread in natural populations. In that case, Rex could be involved in the concerted evolution of the rDNA gene family. If Su(Rex), however, has more than one function, then we would still not know whether Rex is present in natural populations of Drosophila.

Wildtype Lines	B/+ Females	y w spl Males	W.T. Males	W.T. Females	v f B Males	y^+ w spl Males	♀	y w^+ males
Arg-4 III	2943	1670	1907	1	4	22	0	17
Arg-46 IV	3202	1671	2021	2	6	22	0	16
Arg-6 I	2919	1596	1781	3	4	28	0	19
Arg-6 II	3536	1871	2498	3	5	21	0	19
Aust BL-10 I	3042	1539	2004	2	2	19	0	18
Aust BL-17 II	2977	1623	2331	1	4	22	0	15
Aust Bl-17 IV	3487	2075	2223	2	5	29	0	19
Aust BL-17 V	3091	1893	2173	3	5	25	0	24
CAL-15 I	3192	1666	1777	2	4	26	0	19
Cal-15 II	2813	1706	2270	3	5	26	0	22
Taiwan-20 I	3296	1759	2160	0	1	23	0	19
Taiwan-20 II	3550	1888	2522	1	9	30	0	16
Viet-13-1 I	2681	1475	1885	2	3	27	0	22
Viet-13-1 II	3280	1999	2222	2	6	33	0	20
Viet-15-1 I	2734	1317	1556	3	5	23	0	21
Viet-15-1 II	3157	1829	2158	1	3	25	0	20

Table 3 - Test for Su(Rex) in natural populations of D. melanogaster.

	Rex		Su(Rex)	
	Total y^+ w	Fertile y^+ w	Total y^+ w	Fertile y^+ w
Arg-4 III	21	0	22	0
Arg-4 IV	27	0	22	0
Arg-6 I	17	0	28	0
Arg-6 II	21	0	21	0
Aust BL-10 I	21	0	19	0
Aust BL-17 II	26	0	22	0
Aust BL-17 IV	23	0	29	0
Aust BL-17 V	23	0	25	0
CAL-15 I	24	0	26	0
CAL-15 II	16	0	26	0
Taiwan-20 I	21	0	23	0
Taiwan-20 II	23	0	30	0
Viet-13-1 I	24	0	27	0
Viet-13-1 II	17	0	33	0
Viet-15-1 I	33	0	23	0
Viet-15-1 II	21	0	25	0
TOTAL	358	0	401	0

Table 4 - Testing y^+ w males for fertility.

y^+ w males were crossed to C(1)RM, y v bb females. y males and y^+ v females are expected from this cross if the male is a y w/y^+Y detachment product.

APPENDIX

MEASUREMENT OF rDNA COPY NUMBER

Improving the resolution of rDNA maps, as well as being able to construct these maps quickly using Rex-induced exchange, depends on being able to determine rDNA copy number reliably for fairly large numbers of samples. I have, therefore, devoted some time to technology development. At first, a quantitative single-insect squash-blot technique (Betty et al., 1988; Tchen et al., 1985) appeared promising, but I have found it to be too variable. I have, therefore, turned to quantitative dot blots. The results seem to be reliable.

Once the parameters of the technique have been established, twenty-two pairs of chromosomes will be tested; a parental pair and eleven pairs of recombinants from one series of hairpin exchanges and a parental pair and nine recombinant pairs from a second series. The first series is described here.

MATERIALS AND METHODS

I) Separation of parental NO's

Two chromosomes will be tested: In(1)w^{m4} (w^{m4}) and In(1)w^{m51b} (w^{m51b}). As shown in Fig. 8, the breakpoints of w^{m4} and w^{m51b} define the proximal and distal ends of the X ribosomal region (Hilliker and Appels, 1982). In(1)w^{m4} moves the heterochromatin that normally lies distal to the rDNA to a point near the w locus at the tip of the euchromatin; it leaves the bulk of the ribosomal cistrons near the centromere. In(1)w^{m51b} moves most of the rDNA to the tip but leaves a very small portion near the centromere. An inversion chromosome, In(1)$w^{m51bL}w^{m4R}$, bearing both nucleolus organizers was used as a target for Rex-induced recombination. The two ends of this parental chromosome were separated by a single crossover with a deleted chromosome, In(1)$w^{m4L}w^{m51bR}$, to provide chromosomes bearing the parental rDNA arrays (Robbins, unpublished).

II) SEPARATION OF RECOMBINANT NO's

The result of hairpin exchange in In(1)$w^{m51bL}w^{m4R}$ is a normal order chromosome bearing two recombinant rDNA arrays, Dp(1;1)$w^{m51b}w^{m4}$. These recombinant arrays are separated by a crossover between Dp(1;1)$w^{m51b}w^{m4}$ and Df(1)X1 which results in the production of a normal X chromosome and a Tp(1;1)$w^{m51b}w^{m4}$ chromosome (Fig. 9); each bearing a single nucleolus organizer. Eleven pairs of recombinant NO's were generated

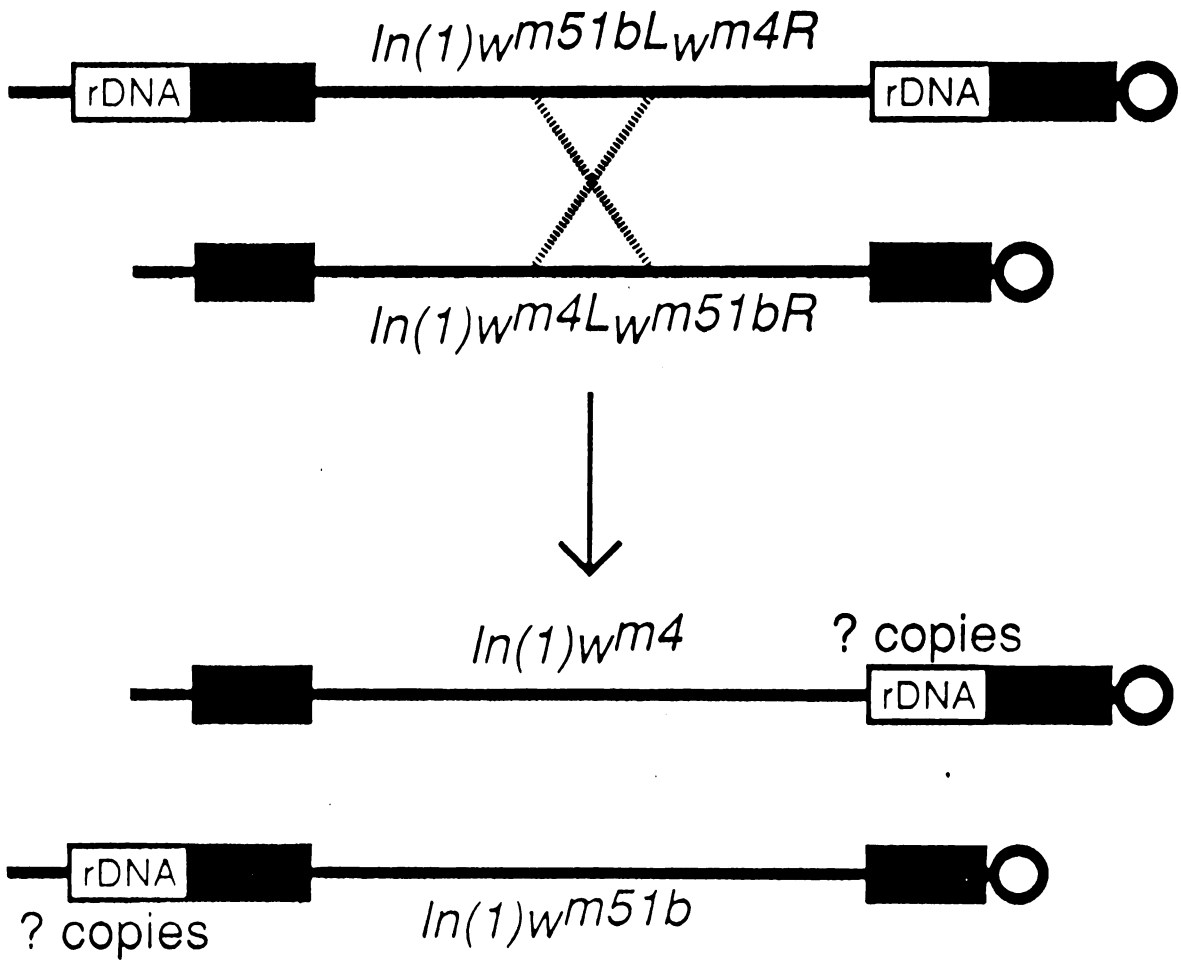


Figure 8 - Separation of parental NO's.

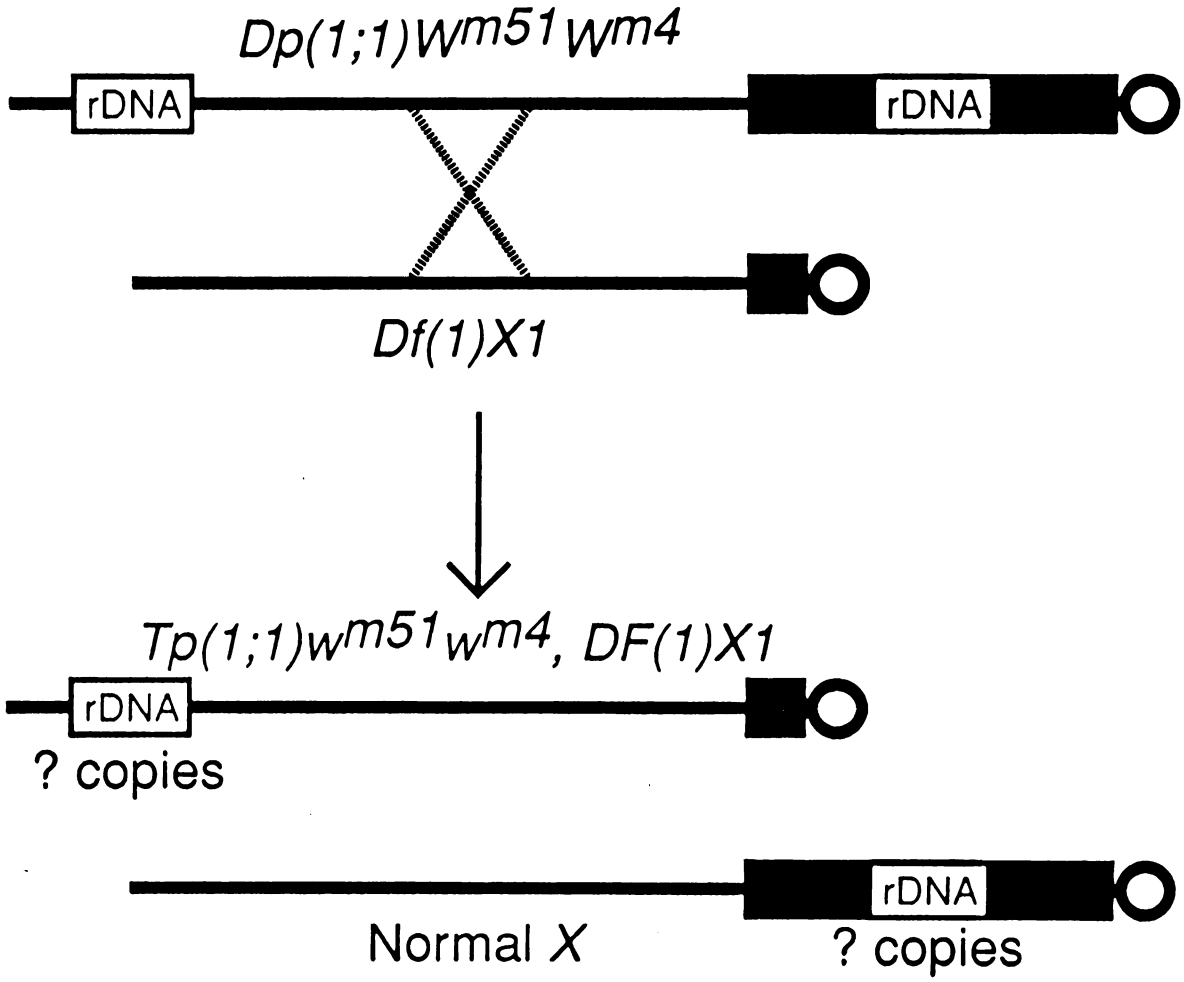


Figure 9 - Separation of recombinant NO's.

from the eleven Dp(1;1)$w^{m51bL}w^{m4R}$ reinversions.

It should be noted that all the chromosomes used have been kept in stock for several years with no sign of breakdown. Swanson (1987) has observed each of these stocks for at least twelve generations and has never seen any products of spontaneous breakdown.

III) DNA DOT BLOTS

Multiple samples of genomic DNA are spotted next to each other on a single filter in dots of uniform diameter. For quantitative analysis, known amounts of DNA are applied. To evaluate the extent of hybridization of the probe, a standard consisting of a dilution series of DNA is applied in an identical way to the same filter. The procedure binds samples quickly so that many samples can be handled at once. rDNA is readily detected in a spot containing as little as 50 ng of total DNA. Dot blots do not distinguish the number and size of the molecules hybridizing, so the hybridization "signal" is the sum of all sequences hybridizing to the probe under the conditions used. A general discussion of dot blot techniques may be found in Hames and Higgins (1985).

Filters are treated with high concentrations of salt prior to binding of nucleic acid. This both improves the efficiency of binding and helps to keep the diameter of the dot small. The salt solution most commonly used is 20x SSC (1 x SSC is 0.15M NaCl + 0.015M trisodium citrate, pH 7.0). For

the dot blots, DNA was covalently bound to the membrane by UV irradiation (Stratalinker UV Crosslinker, 1800). It is important to note that the filter must not at any stage be handled with bare bands. Grease from the fingers will result in poor binding of nucleic acids and high backgrounds. Therefore disposable plastic gloves must be worn at all stages.

IV) SINGLE-INSECT SQUASH-BLOT

Nitrocellulose filters are first treated with high salt solution, prior to squashing. This both improves the efficiency of binding and helps keep the diameter of the squash small. Flies, frozen at -72°C for a brief time, are placed next to each other in the wells, squashed, under vacuum, using a teflon dowel (6 to 8 rotations of rod). The filter is then air dried at room temperature for 5 minutes, denatured and neutralized. The filter is then either baked at 80°C for 2 hours or UV fixed. To digest the proteins and other debris, the filter is treated with chitinase in 10 ml citrate, pH 5.5 for 18 hours and then treated with proteinase K at 38°C for 2 hours. It is important to note that nitrocellulose membranes cannot be readily stripped and reprobed. Note also that disposable gloves must be worn at all stages.

V) RADIOLABELING OF DNA PROBES

The DNA probes used in this study included: (1) an rDNA probe containing a 300 bp sequence flanking the T1/T2 insertion sites in the 28S subunit. This fragment is present once in each rDNA repeat. It has been cloned into the 2.7 kb pUC18 vector (Jakubcak, personal communication) and (2) a probe containing the single copy D. melanogaster urate oxidase gene (UO) (Wallrath et al., 1990).

VI) PROBE-LABELLING AND HYBRIDIZATION

rDNA and urate oxidase (UO) probes were labelled using the oligonucleotide labelling method of Feinberg and Vogelstein (1982) and labelled probe was separated from unincorporated nucleotide by column chromatography using Selphadex G-50.

Pre-hybridization is carried out overnight at 42^0c in heat-sealed bags with 10 mls of pre-hybridization solution (50% formamide; 0.5 mg/ml alkali-sheared salmon sperm DNA; 1x pre-hybridization stock). For measuring rDNA copy numbers, 10-fold excess of unlabelled copies of the ^{32}P-labelled probing sequence and short hybridization times are used to ensure appropriate kinetics. For the rDNA probe, hybridization is carried out at 42^0C in heat-sealed bags with 10^6 dpm/ml of probe in hybridization solution (1x prehybridization stock; 50% formamide; 0.2 mg/ml alkali-sheared salmon sperm DNA). The presence of 10x Denhardt's solution and denatured DNA in the

pre-hybridization and hybridization media, as well as the washing under strigent conditions reduce the background on the filter resulting from non-specific hybridization.

After hybridization, the filters are washed twice with 2x SSC + 0.05% Sarkosyl + 0.02% sodium phosphate at room temperature, followed by three 15 minute washes at 50^{0}C and one 15 minute wash at 60^{0}c in 0.1x SSC + 0.05% sarkosyl + 0.02% sodium phosphate. The filter is then wrapped in plastic wrap to prevent drying, autoradiographed and counted using a Betascan blot analyzer.

Hybridization of the single-copy urate oxidase gene was used as an internal control. rDNA probe must be removed from the hybond-N nylon membranes by incubating the blots at 45^{0}C for 30 minutes in 0.4M NaOH and for another 30 minutes in 0.1x SSC + 0.1% SDS + 0.2M tris-HCl, pH 7.5. After this, the same protocol of pre-hybridization, hybridization and washing described above is used for the UO probe with the exception that hybridization is carried out for a longer time in the presence of 10% Dextran Sulfate to ensure appropriate kinetics.

VII) Hybridization kinetics

Filter hybridization depends on two processes, diffusion of the probe to the filter and hybridization at the filter. It is thought that at low concentration of filter-bound nucleic acid sequences, the hybridization reaction itself is the rate

limiting step, whereas at high concentration of filter-bound nucleic acid sequences, hybridization is so fast that the solution surrounding the filter becomes depleted of probe and the overall reaction is then limited by diffusion of the probe to the filter. Increasing the concentration of the probe in solution will increase the initial rate of hybridization at the filter, and the proportion of the filter-bound sequences in duplex will increase. Thus, for a multi-copy sequence such as rDNA, a high probe concentration and short hybridization times yield a measure of bound sequence. For the single-copy UO gene, in contrast, high specific activity and long hybridization times are appropriate because probe concentration cannot be seriously depleted during the course of the reaction. For the rDNA, therefore, we used a 10-fold excess of un-labelled probe sequence and short hybridizations. For UO, we used only labelled probe and longer hybridizations.

The validity of these conditions can be assessed by examining the linearity and extent of hybridization with variation of the amount of bound DNA.

VIII) Calculation of 10x excess of probe

In Drosophila melanogaster, there is approximately 400ng of DNA in a single fly (Robbins, personal communication). The rDNA represents about 2% of total DNA or 8ng; rDNA= (% DNA in the X chromosome= 20%)x(% of heterochromatin in the X= 30%)x(% of rDNA in heterochromatin= 30%). The total fragment DNA is

determined by the fragment size (= 400bp) divided by the rDNA repeat length (= 11.5kb). Therefore there is approximately 3.3% (= 0.25ng) of fragment DNA in a single fly.

10x excess of probe corresponds, therefore, to 10 x 0.25 ng or 2.5ng of fragment DNA per fly or 6.25 x 10^{-3}ng of fragment DNA per ng of DNA.

The volume of hybridization solution per unit area of membrane required for running a good reaction is 0.05 ml/cm^2 and the membrane size is 100 cm^2 (8.5 cm x 11 cmm). Therefore, 5 ml of hybridization solution are used to hybridize a single membrane.

I normally use 1.0 x 10^6 cpm/ml to do the hybridization, corresponding to 5.0 x 10^6 total counts in 5 ml of hybridization solution. Once the probe is prepared and total counts are determined, the volume of probe needed for a hybridization reaction is determined as follows:

$$\text{volume of probe needed} = \frac{(5.0 \times 10^6)(\text{total volume of probe})}{\text{Total counts of the probe}}$$

IX) COUNTING AND CALCULATIONS

1) - Counting

A betascope (Betagen corp., model 603) was used to quantify the relative amount of ^{32}P present in each DNA dot. Counting of the filter in the betascope for four hours using the rDNA probe and for 18 hours using the single copy UO probe

is sufficient to give intense signals.

2) - Calculations

A) Subtracting the background - the Betascope image is divided into small 9.2 mm squares and small 9.2 mm diameter circles around every DNA dot. The number of counts present in each circle is subtracted from that present in the corresponding square and the result is divided by (Area of square - Area of circle) to determine the amount of background radioactivity per unit area. This value is then multiplied by the area of the circle and the result is subtracted from the number of counts in that circle to determine the net counts for the corresponding DNA dot.

B) Determination of the relative copy number - The gene copy number for a particular genotype is measured relative to our standard genotype, Ore-R. In the graphs, net counts for both probes are plotted as a function of DNA amount for each genotype tested and the slopes for both graphs are determined by linear regression. Let these slopes be S1 and S2 for rDNA and UO, respectively for the genotype tested and Sc1 and Sc2 for rDNA and UO of Ore-R. The relative copy number is, therefore: (Sc1/Sc2)x(S2/S1)

PRELIMINARY RESULTS

I) - SQUASH-BLOT

In order to establish a technology for rapidly determining rDNA copy number using single-insect squash-blots, a number of different parameters have been examined. Here, I present some preliminary results for the following:

1) - Different ways of keeping the dots small and improving the efficiency of binding
2) - Different ways of fixing DNA onto filter.
3) - Linearity

1) - Improving the efficiency of binding and keeping the dots small:

Treating the membranes with high salt, prior to DNA application, improves the efficiency of binding and keeps the dots small. For this experiment, the following concentrations of salts have been applied to a number of filters:

a) - Filter wet in 10 ml of H_2O followed by 10 ml of 20x SSC
b) - Filter treated with 10 ml H_2O followed by 10 ml of 10x SSC
c) - Flies directly squashed in 5M NaOH with no pre-treatment of membrane
d) - No treatment: Filter dry

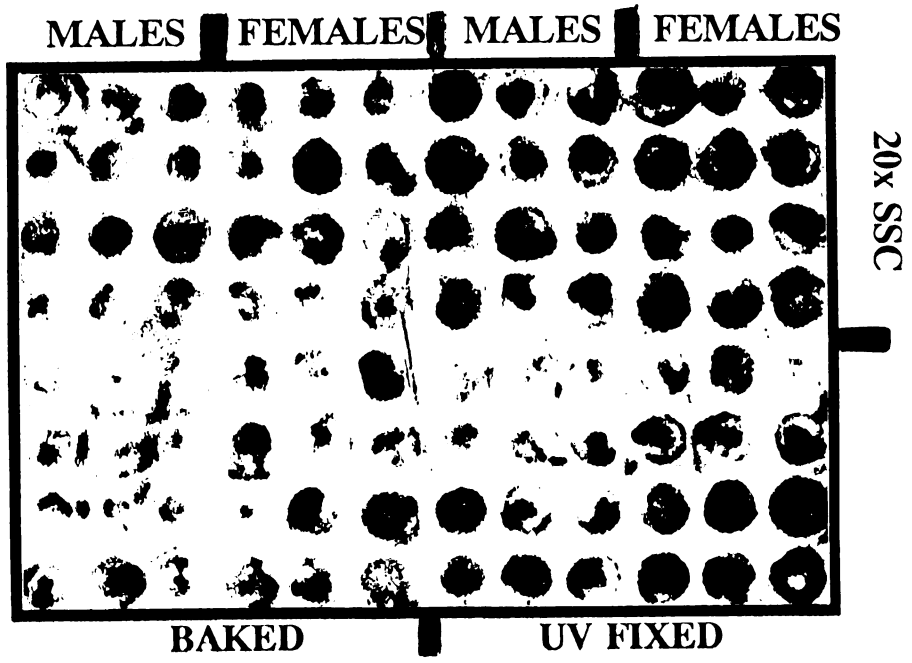


Figure 10 - Hybridization of genomic DNA from $\underline{Y^{S}X\cdot Y^{L}/0}$ males (2 NO's) and $\underline{C(1)RM/0}$ females (one NO) squashed on nitrocellulose filter and probed with rDNA. Filter was cut in half and each half was divided into two portions. One portion was treated with 20xSSC prior to squashing and the other portion was kept dry. One half was then baked at 80^{0}C for 90 minutes and the other half was UV fixed.

Figure 11 - Hybridization of genomic DNA from squashed $\underline{w^c}$ $\underline{m}$ flies probed with rDNA sequences. Prior to squashing, filter was either treated with H_2O (line a) 10x SSC; NaOH or kept dry.

Techniques b and c gave too much variation. and the DNA was spread out (Fig. 11). Filters treated with both techniques a and d gave better results (Figures 10 and 11). Technique d did not, however, give the same results for all the membranes treated (Figures 10 and 11).

2) - Different ways of fixing DNA onto filter:

Two techniques have been tried: baking the membrane at 80°C for one hour 30 minutes versus fixing DNA onto filters by UV exposure. Several membranes were made, and each was cut in half. One half was baked for 90 minutes, and the other half was UV fixed (Fig. 10). In all cases, the results showed that the UV exposure gives equal or better results than the baking technique (Fig. 10).

3) - Linearity

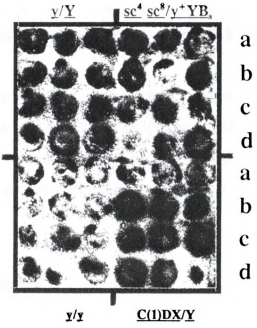
For quantitative analysis, it is important to ensure that the proportion of the probe hybridizing increases linearly with DNA amount. For the squash-blots, visual comparison of the intensity of hybridization signals on an autoradiogram indicates that we have not achieved this. Fig. 12 shows squash blots of genomic DNA from a single fly (line a), two flies (line b), three flies (line c) and four flies (line d). We expect that the autoradiographic signals would increase from one line to the next giving a ratio of 1:2:3:4. Fig. 12B, for example, shows that line a (a single fly) gives as intense

Figure 12 - Test for linearity.

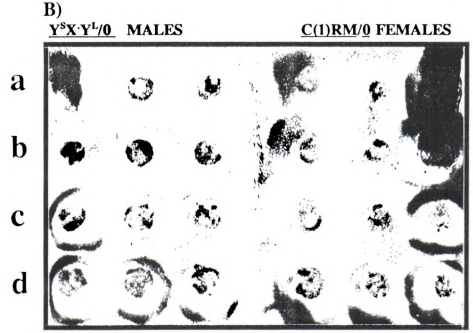
A): Squash blot rDNA hybridization of homozygous y flies (two NO's); and $\underline{IN(1)sc^{4}sc^{8}}/\underline{y^{+}YB^{S}}$ and C(1)DX/Y (one NO)

B): Squash blot rDNA hybridization of $\underline{Y^{S}X{\cdot}Y^{L}}/\underline{0}$ (2 NO's) and C(1)RM/0 (one NO). Single flies (line a), two flies (line b), three flies (line c) and four flies (line d).

A)


y/Y
sc⁴ sc⁸/y⁺YB,
a
b
c
d
a
b
c
d
y/y
C(1)DX/Y

B)


Y^S X·Y^L/0 MALES
C(1)RM/0 FEMALES
a
b
c
d

signals as line b (two flies) or line c (three flies); and in Figure 12A, line a (one fly) and line c (three flies) give more intense signals than line b (two flies) and line d (four flies).

It is also important to note that, although the filter was treated with high salt solution (Figure 12B) which should keep the diameter of the spots small (see section 1), the DNA was spread out. These inconsistencies led us to switch to DNA dot blots.

II) DNA DOT BLOTS

The following parameters and comparisons have been tested using DNA dot blots:

1) Different ways of denaturing the DNA
2) The time period of pre-hybridization
3) The time period of hybridization

1) - Different ways of denaturing the DNA

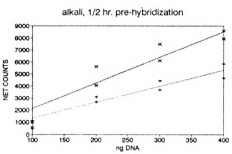
Two techniques have been tried: (1) heating the DNA at 75^{0}C for 20 minutes and application to the filter followed by denaturing and neutralizing in 0.5M NaOH; 1.5 M NaCl and in 0.5M Tris, pH 8; 1.5M NaCl, respectively (Fig. 13A) and (2) denaturing the DNA by heat only (Figure 13B). Although both techniques give strong signals, the first technique appears to be more efficient.

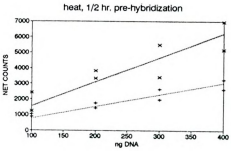
Figure 13 - Denaturation procedure and pre-hybridization time. Ore-R DNA blots denatured by heat only (A) or alkali-denatured (B),were pre-hybridized for 1/2 hour, 2 hours, or 18 hours.

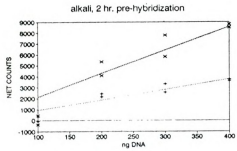
X = pA-56 (rDNA) probe

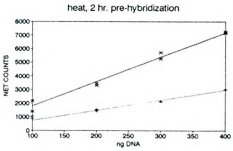
\+ = UO probe, --- = linear regression, y = 0

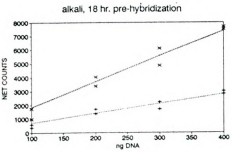
Oregon-R DNA, UO vs. pA-56, 100-400ng

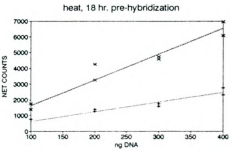












2) - Time period of pre-hybridization

In the pre-hybridization step, the filter was incubated at 42^{0}C for 1/2 hour; 2 hours or 18 hours in a solution which is designed to pre-coat all the sites that would, non-specifically, bind the probe. The results are shown in fig.13. In all cases, strong signals are observed with both the rDNA probe and the UO probe. With half hour pre-hybridization, not only do we get strong signals, but the amount hybridization is proportionate DNA amount.

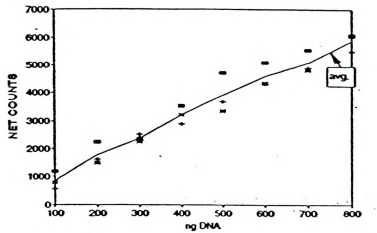
3) - Time period of hybridization

Hybridization with the rDNA probe was carried out at 42^{0}C for one; two; three or four hours (Fig. 14). Since the initial rate of reaction is proportionate to the concentration of bound DNA, we want to use the shortest hybridization time that gives adequate signal. Long hybridization times may result in depletion of the probe and a non-linear response. The results shown in Fig. 14 suggest that one hour hybridization with the rDNA probe is sufficient and that the signal varies proportionally with bound DNA concentration. Prolonged incubation (Fig. 14) does not necessarily increase the extent of hybridization because:

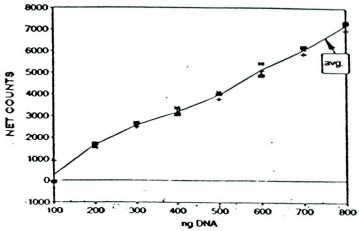
1) more and more probe reassociates
2) the probe concentration is reduced as it hybridizes to the bound DNA.

Figure 14 - Ore-R DNA blots hybridized with rDNA probe for one, two, three or four hours.

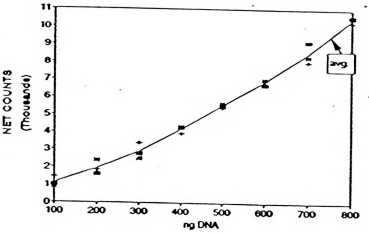
1 HOUR HYBRIDIZATION



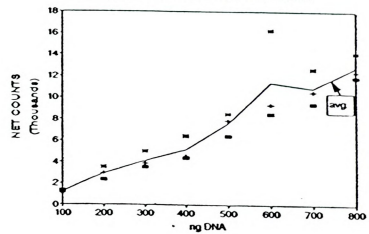
2 HOUR HYBRIDIZATION



3 HOUR HYBRIDIZATION



4 HOUR HYBRIDIZATION



For the single copy UO probe, hybridization was carried out at 42^{0}C for four hours or 18 hours in the presence of Dextran sulfate. Fig. 15 shows that 18 hour hybridization is more efficient than 4 hours, while the preceding graphs (fig.13) showed that the extent of reaction remains proportionate to DNA amount even at this longer time.

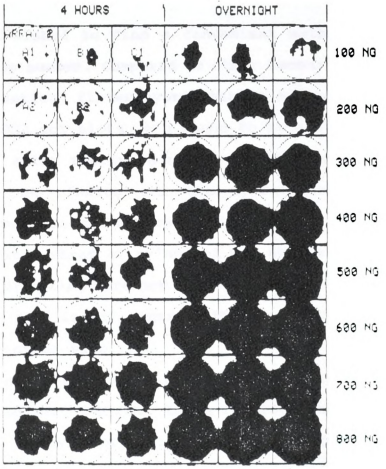


Figure 15 - Ore-R DNA dot blot hybridized with UO probe for four hours or 18 hours.

CONCLUSION

We wished to find a convenient method for determining rDNA copy number. Compared to other techniques, i.e. solution hybridization, filter hybridization or single-insect squash-blots, DNA dot blots were found to be a simple and sensitive method. After testing a number of different parameters that affect DNA binding and the hybridization reaction, the following protocol has been established:

1) Float a sheet of nylon and two sheets of 3MM paper on water taking care not to trap air bubbles underneath. When one side is wet, immerse the membranes completely to wet the other side.
2) Transfer the filter and the 3MM papers to a dish containing 20X SSC. Leave for 10 minutes.
3) Dry at room temperature until completely dry
4) Dissolve DNA in TE (0.01M Tris, 0.001M Na_2 EDTA) at a concentration 0f 5 ug/ml. Heat these samples to boiling for 10 minutes. Adjust each sample to a final concentration of 2.5M NaCl by adding an equal volume of 5M NaCl.
5) Add samples of 50ng, 100ng, 150ng, 200ng, 250ng, 300ng, 350ng, 400ng to wells of Millipore blot apparatus, under vacuum.
6) Air dry for 5 minutes.
7) Denature in 1.5M NaCl; 0.5M NaOH.

8) Neutralize in 1.5M NaCl; 0.5M Tris-HCl, pH 7.2; 0.001M Na2 EDTA.

9) Air dry for 5 to 10 minutes

10) Transfer filter to a Whatman 3MM paper saturated with 10X SSC and expose to UV light for DNA fixing.

11) Pre-hybridize for 1/2 hour at 42^0C in heat-sealed bags with 10 mls of pre-hybridization solution (50% formamide; 0.5 mg/ml alkali-sheared salmon sperm DNA; 1X pre-hybridization stock).

12) Hybridize for one hour with rDNA probe or 18 hours with UO probe at 42^0C in heat-sealed bags with 10^6 dpm/ml of probe in hybridization solution (1X pre-hybridization stock; 50% formamide; 0.2 mg/ml alkali-sheared salmon sperm DNA). The rDNA hybridization mix has cold probing sequence added, while the UO mix has 10% dextran sulfate added

13) Wash filter twice with 2X SSC + 0.05% sarkosyl + 0.02% sodium pyrophosphate at room temperature followed by three 15 minute washes at 50^0C and one 15 minute wash at 60^0C in 0.1x SSC + 0.05% sarkosyl + 0.02% sodium pyrophosphate.

14) Wrap filter in plastic wrap to prevent drying.

15) Autoradiograph and count using Betascan.

BIBLIOGRAPHY

Arnheim, N. 1983. Concerted evolution of multigene families. IN: Evolution of genes and proteins, M. Nei & R.K. Koehn. eds. (Sunderland, Mass., Sinauer Assoc.), pp.33-61.

Coen, E. S., J. M. Thoday and G. Dover. 1982. Rate of turnover of structural variants in the rDNA gene family of D. melanogaster. Nature 295: 564-5568.

Dover, G. 1982. Molecular drive: a cohesive mode of species evolution. Nature 299: 111-117.

Hames B. D. and S. J. Higgins eds., 1985. Nuclei acid hybridization: a practical approach. IRL Press Ltd Oxford: 73-112.

Hilliker, A. J., R. Appels and J. Schalet. 1980. the genetic analysis of D. melanogaster heterochromatin. Cell 21: 607-619.

Indik, K. and K. D. Tartof. 1980. Long spacers among ribosomal genes of D. melanogaster. Nature 284: 477-479.

Kidd, S. J. and D. M. Glover. 1981. D. melanogaster ribosomal DNA containing type II insertions is variably transcribed in different strains and tissues. J. Mol. Biol. 1551: 645-662.

Lindsley, D. L. and E. H. Grell.1968. Genetic variations of D. melanogaster. Crnegie Inst. of Wash., Publ. 627.

Lindsley, D. L. and E. Novitski. 1959. Compound chromosmes involving the X and Y chromsomes of D. melanogaster. Genetics 44: 187-196.

Lindsley, D. L and G. Zimm. 1985. The genome of D. melanogaster, part 1: genes A-K. Dros. Inform. Service 62.

Lindesley D. and G. Zimm. 1987. The genome of D. melanogaster, part 3. Dros. Inform. Service 65.

Long, E. O. and I. B. Dawid. 1979. Expression of rDNA insertions in D. melanogaster. cell 18: 1185-1196.

Long, E. O. and I. B. Dawid. 1980. Repeated genes in eukaryotes. Ann. Rev. Biochem. 49: 727-764.

Long, E. O. and M. Collins, B.I. Kiefer & I.B. Dawid. 1981. Expression of rDNA insertions in bobbed mutants of D. melanogaster. Mol. Gen. Genet. 182: 377-383.

Muller, H. J. 1932. Further studies on the nature and causes of gene mutations. Proc. 6th Intl. Cong. Genetics 1: 213-255.

Peacock, W. J., R. Appels, S. Endow, and D. Glover. 1981. Chromosomal distribution of the major insert in D. melanogaster 28S rRNA genes. Genet. Res. 37: 209-214.

Rasooly, R. S. and Leonard G. Robbins. 1991. Rex and a suppressor of Rex are repeated neomorphic loci in the Drosophila melanogaster ribosomal DNA. Genetics (In Press).

Rebekah, R. 1989. Genetic and molecular mapping of Rex within the rDNA of D. melanogaster. Ph.D. thesis, Michigan State University.

Ritossa, F. M., K. C. Atwood and S. Spiegelman. 1966. On the redundancy of DNA complementary to amino acid transfer RNA and its absence from the nucleolar organizer region of D. melanogaster. Genetics 54: 663-676.

Robbins, L. G. 1981. Genetically induced mitotic exchange in the heterochromatin of D. melanogaster. Genetics 99: 443-459.

Robbins, L. G. and E. E. Swanson. 1988. Rex-induced recombination implies bipolar organization of the ribosmal RNA genes of D. melanogaster. Genetics 120: 1053-1959.

Roiha, H. and D. M. Glover. 1981. Duplicated rDNA sequences of variable lengths flanking the type I insertions in the rDNA of D. melanogaster. Nucleic Acids Res. 9: 5521-5532.

Roiha, H., J. R. Miller, L. C. Woods and D. M. Glover. 1981. Arrangements and rearrangements of sequences flanking the two types of rDNA insertions in D. melanogaster. Nature 290: 749-753.

Schalet, A. and G. Lefevre, Jr. 1976. The proximal region of the X chromosome. IN: The genetics and Biology of Drosophila, Vol. 1b, M. Ashburner and E. Novitski, eds. (London: Academic Press), pp. 847-902.

Sim, B. K. L., P. Romans and S. Harun. 1989. Use of Chitinase to facilitate detection of protozoan, helminth and single copy genes in squashed whole mosquitoes. Mol. Biochem. Paras. 34: 127-134.

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

Smith, G. P. 1973. Unequal crossover and evolution of multigene families. Cold Spr. Harb. Symp. Qant. Biol. 38: 507-518.

Swanson, E. E. 1984. Parameters of the Rex phenotype in D. melanogaster. Ph.D. thesis, Michingan State University.

Swanson, E. E. 1987. The responding site of the Rex locus of D. melanogaster. Genetics 115: 271-276.

Tartof, K. D. 1971. Increasing the multiplicity of ribosomal RNA genes in Drosophila melanogster. Science 171: 294-297.

Tartof, K. D. 1973. Regulation of ribosomal RNA multiplicity in D. melanogaster. Genetics 73: 57-71.

Tautz, D., J. M. Hancock, D. A. Webb, C. Tautz & G. A. Dover. 1988. Complete sequences of the RNA genes of Drosophila melanogaster. Mol. Biol. Evol. 5: 366-376.

Tchen, P., Anxolabehere, D., Nouaud, D. and Periquet, G. 1985. Hybridization on squashed flies: a method to detect gene sequences in individual Drosophila. Anal. Biochem. 150: 415-420.

Terracol, R. 1986. Transcription of rDNA insertions in bobbed mutants of D. melanogaster. Genet. Res. 48: 164-184.

Wallrath, L., J. B. Burnett, and T. B. Friedman. 1990. Molecular characterization of the Drosophila melanogaster urate oxidase gene, an ecdysone-repressible gene expressed only in the Malpighian tubules. Mol. Cell. Biol. 10: 5114-5127.

Wellauer, P. K., I. B. Dawid and K. D. Tartof. 1978. X and Y chromosomal ribosomal DNA of Drosophila: comparison of spacers and insertions. Cell 114: 269-278.

Williams, S. M., G. R. Furnier, E. Fuog and C. Strobeck. 1987. Evolution of the ribosomal DNA spacers of D. melanogaster: different patterns of variation on X and Y chromosomes. Genetics 116: 225-232.

Williams, S. M., J. A. Kennison, L. G. Robbins and C. Strobeck. 1989. Reciprocal recombination and the evolution of the ribosomal gene family of Drosophila melanogaster. Genetics 122: 617-624.

Williams, S. M., L. G. Robbins, P. D. Cluster and R. W. Allard. 1990. Superstructure of the Drosophila ribosomal gene family. Proc. Natl. Acad. Sci. 87: 3156-3160.