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GENETICS AND MOLECULAR MAPPING OF REX WITHIN THE
RIBOSOMAL DNA OF DROSOPHILA MELANOGASTER

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REBEKAH SARAH RASOOLY

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GENETIC AND MOLECULAR MAPPING OF REX WITHIN THE
RIBOSOMAL DNA OF DROSOPHILA MELANOGASTER

By

Rebekah Sarah Rasooly

A DISSERTATION

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ABSTRACT

GENETIC AND MOLECULAR MAPPING OF REX WITHIN THE
RIBOSOMAL DNA OF DROSOPHILA MELANOGASTER

By

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Rex is a maternal-effect dominant of Drosophila melanogaster which induces exchange between 2 blocks of ribosomal DNA on a single chromosome. In this study several features of Rex were characterized. Genetic experiments mapped Rex to the proximal heterochromatin of the X chromosome. Several dominant, maternal-effect suppressors of Rex (Su(Rex)) were identified and also mapped to the same region. Using duplications and deletions, both Rex and one Su(Rex) locus were shown to be neomorphs. Both loci were then mapped to a specific position within the ribosomal DNA array using molecular size variants of the ribosomal DNA intergenic spacer. mei-41 is an X-linked meiotic mutation which induces X-Y exchange and inhibits magnification in males, perhaps because of a defect in post-replication repair. Although Rex did not induce interchromosomal exchange between the X and Y chromosomes, the phenotype of mei-41 males was shown to have other similarities to the Rex phenotype in females. Most notably, mei-41 in males induces Rex-like exchanges. The results support a hypothesis that Rex encodes is a site-specific endonuclease activity cutting within the rDNA and that Su(Rex) loci repress the endonuclease.

To Avi and Irit
for their unquestioning love and support

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Peter, Susan, Lori Wallrath and other members of the Friedman lab also provided intangible assistance. They were ideal office-mates, always ready to listen and advise and share information and know-how. My thanks also go to Nancy Veenstra, who was a confidante and companion through many long hours of fly counting.

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The Talmud counsels a teacher to "respect your student as you do yourself..." My advisor, Lenny Robbins, is the very example of what the rabbis had in mind. Throughout my graduate career, he has shared his enormous insight, always willing to discuss a major result or a minor technical problem. My thanks go to him for teaching me genetics and how to teach and how to make fly food and a dozen other large and small things that I will take with me from here.

Finally, I cannot find the words that will describe my family's contribution to this work. Instead, I have dedicated this dissertation to my husband and my daughter, and here add my thanks to my parents as well.

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CHAPTER 1

INTRODUCTION

Rex is a maternal-effect dominant locus of Drosophila melanogaster that induces exchange between two ribosomal DNA (rDNA) arrays on a single chromosome (Robbins, 1981). The exchange is mitotic and takes place in the early zygote, usually before S of the first division. For example, when a Rex female is mated to a male with an attached-XY chromosome that has two rDNA blocks, 1 to 6% of the XXY daughters will undergo an exchange event that deletes the intervening X euchromatin from the attached-XY chromosome (Fig. 1) (Robbins, 1981). These "detachments" of the Y chromosome change the XXY daughters into XY sons. If the Rex-induced exchange event takes place after S phase of the first cell cycle in the embryo or at the two cell stage then only half the cells will contain the detachment product, and the resulting animal will be a gynandromorph (part male, part female).

Swanson (1987) demonstrated that Rex causes exchanges between any two separated blocks of rDNA on a single chromosome. She tested various chromosomes with two rDNA blocks for their ability to undergo detachment events. Even half a block of rDNA at each end of the chromosome is a target. Furthermore, Rex can induce both "spiral" exchanges that cause deletions, and "hairpin" exchanges that invert the intervening material in the same target chromosome (Fig. 2) (Swanson, 1987; Robbins & Swanson,

Figure 1 - Detection of Rex activity

A: 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. Although non-disjunctional sons are also y^+ , they have all of the paternal markers (y f B).

B: 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 (or nucleolus organizers, NO) are indicated by open boxes.

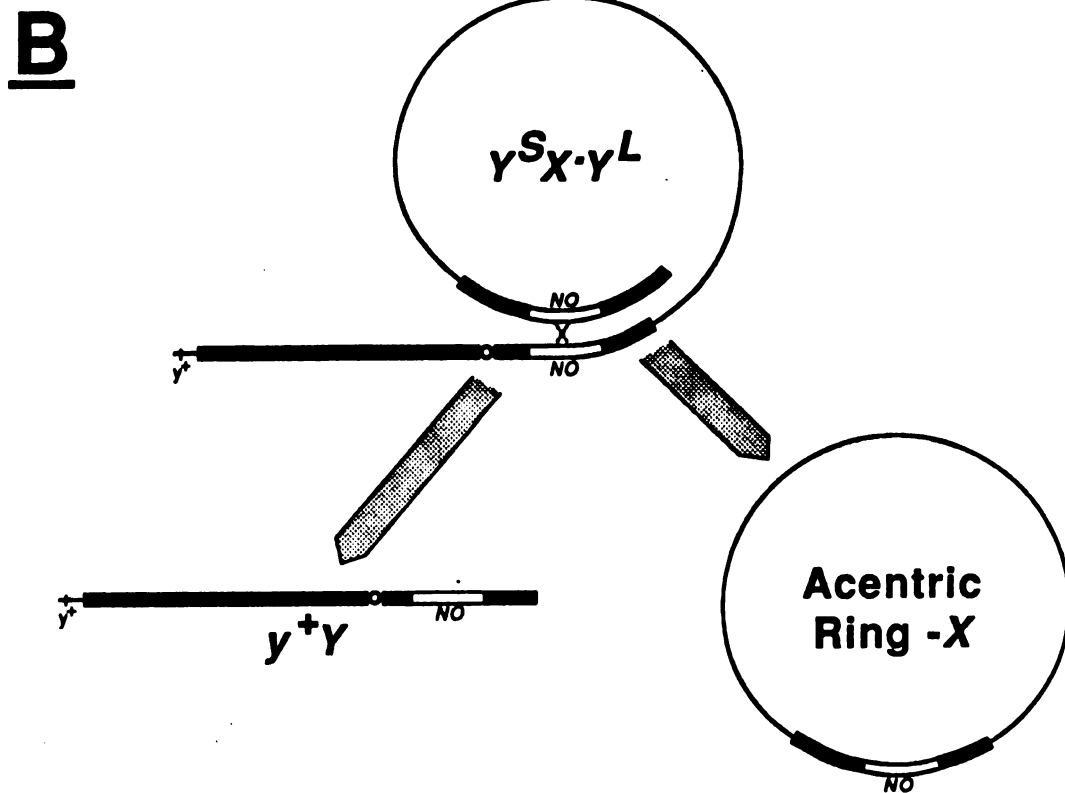
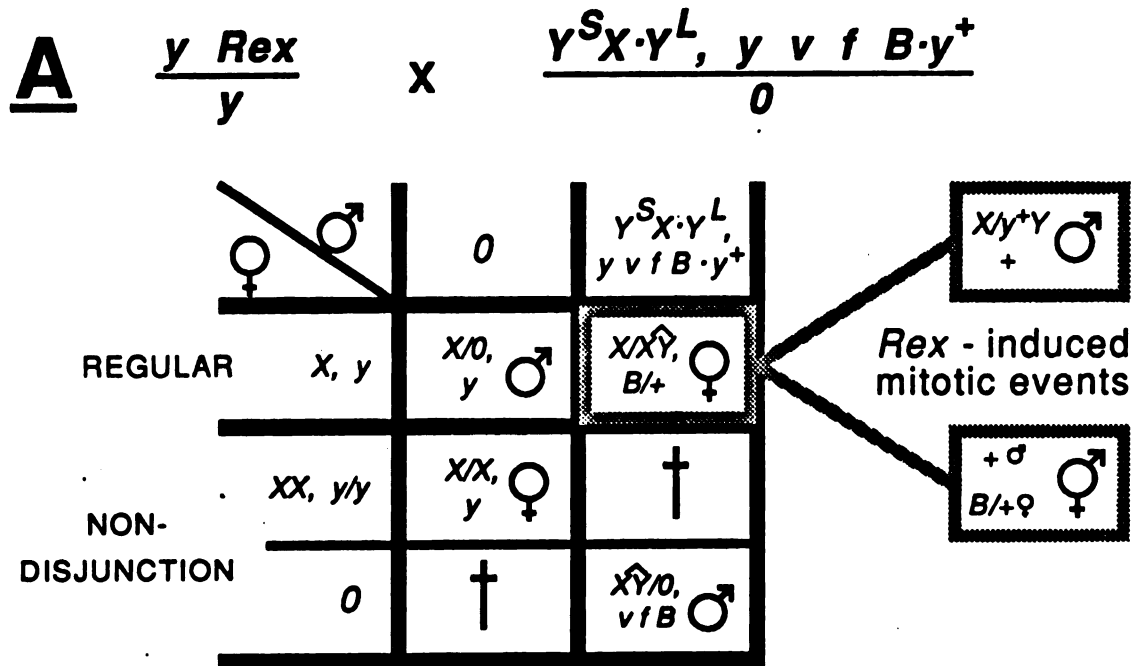


Figure 1

Figure 2 - Two types of Rex-induced exchange (adapted from Robbins & Swanson, 1988)

The target shown is an \bar{X} chromosome duplicated for the NO and surrounding heterochromatin (such as $\text{In}(1)\text{sc}^{\text{S1}}\text{sc}^{\text{A}}$). 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 \bar{X} euchromatin, changing \bar{X}/\bar{X} daughters into sterile \bar{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.

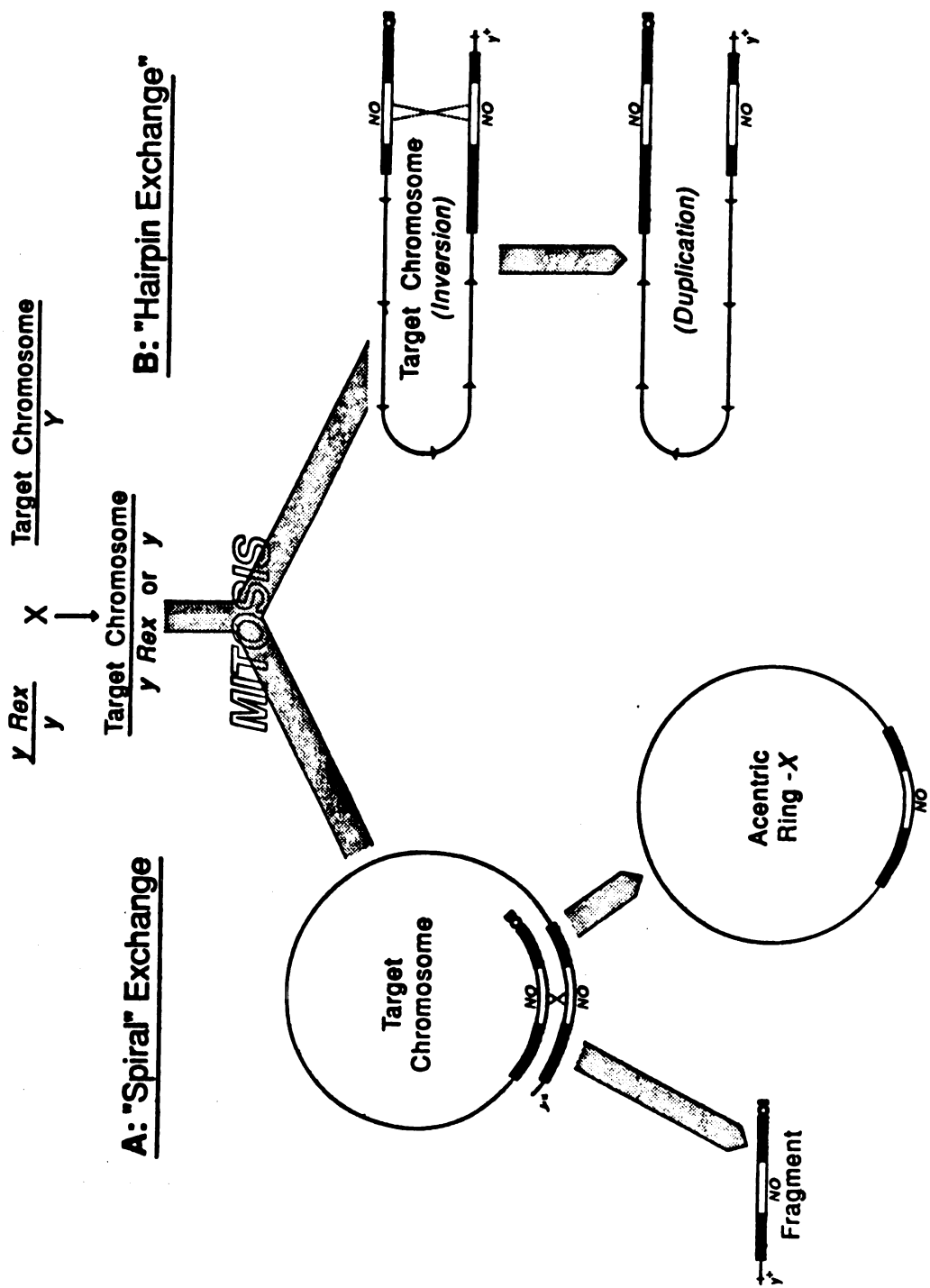


Figure 2

1988). Rex was mapped to the proximal portion of the X chromosome, between car and the centromere (Fig. 3) (Robbins, 1981).

Rex is fascinating for two reasons. First, it induces a high frequency of exchange in the rDNA, which rarely undergoes meiotic exchange. The genetic length of the entire centric heterochromatin is approximately 0.01 map units (Schalet & Lefevre, 1976), while the frequency of Rex-induced exchange is approximately 5%. Even though the rDNA appears to be a relative "hot-spot" for recombination within the heterochromatin, with most of the exchanges within the heterochromatin actually taking place in the rDNA (Williams et al., 1989), Rex-induced exchange is still at least two orders of magnitude more frequent. Second, Rex-induced exchange is mitotic and takes place specifically during the earliest stages of embryogenesis. Although a few other loci are known to be active at this time, including pal, abo, mit and cand, all of these involve loss of chromosomes or lethality, and not exchange (Baker, 1975; Davis, 1969; Gelbart, 1974; Sandler, 1970).

The Nucleolus Organizer - The Rex target is the nucleolus organizer (NO) which contains the 18S and 28S rDNA (Ritossa et al., 1966). In D. melanogaster, as in many other organisms, the NO is organized as an array of repeats. There are two rDNA arrays, which correspond genetically to the bobbed (bb) loci. One is in the centric heterochromatin of the X chromosome and one is in the short arm of the Y chromosome. Each contains approximately 225 repeats (Tartof, 19713). Mutations at the bb loci correspond to deficiencies of rDNA. The phenotype of a bb mutations ranges from lethality to a more or less severe "bobbed" phenotype characterized by reduced fertility, delayed

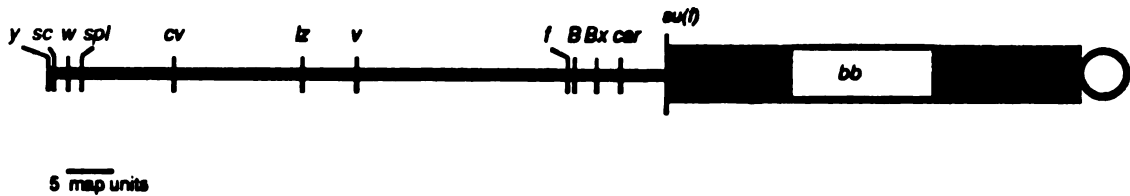


Figure 3 - Schematic representation of the X chromosome

The X chromosome is 66 map units long, and the map positions of many of the phenotypic markers used in these experiments is shown. Although the basal heterochromatin (heavy lines) is approximately 40% of the physical length of the X chromosome, it is less than 0.1 map units in genetic 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.

development, thinning of thoracic bristles and abdominal etching (Lindsley & Zimm, 1985).

The individual rDNA genes within a cluster are not identical (Fig. 4). There is a variety of intergenic spacer (IGS) lengths, ranging from 4 kb to as large as 20 kb (Coen et al., 1982; Indik & Tartof, 1980; Terracol, 1986). There are two sources of the IGS length heterogeneity. The 5' portion of the IGS contains variable numbers of a 340 bp sequence bounded by DdeI sites (Williams et al., 1987). The central portion of the IGS is entirely composed of integral numbers of a 240 bp sequence bounded by AluI sites (Coen et al., 1982; Simeone et al., 1985).

The other source of heterogeneity within an rDNA array is the insert sequences which interrupt the 28S coding region. Insert sequences are found in approximately 60% of X chromosome and 15% of Y chromosome repeats (Pelligrini et al., 1977; Wellauer et al., 1978; White & Hogness, 1977). The insertion sequences fall into two classes designated as Type I and Type II (also referred to as R1Dm and R2Dm, respectively; Burke et al., 1987). Type I inserts have only been reported in the X NO, while Type II inserts are found in both the X and Y NO's (Wellauer et al., 1978). There is considerable length heterogeneity among the members of each class, with Type I ranging from 0.5-7.5 kb (Pelligrini et al., 1977) and Type II ranging from 1.5-4 kb (Wellauer et al., 1978). Repeats containing insertions appear to be inactive, based on the fact that there are virtually no primary transcripts from the interrupted repeats (Franz & Kunz, 1981; Glatzer, 1979; Jamrich & Miller, 1984; Kidd & Glover, 1981; Long & Dawid, 1979).

It has been suggested that the D. melanogaster Type I and Type II insertion sequences are transposable elements (Glover, 1981; Dawid et

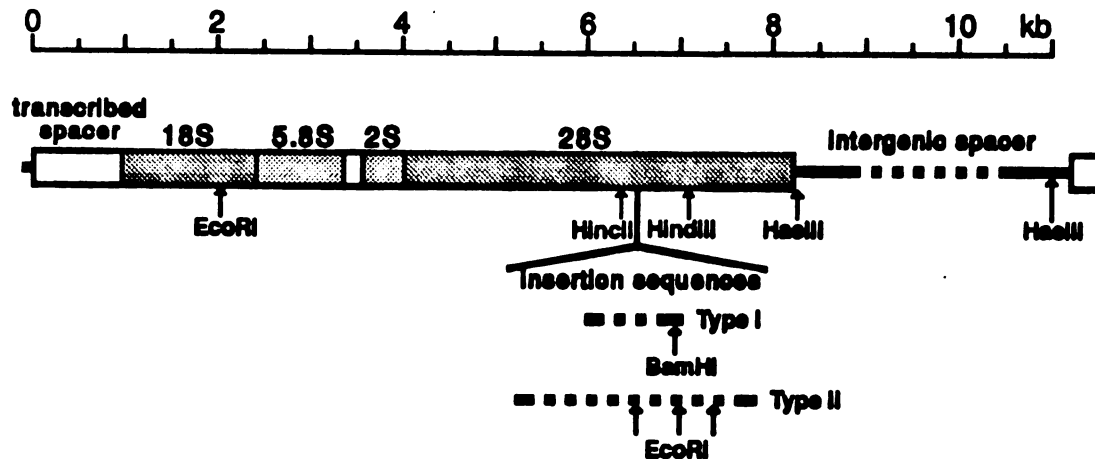


Figure 4 - Map of a single rDNA repeat unit

The NO contains approximately 225 rDNA repeats or cistrons (Tartof, 1971). A single cistron is shown schematically, with the transcribed sequences in boxes. There are coding regions for four species of rRNA, and two transcribed spacers shown as open boxes (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 (or non-transcribed spacer) indicate the variable lengths of these regions. The modal lengths are approximately 5 kb for the IGS (Coen et al., 1982), 5 kb for Type I inserts and 3.2 for Type II inserts (Wellauer et al., 1978). All EcoRI restriction sites and relevant sites for other enzymes are shown.

al., 1981). In fact, based on several lines of evidence, both classes of elements appear to be "non-LTR retrotransposons" or "retroposons", elements which transpose via an RNA intermediate, but lack the characteristic long terminal repeats of retroviruses (Rogers, 1985; Xiong & Eickbush, 1988a). Firstly, Type I insertions are flanked by duplications or deletions of the 28S coding region (Dawid & Rebbert, 1981; Roiha & Glover, 1981; Roiha et al., 1981), a characteristic of many transposon insertion events. Secondly, Type I insertions are found as tandem arrays flanked by rDNA at multiple sites in the X heterochromatin and the 4th chromosome. Thirdly, Type II elements have a stretch of polyA at the 3' end and the shorter copies of each element found in the genome always appear to be truncated elements containing just the 3' ends. All these features suggest transposition via a complete or incompletely transcribed RNA intermediate. Lastly, each element has an ORF with regions of close homology to reverse transcriptase (Eickbush, personal communication).

Beyond this, little is known about the organization of this large gene family because it is located in heterochromatin. The heterochromatin makes up 40% of the length of the X chromosome, and all of the X (Hilliker et al., 1980). Nevertheless, there is very little recombination (0.02% for the X heterochromatin) (Schalet, 1972), no conventional genetic loci (Hilliker et al., 1980), and few cytological landmarks (Gatti et al., 1976). As a result, the bb locus, like the few other heterochromatic loci known, has been refractory to genetic analysis. The NO is also difficult to dissect with molecular techniques, because each repeat unit is at least 11 kb, preventing the

cloning of more than two or three in any given fragment using a conventional vector.

Although there is little meiotic recombination within the NO, there are unique mechanisms that maintain the number of intact rDNA cistrons in this large gene family. One such process is magnification (Ritossa, 1968), a heritable increase in rDNA copy number which takes place both pre-meiotically and meiotically in the male germ-line (Hawley and Tartof, 1985). Males with a bb X chromosome and one of several aberrant X chromosomes produce gametes with bb⁺ X chromosomes at a high frequency ranging up to 35%. Hawley et al. (1985) propose that pre-meiotic magnification involves extrachromosomal copies of the rDNA in the germline of magnifying males. These extrachromosomal copies form an amplified "onion-skin" structure, which is resolved by successive exchanges into a magnified bb locus. They suggest that meiotic magnification is the result of unequal sister-chromatid exchange. The evidence that the two processes occur by different mechanisms comes from the fact that various meiotic mutants can inhibit pre-meiotic magnification without affecting meiotic magnification. Furthermore, reduction (a heritable decrease in rDNA expression) is only seen as a meiotic event, presumably as the reciprocal result of uneven sister-chromatid exchange.

Another phenomenon, known as compensation also affects rDNA copy number. When a fly is missing one NO, there is an increase in the somatic copy number of rDNA cistrons apparently by amplification of the single NO present (Tartof, 1971). Compensation appears to be under the control of a locus known as cr (compensation response) (Procunier & Tartof, 1977). On the X chromosome, cr is located within the

heterochromatin distal to the NO. The location of the Y chromosome cr is not known. The X-linked cr locus acts in cis to trigger amplification of the adjacent bb locus, and acts in trans to signal presence of an NO. Although the Y chromosome cr can induce compensation in the X, the Y NO does not itself compensate.

Magnification and compensation are both intrachromosomal events and cannot explain another interesting feature of rDNA: despite the low frequency of exchange in the heterochromatin, there is striking homogeneity among the rDNA repeat units on the X and Y chromosomes, a phenomenon that has been termed "concerted evolution" (Tartof & Dawid, 1980; Zimmer et al., 1980; reviewed in Arnheim, 1983). Unequal reciprocal exchange does not appear to be sufficient to explain the pattern of homogeneity (Williams et al., 1989) and so the question remains as to what forces operate in the population to prevent excessive heterogeneity.

Areas of investigation - There are a number of questions that can be asked about Rex, and, more importantly, a number of questions about rDNA that Rex can be used to answer. In the subsequent chapters, various questions are addressed, using a common set of materials and methods described in Chapter 2:

- 1) Where is Rex located with respect to its target, the NO? In both Chapters 3 and 4, genetic experiments are used to map Rex with respect to both su(f) and the bb locus (Fig. 3). The results are that Rex maps within the rDNA. In addition, in Chapter 4, Rex-induced recombination and a novel molecular approach are used to map Rex with respect to molecular variants within the rDNA array.

2) What is the nature of the Rex locus? In Chapter 3 deletions and duplications of the proximal portion of the X chromosome are tested to see whether Rex is a hyper-,hypo- or neomorph. The results suggest that Rex is a neomorph.

3) Are there other loci which interact with Rex? In Chapter 3 several suppressors of Rex are described and mapped proximal to car. Two other Su(Rex) loci are mapped to the NO, one by genetic experiments (Chapter 3), and the other at the molecular level using the same approach taken for mapping of the Rex locus (Chapter 4).

4) What is the mechanism of the Rex exchange and is it generally applicable for large scale mapping of the NO? Both these questions are addressed in Chapter 4, where Rex-induced exchange events are analyzed on a molecular level. Rex exchange appears to be conventional, and is used to generate a map of molecular variants in the NO.

5) What is the nature of the Rex target? In Appendix A, a special chromosome with a single distal ribosomal repeat and a normal, proximal NO is tested as a target. However the chromosome appears to be unstable, making the experiment inconclusive.

6) Does Rex play a role in the homogenization of rDNA repeats on the X and Y chromosomes? In Appendix B, the rate of Rex-induced interchromosomal exchange is measured. Although the experiment is difficult to interpret, because one of the target chromosomes appears to be somewhat unstable, Rex does not induce X-Y exchange at anything approaching the rate of intrachromosomal exchange, and may not induce X-Y exchange at all.

7) How is Rex involved in magnification, an intrachromosomal exchange event specific to the rDNA? This question is not addressed

directly. In Appendix C, however, mei-41, a mutation which severely inhibits magnification in males, is found to have Rex-like activity.

8) What is the product of the Rex locus? In the Discussion, data from all the experiments are drawn together to suggest a working hypothesis for Rex action. I propose that Rex either encodes an endonuclease activity, or is a mutation that has activated the silent open reading frame of a retroposon which encodes an endonuclease. Several experiments are also proposed to test that hypothesis.

9) What implications do the study of Rex and its effect on the NO have and what are possible future lines of research? These questions are addressed in the Discussion.

CHAPTER 2

MATERIALS & METHODS

Stocks and Crosses - All matings were done on a cornmeal, molasses and brewer's yeast medium at 25°C. Five days after mating, parents were transferred to fresh food, and progeny were scored at regular intervals. Phenotypic markers and standard chromosomes are described in Lindsley & Grell (1968) and Lindsley & Zimm (1985, 1987). A variety of rearranged chromosomes were used extensively in this work, several of which were newly constructed. Their origins and relevant properties are listed in Table 1.

Measurement of Rex activity - Rex activity was detected as shown in Fig. 1. Briefly, maternal presence of Rex causes mitotic exchange between the two blocks of rDNA on a paternally-derived target chromosome, in this case an attached-XY. The standard tester chromosome used was $y^{Sc}X-Y^L, In(1)En, Y v f B-y^+$. The result is a change of XXY daughters into XY sons, by "detachment" of the Y chromosome from the X euchromatin. The event takes place in the first or second division of the zygote, generating whole-body "detachment" males or gynandromorphs, respectively, which can be identified because they have y^+ male tissue without any other paternal X-linked markers. Rex activity is calculated as: detachment progeny/(regular females + detachment progeny). There are some variations on this calculation in certain cases because of the

Table 1 - Description of chromosomes used in experiments

Chromosome	Relevant Properties	Ref.
Standard chromosomes		
<u>Df(1)R35</u>	Deficient for most of \bar{X} basal heterochromatin, including NO, and proximal euchromatic loci (Fig. 8)	(1)
<u>Df(1)X-1</u>	Deficient for most of basal \bar{X} heterochromatin, including NO, and proximal euchromatic loci (Fig. 8)	(1)
<u>Dp(1;1)sc^{VI}</u>	Duplicated material from the tip, including y^+ , to the \bar{X} centromere (Fig. 5)	(1)
<u>In(1)dl-49</u>	Middle third of \bar{X} euchromatin inverted used to balance inversions of entire \bar{X} euchromatin	(1)
<u>In(1)FM7</u>	Multiply inverted balancer of the \bar{X} chromosome	(1)
<u>In(1)sc^{4L}sc^{8R}</u>	Deficient for middle 3/4 of \bar{X} basal heterochromatin including NO (Fig. 8)	(1)
<u>In(1)sc^{S1L}sc^{4R}</u>	Duplicated for middle 3/4 of \bar{X} basal heterochromatin, including NO (Fig. 8)	(1)
<u>In(1)w^{m4L}w^{m51bR}</u>	Deficient for NO (Fig. 11)	(1)
<u>In(1)w^{m51bL}w^{m4R}</u>	Duplicated for NO	(1)
<u>T(1;Y)B34,Xy^S</u>	One piece of \bar{X} -Y translocation, includes all basal \bar{X} heterochromatin (Fig. 8)	(1)
<u>y^{Sx}·y^L, In(1)En</u>	\bar{X} chromosome inserted between two arms of Y (Fig. 1)	(2)
Novel chromosomes		
<u>Dp(1;1)w^{m51bwm4}·23</u>	Non-inverted chromosome duplicated for NO at tip free of surrounding heterochromatin. Generated by <u>Rex</u> -induced hairpin exchange in <u>In(1)w^{m51bL}w^{m4R}</u>	(3)
<u>Dp(1;1)w^{m51b}Rex^R</u>	Target chromosome for <u>Rex</u> -induced hairpin exchange with <u>Rex</u> -bearing basal heterochromatin, and duplicated for the NO at the tip (Fig. 10)	(4)
<u>In(1)w^{m51bL}w^{m4R}·#</u>	Inverted chromosomes generated by <u>Rex</u> -induced hairpin exchange in <u>Dp(1;1)w^{m51bL}Rex^R</u> (Fig. 10)	(4)

Table 1 (cont'd)

<u>In(1)w^{m51b},#</u>	Distal end of <u>In(1)w^{m51bL,m4R},#</u> , including distal NO (Fig. 11)	(4)
<u>In(1)w^{m4},#</u>	Proximal end of <u>In(1)w^{m51bL,m4R},#</u> , including proximal NO (Fig. 11)	(4)
<u>Tp(1;1)NO.23L</u>	Distal portion of <u>Dp(1;1)w^{m51bwm4}.23</u> , with single NO at the tip free of surrounding heterochromatin, and base of <u>Df(1)X-1</u> .	(4)

- References: (1) Lindsley & Zimm, 1987
 (2) Lindsley & Grell, 1966
 (3) Robbins & Swanson, 1988
 (4) this study

use of different maternal genotypes. These modified calculations are noted both in the text and in the tables where relevant.

bb phenotype measurement - The **bb** phenotype, reflecting rDNA expression, was measured using either of two crosses. In both assays, the **bb** phenotype was evaluated in In(1)sc⁴sc⁸ heterozygotes since In(1)sc⁴sc⁸ is completely deficient for rDNA. Either individual males bearing the chromosome of interest were crossed to individual In(1)dl-49,y ct v B/In(1)sc⁴sc⁸,y daughters or individual females who were chromosome/FM7 were crossed to In(1)sc⁴sc⁸,y/y-tyb^S males. In both cases, the **bb** phenotype was expressed as penetrance, the percentage of chromosome/In(1)sc⁴sc⁸,y daughters showing any level of **bb** phenotype [(phenotypically **bb**)(100)/total].

Rate of non-disjunction - The rate of non-disjunction in all crosses is calculated as:

$$\frac{(2 \times \text{non-disjunctional progeny})}{\text{regular progeny} + \text{detachment progeny} + (2 \times \text{non-disjunctional progeny})}.$$

DNA extractions - To analyze a particular **bb** locus, X/0 males, homozygous females or X/bb⁻ females were used. X/0 males were generated by crossing X/Y males to C(1)RM,y y/0 females en masse. At least five individual sons were checked for fertility from each mating to ensure that no free Y chromosome was segregating.

Total Drosophila melanogaster genomic DNA was prepared by a modification of the procedure of Bender et al. (1983). Flies were stored frozen at -70°C. 50-100 flies were ground quickly at room temperature in a ground-glass homogenizer in 2 mls of grinding buffer

(0.1M NaCl; 0.2M sucrose; 50mM Na₂EDTA; 0.1M Tris, pH9.1; adding 0.5% SDS and 1% dimethyl pyrocarbonate just before grinding). The extract was incubated at 65°C for 30 minutes, then 0.3 ml of 8M KOAc was added, followed by incubation on ice for 30 minutes. The extract was centrifuged for 10 minutes at 10,000 x g and the supernatant was carefully drawn off. One volume of ethanol was added, the extract was mixed and allowed to stand for 5 minutes at room temperature, and then centrifuged for 5 minutes at 10,000 x g. The pellet was washed with 80% ethanol and dried under vacuum. After dissolving the pellet in an appropriate buffer, the DNA was further purified by passage through an Elu-Tip (Schleicher & Schuell) or NENSORB (New England Nuclear) column, according to the manufacturer's protocol. The final DNA pellet was redissolved in 0.1-0.4 ml of 10mM Tris, pH8.0; 1mM EDTA and the concentration was determined spectrophotometrically.

Plasmid DNA was prepared by a modification of the boiling mini-prep method as described in Maniatis et al. (1982). 1.5 mls of an overnight culture grown in LB were spun in an Eppendorf microfuge for 2 minutes and the bacterial pellet was resuspended by vortexing in 350 µl of STET buffer (50mM EDTA; 50mM Tris, pH8.0; 5% Triton X-100; 8% (w/v) sucrose). 25 µl of a fresh 10 mg/ml lysoyme solution was added the tube was capped and mixed gently. A hole was poked in the cap with a needle and the tube was placed in boiling water for 45 seconds. The tube was immediately centrifuged at room temperature for 10 minutes and placed on ice. 200 µl of the supernatant was removed to a fresh microfuge tube, 200 µl of isopropanol was added, and the tube was vortexed and placed on ice for 5 minutes. The DNA was pelleted by centrifugation at 4°C for 10 minutes and resuspended in 100 µl water.

Salmon sperm DNA (Type III, Sigma) was alkali sheared to fragments of approximately 300-1000 bp long. The DNA was dissolved to a concentration of 33 mg/ml in water at 65°C. One-tenth volume of 10N NaOH was added and the solution was boiled for 10 minutes and then cooled. Two volumes of 2M Tris-HCl were added and the pH of the resulting solution was checked to ensure that it was roughly 8.0.

Restriction digests and electrophoresis - Restriction digests were carried out in the supplier's recommended buffer, using approximately 10 units per microgram of genomic DNA. The digests were separated on agarose (Seakem, ME) gels ranging from 0.7-1.0% run at constant voltage (35-50 volts) in TBE (89mM Tris; 89mM boric acid; 2mM EDTA) (Maniatis et al., 1982).

Molecular analysis of rDNA - IGS length variants were visualized by digesting genomic DNA with HaeIII (Fig. 4) and probing with pDm103HH2, which is a HindIII-HaeIII clone of IGS sequence in pAT153 (Coen et al., 1982). The fraction of rDNA repeats interrupted by insertion sequences was assessed by digesting total genomic DNA with HincII and HindIII and probing with PA56 (Jakubczak & Eickbush, personal communication). pPA56 is a 307 bp BamHI-HindIII fragment from the right-hand junction of a Type I insert with the 28S coding region cloned in pUC18. It contains 30 bp of Type I insert sequence. When genomic DNA is digested with HincII and HindIII, all uninterrupted rDNA repeats will yield a 373 bp fragment homologous to PA56 that spans the insertion sites of both Type I and Type II elements (Fig. 4) (Roiha et al., 1981) homologous to PA56. Interrupted cistrons yield much larger fragments whose size depends on

the position of whatever HincII or HindIII site in the insert sequence is closest to the right end. The modal length Type I will produce a 3.0kb fragment, while the modal length Type II will produce a 1.2 kb fragment (Jakubczak & Eickbush, personal communication), however, heterogeneity among the insert classes yields a wide array of fragment sizes.

Probe labelling and hybridization - Probes were labelled using the oligonucleotide labelling method of Feinberg & Vogelstein (1982). Labelled probe was separated from unincorporated nucleotide by column chromatography using Sephadex G-50. For the IGS clone, the source of DNA was the entire plasmid clone. In the case of PA56, however, purified insert DNA was labelled. The plasmid was digested with BamHI/HindIII, the digest separated on a 0.8% mini-gel (with 1 μ g ethidium bromide). The DNA was visualized with long-wave UV and a channel was cut in front of the smaller (insert) band. A low-melting agarose patch (1.5% NuSieve) was put into the channel and the insert band was electrophoresed into the patch. The low-melting agarose patch was scooped out, weighed to determine its volume, and used as the source of DNA for labelling (Feinberg & Vogelstein, 1984).

Gels to be probed were Southern blotted onto Hybond-N (Amersham) nylon membranes according to manufacturer's specifications. The DNA was fixed onto the membrane by 3 minutes exposure on a UV transilluminator (Fotodyne 3-3000, 300 nm). Pre-hybridization was carried out overnight at 42°C 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) (2.5X pre-hybridization stock=250mM Pipes; 2M

NaCl; 0.5% sarkosyl; 0.25% Ficoll; 0.25% PVP-40; 0.25% BSA, fraction V; pH 6.8). Hybridization was carried out for 18-24 hours at 42°C in heat-sealed bags with 10^6 dpm/ml of probe in hybridization mix (1X pre-hybridization stock; 40% formamide; 0.2 mg/ml alkali-sheared salmon sperm DNA; 10% dextran sulfate).

After hybridization, the filters were washed twice with manual rubbing in 2X SSC + 0.05% sarkosyl + 0.02% sodium pyrophosphate at room temperature, followed by three or four 30 minute washes at 50°C in 0.1X SSC + 0.05% sarkosyl + 0.02% sodium pyrophosphate. The filters were wrapped in Saran Wrap to prevent drying and autoradiographed, usually without an intensifying screen in order to enhance the resolution of the bands.

CHAPTER 3

REX IS A NEOMORPHIC LOCUS IN THE HETEROCHROMATIN

Introduction

In order to further understand Rex, two related questions were asked. The first question was where does Rex map? Preliminary mapping data placed Rex proximal to car on the X chromosome (Fig. 3), either in the 5% of X euchromatin between car and su(f), or in the heterochromatin (Robbins, 1981). There are very few heterochromatic loci on the X chromosome; the best-characterized is the bb locus encoding the 18S and 28S ribosomal RNA (reviewed in Hilliker et al., 1980). Fortunately, the chromosome which carries the Rex locus also carries a strong bb mutation. This allowed mapping of Rex with respect to bb and to su(f), the most proximal euchromatic locus. The mapping data showed that Rex is, in fact, proximal to su(f).

The second question was whether Rex behaves as a loss of function, gain of function or novel function mutation. Conventionally, mutations are classified according to the scheme first described by Muller (1932). A mutation is either an amorph, with no expression of the normal function; a hypomorph, with lower than normal expression; a hypermorph, with greater than normal expression; a neomorph, with a novel function; or an antimorph, with expression exactly opposite of normal. Three of the classes, moderate hypermorph, neomorph and hypomorph/amorph, can be

distinguished by a series of dosage tests, using complete deficiencies and duplications of the region where the mutation is located (Table 2).

Rex was initially isolated on a bb chromosome, raising the possibility that Rex is actually a bb allele, a loss of function of some ribosomal cistrons. Tests of deficiencies and duplications of the proximal heterochromatin were used to test whether Rex produces a loss or gain of function, or is a novel function. The results demonstrate that Rex is a neomorph.

In the course of these studies, several suppressors of Rex were also identified. Two were mapped to the proximal portion of the X chromosome. A third was mapped to the rDNA itself, and, the results of the dosage experiments show that this locus, too, is a neomorph.

Results

Mapping of Rex with respect to su(f) - Spontaneous crossovers between su(f) and the centromere of a Rex chromosome were recovered from among the progeny of $y \text{ cv } y \text{ f } \underline{Rex}/\underline{Dp(1;1)sc^{VI}}, y \text{ lz f } \underline{car} \text{ su(f)} \cdot y^{+}$ females by looking for phenotypically $y \text{ f}^{+}$ sons (Fig. 5). If Rex is proximal to su(f), at least some of the recombinants should contain Rex. If Rex is distal to su(f), none of the recombinants are expected to have Rex.

Two independent recombinants (from different matings) were recovered among approximately 15,000 male progeny screened (0.01%). Each recombinant was outcrossed twice to C(1)DX,y f/Y females, to reduce the effect of autosomal background, and then tested for Rex and bb phenotype. The results are shown in Tables 3 and 4.

It is apparent that one recombinant, Line 31, is similar to the original Rex chromosome both in terms of bb phenotype and Rex activity.

Table 2 - Use of duplications and deficiencies to classify mutation

<u>Type of mutation</u>	<u>name</u>	<u>mimicked by</u>	<u>suppressed by</u>
Loss of function	amorph/hypomorph	Deficiency/+	Duplication/mutant
Gain of function	hypermorph	Duplication/+	Deficiency/mutant
Novel function	neomorph	-	-

Table 3 - Rex activity of parental and recombinant chromosomes

CHROMOSOME	REGULAR PROGENY		NON-DISJUNCTIONAL PROGENY			DETACHMENT PROGENY gynandro-		
	female	male	female	male	percent	males	morphs	Percent
PARENTAL CHROMOSOMES								
y <u>cy</u> y f Rex	1818	3431	4	7	0.41	79	23	5.31
<u>Dp(1:1)sc^{VI}</u> y lz f car su(f)+y [±]	1970	943	0	0	0	0	0	0
RECOMBINANT CHROMOSOMES								
Line 27 y lz f car su(f)	3460	3835	0	0	0	6	0	0.17
Line 31 y lz f car su(f)	2434	3529	3	23	0.86	38	5	2.25

Individual X-chromosome/y females were mated to y^{Sy+y^L}, In(1)En.y v f B+y[±] males. In the case of Dp(1:1)sc^{VI}, Rex activity was measured in Dp(1:1)sc^{VI}/y[±] cy y f car females.

Table 4 - bb phenotype of parental and recombinant chromosomes

Regular X-Chromosome	<u>Chromosome/sc⁴sc⁸</u>			sc ⁴ sc ⁸ /FM7	males
	bb ⁺	bb	bbb		
PARENTAL CHROMOSOMES					
y lz f <u>car</u> <u>Rex^a</u>	19	195	96%	233	213
Dp(1;1)sc ^{VI} ₁					
y lz f <u>car</u> <u>su(f)-y⁺</u>	173	3	2%	158	213
RECOMBINANT CHROMOSOMES					
Line 27					
y lz f <u>car</u> <u>su(f)</u>	150	25	14%	215	221
Line 31					
y lz f <u>car</u> <u>su(f)</u>	55	131	70%	228	223

Individual males carrying the indicated chromosome were mated to In(1)sc⁴sc⁸,y/FM7 females. The percent bb is calculated as bb sc⁴sc⁸ daughters/total sc⁴sc⁸ daughters.

^aThe parental y cv y f Rex chromosome was not tested for bb phenotype contemporaneously. Instead, the bb phenotype of the parental Rex chromosome was tested in a different recombinant, where the exchange event took place outside the heterochromatin, recovered from the same cross.

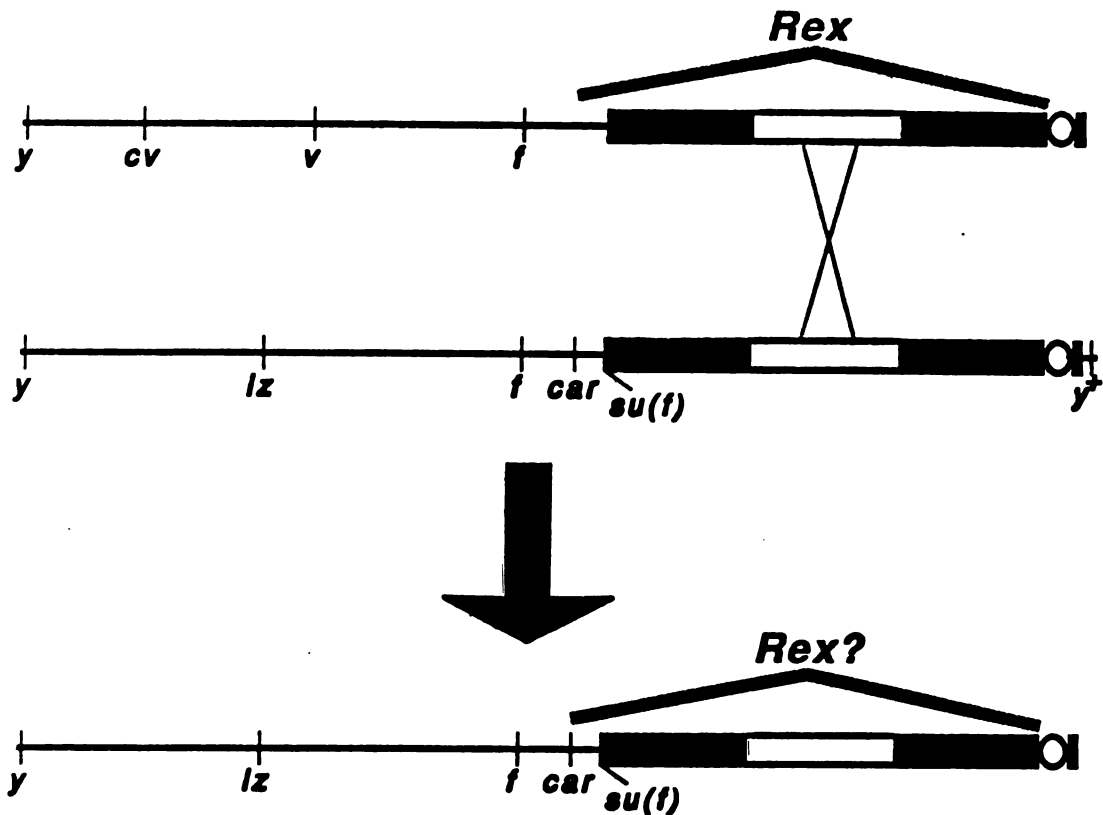


Figure 5 - Mapping of *Rex* with respect to proximal markers and the centromere

One parental chromosome, *Dp(1;1)sc^{VI}*, is duplicated for the tip of the *X* chromosome, including the *y⁺* body-color marker. The centromere and *y⁺* are inseparable by recombination. Recombination within the heterochromatin, between *su(f)* (suppressor of forked) and the centromere, produces *y lz f car su(f)* crossovers which are readily distinguished as phenotypically *y f⁺* sons who are used to establish stocks. The recombinant chromosomes can then be tested for *Rex* activity, *bb* phenotype and rDNA composition.

The other recombinant, Line 27, has significantly less Rex activity than the Rex parental chromosome, and is also significantly less bb. These results place Rex proximal to su(f), since both recombinants bear su(f) and have Rex activity.

Molecular analysis of recombinants - In order to determine where the exchange event took place with respect to the bb locus, the array of ribosomal intergenic spacer (IGS) lengths was examined in the parental and recombinant chromosomes (Materials & Methods, Fig. 4). HaeIII digested genomic DNA was probed with cloned IGS sequence, revealing a distinct array for each parental chromosome (Fig. 6). Line 27, which is intermediate in both Rex and bb phenotype between the two parental chromosomes, is clearly a recombinant within the rDNA array, since it contains bands from both parents (arrows). On the other hand, Line 31, which is phenotypically similar to the Rex parental chromosome, also has the same rDNA array as the Rex parental chromosome. The exchange event which generated Line 31 took place distal to all of the diagnostic variants within the rDNA which distinguish the two parental chromosomes, or possibly distal to the entire NO.

A Su(Rex) locus in Dp(1;1)so^{VI} - There were two possible explanations for the reduced Rex activity seen in Line 27. One was that Rex was split by the exchange event, yielding partial Rex activity. The other was that the other parental chromosome carried a suppressor of Rex locus which is present in Line 27. Maternal-effect dominant suppressors of Rex (Su(Rex) loci) have been found in many lab stocks (see below). This possibility was checked by testing

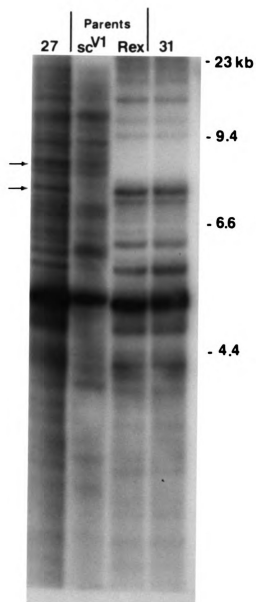


Figure 6 - IGS length arrays of parental and recombinant chromosomes
Genomic DNA was prepared from X/Y males bearing the indicated X chromosome and digested with HaeIII. The blot was probed with cloned IGS sequence (pDm103HH2). Line 27 contained variants from each of the parents, two of which are indicated by the arrows. Line 31 appears to be identical to the Rex parent.

y cv y f Rex/Dp(1;1)sc^{VI} y lz f car su(f)·y[±] females for Rex activity in a cross to attached-XY males. Note that only one half of detachment males (those which are y[±] f) will be identified in this cross, since the other half will be phenotypically y[±] f[±], and indistinguishable from one class of regular sons.

The results (0 detachments among 975 female progeny) clearly indicate that the Dp(1;1)sc^{VI} does, in fact, contain a Su(Rex) locus. Since Line 31 has normal Rex activity, the Su(Rex) locus must be proximal to the Line 31 exchange point in Dp(1;1)sc^{VI}, and thus is also located in the basal heterochromatin.

Line 27 was then tested for Su(Rex) by mating Line 27/y w spl cv y f Rex females to attached-XY males. The results (data not shown) indicate that Line 27 does, in fact, carry Su(Rex) in addition to some Rex activity.

The nature of the Rex locus - Having mapped Rex proximal to su(f), tests of duplications and deficiencies of the heterochromatin permit functional classification of Rex (Table 2). If a deficiency of the heterochromatin mimics Rex activity, then Rex must be a hypomorph or amorph. In that case, a duplication would be expected to suppress Rex activity. Conversely, if Rex is a moderate hypermorph, a duplication would mimic Rex, while a deficiency would suppress it. A neomorph would not be mimicked or suppressed by any change in dosage of the wild-type region. An extreme hypermorph, a mutant with many-fold increased activity, would behave as a neomorph in this test.

Three deficiencies and two duplications of the heterochromatin were tested for their ability to mimic or suppress Rex (Fig. 7). In these

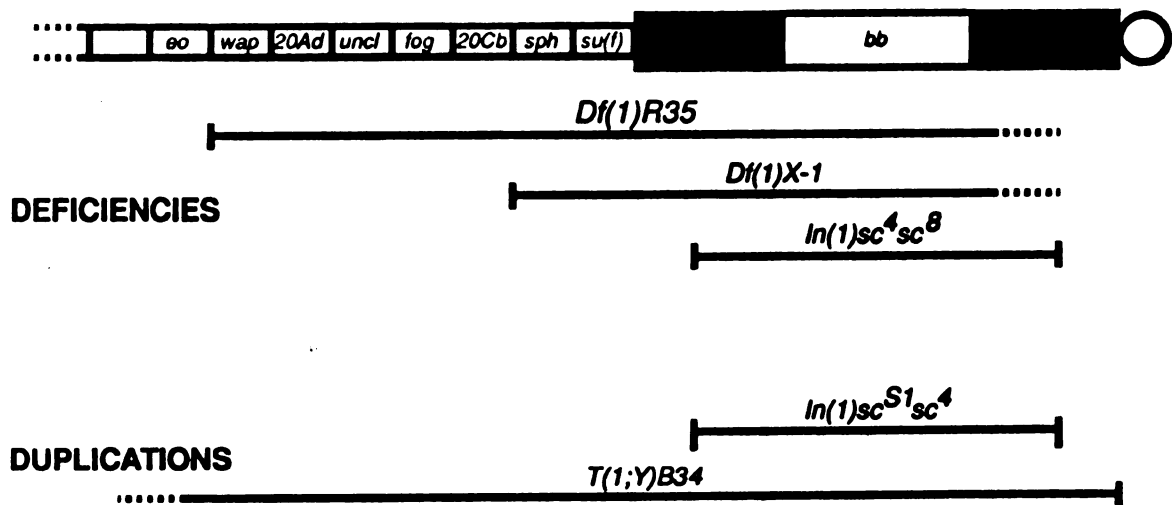


Figure 7 - Duplications and deficiencies used for functional classification of Rex

The proximal portion of the X chromosome is shown. The heterochromatin is shown as heavy lines and the NO as an open box within the heterochromatin. The eight most proximal euchromatic lethal loci are shown as small open boxes. The proximal and distal limits of each rearranged chromosome are shown. Dashed lines are used when the precise boundary is not known. (Lindsley & Zimm, 1987).

tests, Rex activity is evaluated qualitatively (present/absent), since previous experiments have shown that Rex activity is sensitive to background and quantitative comparisons among experiments may not be meaningful. The results (Table 5) indicate that Rex must be a neomorph or extreme hypermorph since none of the aberrations mimic Rex (in crosses which are aberration/y) and none suppress Rex (in crosses which are aberration/Rex). Rex activity is only found in Rex-bearing females, regardless of the dosage of wild-type material.

Multiple suppressors of Rex - The test for dominant suppressors of Rex is to test Rex/chromosome females for Rex activity (see Chapter 2). Females from the stock being tested are crossed to Rex males, or Rex/FM7 females are crossed to males from the stock being tested. Rex activity is quite variable, but usually Rex induces detachments in 1% or more of the target-bearing progeny. When a Su(Rex) is present, many fewer detachments are produced.

In order to map a Su(Rex), the first step is to map it to a chromosome. One way to do this is to see if it segregates from a particular marked balancer autosome. Another way is to see whether it cosegregates with the X chromosome when the autosomes from the stock are allowed to segregate at random. Once the Su(Rex) has been mapped to a particular chromosome, recombinants containing various portions of the Su(Rex) chromosome can be tested to see if they retain the Su(Rex) phenotype.

Su(Rex) in y ; SM1 ; TM2/T(2;3)S9,bw e ; sca^{pol}/sca^{pol} - This stock (abbreviated as "y etc.") has markers on each of the chromosomes, and

Table 5 - Results of dosage experiments

CHROMOSOME	REGULAR		NON-DISJUNCTIONAL			DETACHMENT PROGENY		
	PROGENY		PROGENY			gynandro-		
	female	male	female	male	percent	males	morphs	Percent
DEFICIENCIES								
<u>Df(1)X-1.y</u> <u>BC BC/Y</u>	4098	1397	3	2	0.1% ^a	0	0	0%
<u>Df(1)X-1.y</u> <u>BC BC/Rex</u>	646	477	0	4	0.5% ^a	14	3	5.0% ^a
<u>Df(1)R35.y</u> <u>lz f/Y</u>	3418	1965	0	3	0.1% ^a	0	0	0%
<u>Df(1)R35.y</u> <u>lz f/Rex</u>	1484	1022	6	11	0.9% ^a	39	9	6.1% ^a
<u>In(1)sc⁴sc⁸.y/y</u>	3569	3338	0	87	b	0	0	0%
<u>In(1)sc⁴sc⁸.y/Rex</u>	2477	2395	1	116	b	18	4	0.9-1.7% ^c
DUPLICATIONS								
<u>y/y/T(1;Y)B34.P^S</u>	1404	1781	17	3	1.2%	0	0	0%
<u>y/Rex/T(1;Y)B34.P^S</u>	1839	2340	7	18	1.2%	26	3	1.6%
<u>In(1)sc¹sc⁴.y y/y</u>	2177	1671	1	23	b	0	0	0%
<u>In(1)sc¹sc⁴.y y/Rex</u>	3311	2876	2	123	b	21	2	0.7%

Individual females of the indicated genotype were mated to $y^{Sc^4}y^L$, In(1)En.y y f B.y[±] males or to In(1)sc¹sc⁴.y y B/Y males (in crosses involving In(1)sc¹sc⁴ and In(1)sc⁴sc⁸). In all cases Rex refers to a y cv y f Rex chromosome. The data for In(1)sc⁴sc⁸ and In(1)sc¹sc⁴ were taken from Swanson (1984).

^aThe deficiency chromosomes are hemizygous lethal, so the detachment frequency is calculated by doubling the number of all detachments, including gynandromorphs, and then dividing by (regular females + numerator). Similarly, the frequency of non-disjunction was calculated by doubling the number of regular males and all detachment products in the denominator.

^bThe large number of exceptional males resulted in part from non-disjunction, and in part from four-strand double exchange events in the mother who is an inversion heterozygote.

Table 5 (cont'd)

^aDeficiency/fragment detachment males will not survive unless the y^+ X chromosome fragment generated has enough ribosomal cistrons to be viable. The first detachment frequency shown is calculated on the assumption that all the deficiency/fragment males survive, the second on the assumption that none survived. The results of Robbins (1981), and the recombinants recovered in Chapter 4, suggest that only a small portion of the detachment products are bb^1 , so the true frequency is an intermediate value.

has itself often been used for mapping. Initial experiments (W. Chen, unpublished data) mapped the suppressor in this stock to the X or 4th chromosomes, since it did not segregate with either the balancer autosomes or the translocation (Table 6).

A second experiment (Swanson, 1984) where SM1, TM2, and the fourth chromosome (marked with spa^{pol}) were followed individually, eliminated the possibility that the suppressor was on the fourth chromosome. Females from the "y etc." stock were mated to y males from a non-suppressing stock. SM1; TM2 (phenotypically Cy Ubx) daughters were then crossed to y² Df(1)w^{rJ1} sn Rex/w⁺ males. The resulting females were heterozygous for Rex and had various combinations of the "y etc." autosomes SM1, TM2, and spa^{pol}. The females were scored directly for SM1 and TM2, since they carry dominant markers. The presence of the fourth chromosome marker spa^{pol} was scored in the next generation when each female was mated to y^{Sx}·y^L, y v f B·y⁺/0; spa^{pol}/spa^{pol} males to test for Rex activity. As in the first experiment (Table 6; above), the suppressor did not map to SM1 or TM2 (data not shown). Furthermore, the suppressor did not segregate with the fourth chromosome. Females who were spa^{pol}/+ had 2211 regular daughters and 14 detachment progeny (0.6% Rex activity) while +/+ females had 2921 regular daughters and 15 detachment progeny (0.5% Rex activity). This ruled out an autosomal location for the suppressor. The low level of Rex activity observed in this experiment is also consistent with presence of an X-linked suppressor since one-half of the females would carry an X-linked suppressor and one-half would not.

This suppressor was mapped to the proximal portion of the X chromosome by generating recombinants with non-suppressing X chromosomes

Table 6 - Suppression of Rex activity

MATERNAL GENOTYPE	REGULAR		NON-DISJUNCTIONAL		DETACHMENT PROGENY	
	female	male	female	male	percent	males gynandro- percent
Y CV Y f Rex/Y	1818	3431	4	7	0.41	79 23 5.31
Y CV Y f Rex/Y : SM1/+; TM2/+; spa ^{pol} /+ ^a	3800	4276	2	0	0.05	2 0 0.05
Y CV Y f Rex/Y : T(2;3)S2, bw e/+; spa ^{pol} /+ ^a	4440	5096	2	3	0.10	0 0 0.00
Y CV Y f Rex/Y (Amherst) ^b	5257	5176	2	0	0.04	2 1 0.06

Females of the indicated genotype were mated to Y^{SX}·Y^L, In(1)En, Y Y f B·y[±] or Y^{SX}·Y^L, Y Y f B·y[±] males. Both percent non-disjunction and percent detachment (measuring Rex activity) are calculated as described in Chapter 2.

^aBoth of these genotypes were recovered from a single cross, Y CV Y f Rex/Y x Y/Y : SM1 : TM2/T(2;3)S2, bw e : spa^{pol}/spa^{pol} (W. Chen, unpublished data). SM1 and TM2 are multiply inverted balancers of the second and third chromosome respectively, and T(2;3)S2 is a reciprocal translocation involving the second and third chromosomes.

^bSummary of data from 10 independent lines

(Fig. 8), with the fourth chromosome, marked with spa^{Dol}, segregating at random among the recombinants. We used a chromosome with no euchromatic suppressors (see below), Dp(1;1)sc^{V1}, y cv y f car su(f)·y[†], to generate recombinants bearing distal euchromatin from a non-suppressing X chromosome (Fig. 8a). In this chromosome, the centromere is marked unambiguously by su(f) and y[†]. This is important when generating distal crossovers because a second crossover event in the proximal euchromatin would go undetected without centromere-linked markers. We did not use this chromosome as the source of a non-suppressing X-chromosome base (Fig. 8b), however, because it contains a suppressor in the heterochromatin (see below).

All recombinants that have the centromeric region of the suppressor X chromosome suppress Rex (Table 7). This Su(Rex) thus maps to the base of the X chromosome, and not to the 4th chromosome, or the 95% of the X euchromatin from y (0 map units) to car (62.5 map units). This was also demonstrated by the reciprocal experiment in which the proximal portion of the suppressor X chromosome was replaced with a segment from a non-suppressing chromosome by recombination (Fig. 8b). In all the resulting recombinants suppressor activity is lost (y car; Table 7).

Interestingly, however, there is a significant difference between one of the proximal recombinants (Line 4) and the 3 others ($\chi^2=29$, $p<0.001$), as if Line 4 carried a partial suppressor of Rex. Two generations of outcrossing did not relieve the partial suppression (data not shown). It was determined however, that Line 4 had a bb locus unlike that of either of the two parental chromosomes. The parental y² cv y f car chromosome was bb¹, and as expected, the other proximal

Figure 8 - Recombinants used to map X-linked suppressor in "y etc."

The X chromosome is shown schematically, with the heterochromatin outlined by heavy lines, and relative genetic positions of various markers indicated.

A: To replace the distal portion of the y etc. X chromosome with euchromatin from a non-suppressing stock, males from the y etc. stock (y/Y ; SM1 ; TM2/T(2;3)S9,bw e ; spa^{pol}/spa^{pol}) were crossed to Dp(1;1)sc^{V1}, y cy y f car su(f)·y⁺ homozygous females. F1 females were mated to y/Y males, and F2 males were scored for their X chromosome genotype. Individual F2 males with varying portions of the Dp(1;1)sc^{V1} chromosome replacing y etc. X euchromatin were used to establish stocks. In the y cy recombinant shown, approximately 33% of the euchromatin has been replaced.

B: The proximal portion of the y etc. X chromosome was replaced by crossing y etc. females to y² cy y f car/Y males. F1 females were mated to y/Y males. Individual y car and y f car recombinant F2 males were recovered and used to establish stocks.

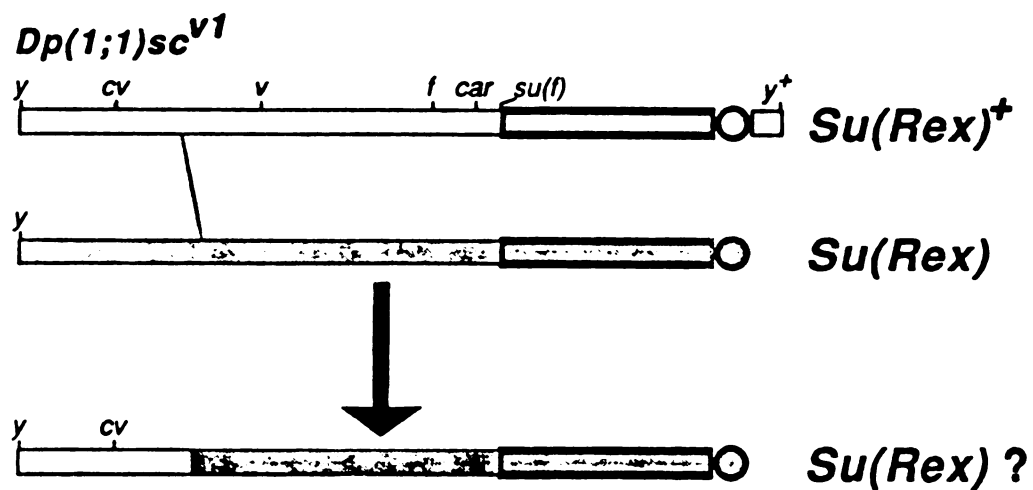
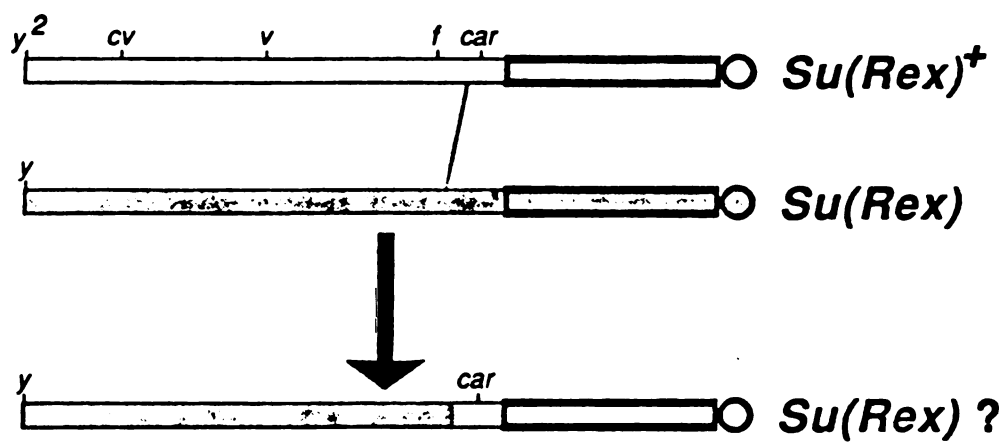
A**B****Figure 8**

Table 7 - Mapping suppressor in "y etc." X chromosome

X	NUMBER	REGULAR		NON-DISJUNCTIONAL			DETACHMENT PROGENY		
		PROGENY		PROGENY			gynandro-		
CHROMOSOME	TESTED	female	male	female	male	percent	males	morphs	Percent
Distal recombinants (Fig. 8a)									
y	3	1627	1444	1	1	0.13	1	0	0.06
y <u>cv</u>	2	603	695	0	2	0.31	0	0	0.00
y <u>cv</u> y	4	1704	1592	0	2	0.17	0	0	0.00
y <u>cv</u> y f	4	1169	1160	1	3	0.34	0	0	0.00
y <u>cv</u> y f <u>car</u>	3	677	686	0	1	0.15	0	0	0.00
Proximal recombinants (Fig. 8b)									
y f <u>car</u>	1	792	562	0	0	0	29	9	4.58
y <u>car</u>	3	1758	1269	0	7	0.45	81	16	5.23
y <u>car</u> , Line 4	1	859	927	1	0	0.11	8	0	0.92

Recombinant males with SM1, TM2 (Cy, Ubx) and various portions of the y etc. X chromosome were obtained as shown in Fig. 1, and stocked by mating to C(1)DX,y f attached-X females. Progeny which had wild-type autosomes (Cy⁺, Ubx⁺) were selected to maintain the stocks. Males from each stock were mated to y cv y f Rex/FM7 females (FM7 is an X chromosome balancer carrying the dominant marker B). Rex/recombinant (B⁺) daughters were mated to Y^SX·Y^L,In(1)En,y v f B·y⁺/0 males to measure Rex activity.

crossovers were bb^1 . The y etc. parental chromosome was bb^+ , and all of the distal crossovers were bb^+ as well. Line 4, however, which should also have been bb^1 were it a simple crossover, bears a 35% penetrant bb locus, intermediate between the two parental chromosomes. Clearly, Line 4 is not merely a euchromatic crossover, but has also undergone a magnification event, suggesting that the change in Line 4's Su(Rex) phenotype was concomitant with an alteration in its rDNA.

Su(Rex) in Amherst wild-type stock - The suppressor in the Amherst stock (Table 6) was mapped to the X chromosome in two experiments. Initially, several independent lines were generated with the Amherst X chromosome (re-marked with y) and an average of 1/4 Amherst autosomes. All of these suppressed Rex (pooled data shown in Table 6). One line was further outcrossed two times to attached- X (C(1)DX) females from a non-suppressing stock, in order to further dilute the Amherst autosomes. Ten males from the progeny of the second outcross were stocked individually with C(1)DX. These stocks contained the Amherst X chromosome, and various combinations of Amherst and non-Amherst autosomes. All the stocks retained Su(Rex) (data not shown). The probability that all ten stocks contained a particular Amherst autosome is $(1/2)^{10}$, so the suppressor appeared to be X -linked.

A recombinant was then constructed that contained the proximal portion of the Amherst X chromosome and the euchromatin of a non-suppressing chromosome (y lz f car Amherst). This recombinant still suppressed Rex (Table 8). The converse recombinant, with the euchromatin of the Amherst chromosome and the proximal portion of a non-suppressing chromosome (y Amherst car), lost suppressor activity (Table

Table 8 - Mapping suppressor in Amherst X chromosome

X CHROMOSOME	REGULAR PROGENY		NON-DISJUNCTIONAL PROGENY			DETACHMENT PROGENY gynandro-		
	female	male	female	male	percent	males	morphs	Percent
y lz f car Amherst	2213	2496	1	0	0.04	0	0	0.00
y ² Amherst car	2375	2757	4	6	0.39	22	4	1.08

Amherst females were mated to males from either of two non-suppressing stocks. The proximal portion of the Amherst X chromosome was recovered in a y lz f car recombinant son from Amherst/Dp(1;1)sc^{V1}, y sc^{V1} lz f car su(f)·y² females crossed to y/Y males. Most of the distal portion of the Amherst X chromosome was recovered in a y² car double-crossover son from Amherst/y² cv y f car mothers crossed to y/Y males. Recombinant males were mated to y cv y f Rex/FM7 females. The resulting recombinant/Rex daughters were mated individually to y²·y², In(1)En, y y f B·y⁺/Q males to test for suppression of Rex.

8). This suppressor is therefore in the proximal portion of the \bar{X} chromosome, in the heterochromatin or in the 5% of \bar{X} -euchromatin that is proximal to car.

Other Su(Rex) loci - Table 9 lists other stocks which contain suppressor activity and preliminary mapping data for some. The suppressor strength is measured as in Table 6. At least one suppressor (y cv y f car) appears to be autosomal (L.G. Robbins, unpublished data). Other suppressors cannot be readily mapped (In(1)dl-49,y ct y B and FM7) because they are in inverted chromosomes.

Discussion

These experiments lead to two conclusions about Rex. One is that Rex is within the proximal heterochromatin of the \bar{X} chromosome and the other is that Rex produces a novel function (a neomorph) or is a extreme hypermorph (with many-fold higher expression of wild-type function). We can further localize Rex by analyzing Line 27. The exchange that generated Line 27 was clearly in the rDNA because Line 27 contains diagnostic IGS variants from both parents. Since Line 27 also has partial Rex activity, at least some Rex sequences must lie proximal to the exchange point. Because Line 27 has a Su(Rex), we cannot determine if Rex is a repeated element split by this exchange, or is entirely proximal to the exchange. In any case, Rex is either within the rDNA or entirely proximal to it, because if Rex were distal to the rDNA, Line 27 would have no Rex activity.

Some other interesting results have also emerged. One is that there is more than one chromosome with a suppressor of Rex. Although

Table 9 - Other suppressors of Rex

<u>X Chromosome Stock</u>	<u>Detachment</u>	<u>Location</u>
FM7/ <u>un Rex</u> ^{2a}	0.12	FM7
<u>y cv y f car</u> ^b	0.07	autosomal
<u>y w^a ct⁶ m f</u>	0.24	?
<u>y w</u>	0.10	proximal to <u>car</u> on <u>X</u>
<u>In(1)dl-49.y ct y B</u>	0.08	?

Several other X chromosome stocks have been found to carry dominant suppressors of Rex. Some of the loci have been mapped, and the results are shown in the last column. Other X chromosome stocks have been tested, and do not suppress Rex, including:

y
In(1)dl-49.y w lz^S
Df(1)X-1.y ac sc
Df(1)R42.y lz f
In(1)sc⁴sc⁸.y^a
y w spl

^aSwanson, 1984

^bL.G. Robbins, unpublished data

most suppressors are X-linked, an autosomal Su(Rex) has also been found. Several of the X-linked Su(Rex) loci have been mapped to the base of the chromosome, proximal to car. Of most interest, several map to the basal heterochromatin.

Based on the molecular data, it is likely that the Su(Rex) in Do(1;1)sc^{VI} is actually within the NO. Line 31, where the exchange was either distal to the rDNA or distal to all diagnostic variants in the rDNA, lacks this Su(Rex), while Line 27, where the exchange was within the rDNA, has it.

Having thus mapped Su(Rex) to the proximal heterochromatin, we can ask about the nature of Su(Rex). If Su(Rex) was a hypomorph/amorph it would be mimicked by a deficiency. But none of the deficiencies tested (Table 5) suppress Rex. Alternatively, if Su(rex) was a hypermorph, it would be mimicked by a duplication. Neither of the duplications tested suppressed Rex (Table 5). Therefore, we can conclude that Su(Rex), like Rex itself, is also a neomorph or an extreme severe hypermorph.

Finally, there remains the question of locating these two loci more precisely with respect to the NO. Are one or both actually within the rDNA? If so, this suggests that Rex is part of a multi-element system, located in, and affected and suppressed by different bb loci. The experiments described in the next chapter address whether both Rex and Su(Rex) are actually within the rDNA.

CHAPTER 4

MAPPING OF REX AND SU(REX) WITHIN THE bb LOCUS

INTRODUCTION

Both the genetic and molecular data presented in the previous chapter suggested that Rex and at least one Su(Rex) are located within the bb locus. How can these loci be mapped more precisely? Are they within the rDNA or at one end of the rDNA array? Are these loci composed of repeated elements, divisible by recombination, like the rDNA itself?

Two novel techniques were adopted to address these points. First, to recover more exchanges within the rDNA, Rex itself was used to induce recombination in a Rex-bearing target chromosome. The rate of spontaneous exchange in the heterochromatin was approximately 0.01%; Rex activity varies considerably but generally induces rDNA recombination at least 100 times more frequently. Rex-induced "hairpin" exchange also allows the recovery of both products of each exchange event, the mitotic equivalent of a full tetrad.

Secondly, the exchange events were analyzed molecularly, using a new technique, recently developed by Williams et al. (submitted for publication). The technique uses the polymorphic length variants of the rDNA intergenic spacer (IGS) as genetic markers. Each distinct length variant class is dispersed differently throughout the rDNA. Some IGS spacer length classes are clustered, while others are dispersed over

most of the rDNA. Each exchange breakpoint is mapped with respect to the length variants of the IGS, generating a map of the limits of each IGS length variant within the rDNA. The Rex phenotype can then be positioned with respect to the IGS variants.

The results of these experiments demonstrated that Rex is a mappable function with discrete limits within the rDNA. Somewhat inadvertently, a Su(Rex) locus was also mapped to a discrete position within the rDNA, and was shown to be divisible by recombination.

EXPERIMENTAL DESIGN

The first step in generating exchanges is to recover an appropriate target chromosome by a single crossover between a Rex-bearing chromosome and Dp(1;1)_{w^{m51b}}_{w^{m4}},23 (Fig. 9). The Dp chromosome is a regular sequence chromosome duplicated for the rDNA, which was generated by Rex-induced "hairpin" exchange of In(1)_{w^{m51b}}_{w^{m4R}} (Robbins & Swanson, 1988). The resulting target, Dp(1;1)23^{L_{Rex}R}_{car}, has a distal nucleolus organizer, devoid of other heterochromatin, and structurally normal, Rex-bearing, centromeric heterochromatin (Fig. 10).

Males with this target chromosome are mated to y cv y f Rex/y females. In some of the progeny the target chromosome will undergo spiral or hairpin exchanges (Fig. 2) between the two bb loci. Spiral exchanges delete the material between the two NO's, yielding X-fragment chromosomes. Progeny containing such chromosomes are readily detected as sterile y⁺ car⁺ X/X-fragment males. Hairpin exchange inverts the material between the two NO's, in this case, converting a Dp(1;1)23^{L_{Rex}R}_{car} chromosome back into an In(1)_{w^{m51b}}_{w^{m4R}} chromosome (Fig. 10). The progeny containing hairpin exchanges are phenotypically

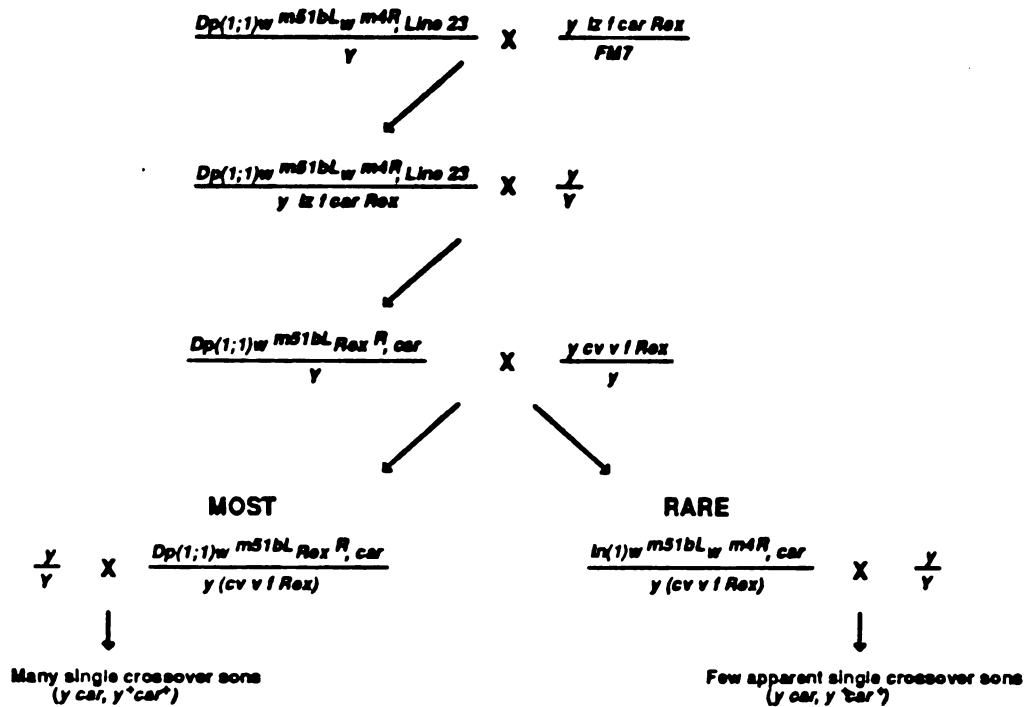


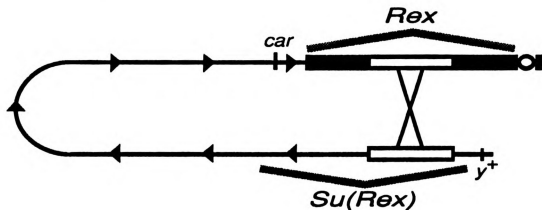
Figure 9 - Mating scheme for recovery of Rex-mediated inversions of a Rex-bearing target chromosome

The first three steps generated the appropriate Rex-bearing target chromosome with a duplication of the rDNA at the tip. This chromosome was stocked (not shown) and then target-bearing males were mated to Rex females. As shown, the regular daughters of this mating carried either the uninverted target or a target which has undergone Rex-mediated inversion of the material between the two NO's. Because the two X chromosomes of the mother in generation 3 recombined freely, the generation 4 daughters had various combinations of the markers cy, y, f and Rex, but in all cases had one $y \ car^+$ and one $y^+ \ car$ X chromosome allowing detection of single-crossovers over nearly the entire length of the chromosome. They were individually mated either to the y males shown or to $y \ cy \ y \ f \ Rex$ or $y^2 \ cy \ y \ f \ car$ males.

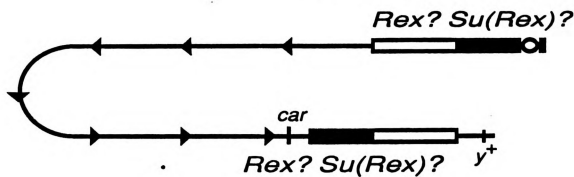
Figure 10 - Rex-induced hairpin exchange in a target chromosome containing both Rex and Su(Rex)

The effect of the hairpin exchange is to invert all the material between the two NO's. The effect of the exchange event on the positions of Rex and Su(Rex) depends on their locations with respect to each NO. For example, if Su(Rex) is distal to the NO at the tip, near y^t , its position will not be altered by the exchange events. Alternatively, if it is within the NO, some exchanges will move Su(Rex) to the proximal end, while others do not affect its position. Clearly, if either Rex or Su(Rex) is a repeated element within the NO, it is possible that an exchange event will split them, giving activity at both recombinant ends.

$Dp(1;1)_w^{m51bL} \text{Rex}^R, \text{car}$



Rex - induced
mitotic exchange



$ln(1)_w^{m51bL} \text{W}^{m4R}, \text{car}$

Figure 10

identical to regular females. The only difference is that these daughters are inversion heterozygotes for virtually the entire X euchromatin. Thus, to detect hairpin products the frequency of crossovers between y and car is measured in each daughter individually. A female bearing a hairpin exchange chromosome will have markedly reduced recombination because of the absence of single crossovers.

Once the inverted exchange products (designated In(1)w^{m51bL}w^{m4R},#,car) are recovered and stocked, the two recombinant bb loci on the chromosome are separated by single crossovers with In(1)w^{m4L}w^{m51bR},y ct m f which contains virtually no rDNA (Appels & Hilliker, 1982) (Fig. 11). The chromosomes bearing the separated NO's are referred to as "recombinant #-distal" and "recombinant #-proximal", in order to indicate the source of the rDNA. The ends of the parental Dp(1;1)23^LRex^R,car chromosome, which itself is not inverted, are separated by single crossovers with Df(1)X-1,y ac sc f Bx, which is deleted for most of the basal heterochromatin including all of the rDNA and two proximal euchromatic lethals.

The next step is to measure Rex activity and bb phenotype of each end with the standard assays (Chapter 2). Then DNA of each end is analyzed for IGS length polymorphisms (HaeIII digest probed with cloned IGS) and insert sequences (HincII/HindIII digest probed with PA56) (see Chapter 2). The HaeIII blots are scored for the presence of each IGS length variant unique to one or the other NO of the parental target chromosome. To avoid ambiguity, only easily differentiated variants are used.

Maps of the rDNA are then be generated by a procedure similar to that described in Williams et al. (submitted). When approaching this

Figure 11 - Separation of recombinant NO's of hairpin exchange products

The inverted products of hairpin exchange events have two recombinant NO's. In order to study each individually, the two ends were separated by recombination with a chromosome nearly devoid of rDNA, In(1)w^{m4L}w^{m51bR} (Appels & Hilliker, 1982). Males containing the hairpin exchange product were mated to In(1)w^{m4L}w^{m51bR}, y ct m f / In(1)dl-49, y ct v B females. The resulting In(1)w^{m4L}w^{m51bR} / In(1)w^{m51bL}w^{m4R} daughters were then mated to y/Y males. Three y⁺ ct car sons containing the distal NO and three y m f sons containing the proximal NO were recovered and used to establish stocks. All three stocks of each NO were tested for Rex, Su(Rex) and bb phenotype.

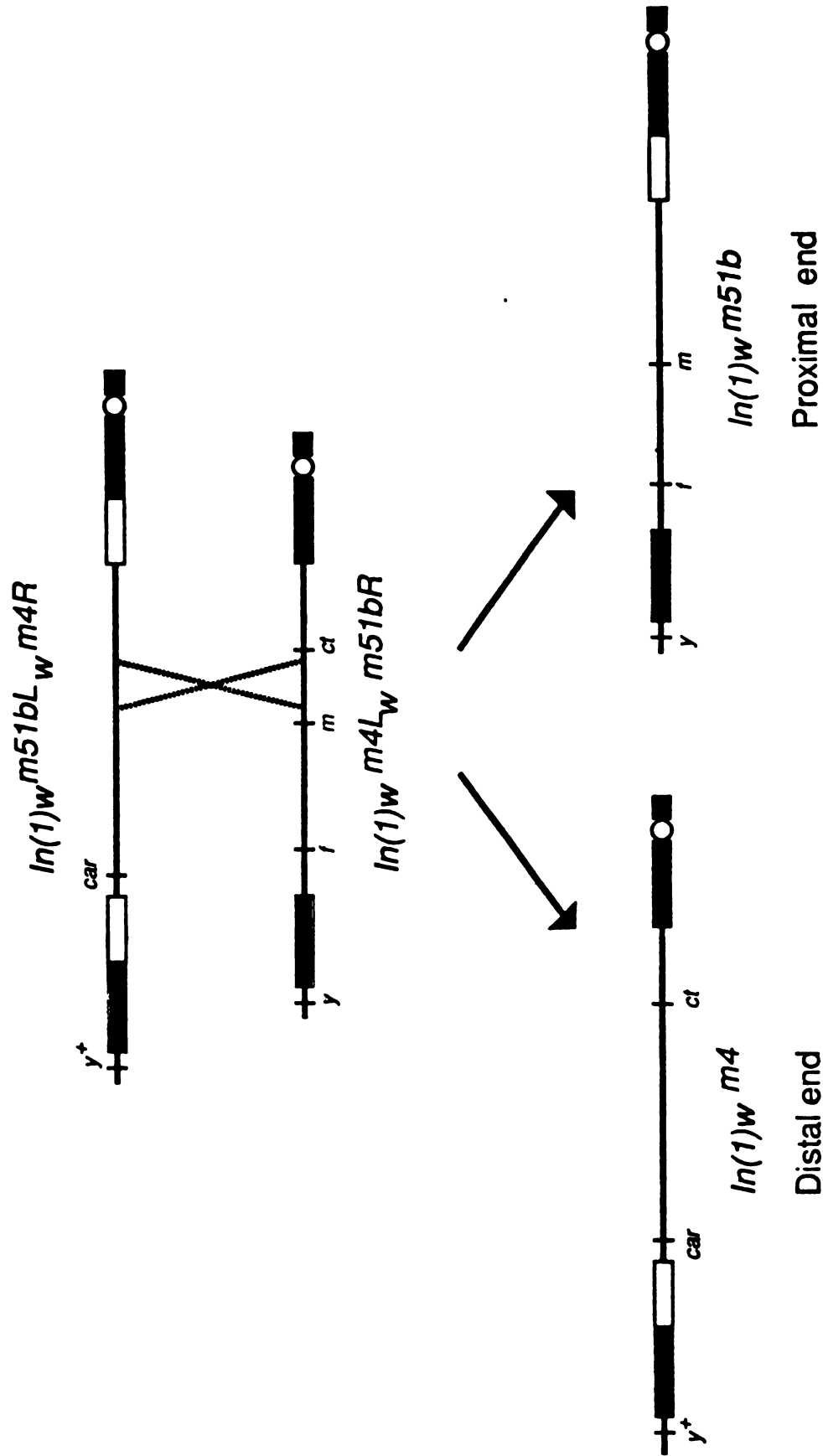


Figure 11

analysis, however, it is important to note that when hairpin exchanges are used each recombinant end has variants from both of the original parental hb loci. Furthermore, unless deletions were generated by the exchange event, all of the original parental variants should be present in one or both recombinant ends.

A diagram of the procedure is shown in Fig. 12, which outlines the procedure using a simplified array. A detailed example of this mapping procedure, using data for one of the IGS length variants, is described in the Results section.

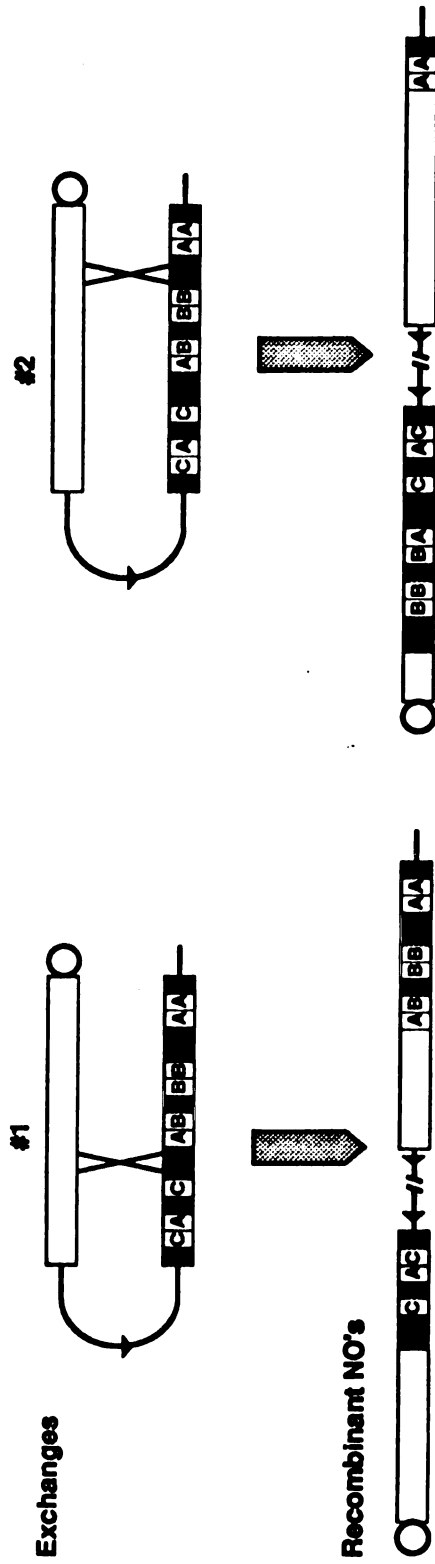
In Figure 12, three length variant classes are shown in the distal parental NO. The four copies of a are widely dispersed throughout the rDNA, while both b and c are clustered. First, exchange points in the distal parental rDNA are ordered according to the number of parental distal-end variants remaining in each recombinant's distal NO. For example, if all the distal-end variants are still present in a recombinant's distal rDNA array, that exchange point is proximal to at least one copy of each of the variants. After ordering the exchange points, the distal limits of these variants was determined. That is, if the distal end of a recombinant lacks a variant that was originally in the parental distal NO, the exchange must have taken place distal to all copies of that length variant. Proceeding from the telomere, the last exchange event that removes all of a given variant defines the distal limit of its distribution. The same logic applies to ordering the exchange points in the proximal parental NO and finding the proximal limit of each variant by use of the proximal rDNA arrays of the recombinants.

Figure 12 - Molecular mapping of rDNA array with IGS length variants

Two hairpin exchanges in the same target chromosome are shown schematically. For simplicity only three IGS length variants are shown, all in the distal parental NO.

First, the two exchange events are ordered with respect to each other within the distal parental NO. Looking at the distal rDNA arrays of the recombinants, #2 clearly took place distal to #1 because fewer of the variants remain at the distal end (only a in #2, while #1 has a and b). These exchange points are then used to map the distal limit of the variants' distributions. Note that the distal limit of a is beyond these two exchange points, since both retain some copies of a in their distal NO's. Exchange event #2 moves all copies of b to the other NO, but exchange #1 does not. Proceeding from the telomere, #2 defines the distal limit of variant b since it is the last exchange event to move all copies of b. Both exchange events moved all copies of c to the proximal NO. Therefore, #1 defines the distal limit of variant c, since it is the last exchange event to move all copies of c.

The next step is determining the order of the two exchange events with respect to each other by looking at the proximal NO's of the recombinants. Again, within the distal parental NO, #1 must be more proximal since fewer variants have been moved to its proximal NO (only a and c in #1, while #2 has all three). The ordered exchange points are then used to map the proximal limit of the variants' distributions. The proximal limits of neither a nor c are defined by these two exchanges, since a and c were moved to the other NO by both exchanges. However, exchange #2 moved b to the other NO, while exchange #1 did not move any copies of b. Proceeding from the centromere, #1 defines the proximal limit of b since it is the last exchange event that does not move any copies of b.



RECOMBINANT NO	A	VARIANT B	C
DISTAL NO 1	+	+	-
2	+	-	-
PROXIMAL NO 1	+	-	+
2	+	+	-

Data

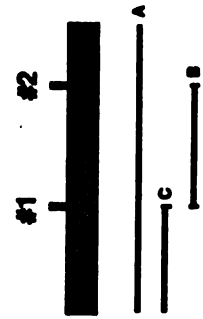


Figure 12

The next step is to determine the other limit of each variant's distribution. To determine the proximal limit of a variant unique to the parental chromosome's distal NO, the proximal recombinant ends are scored for variants that have been moved from the distal parental NO. Once again, the exchange points are ordered by the number of originally distal variants present. The most distal exchange point is that which moves all the distal NO variants to the proximal end of a recombinant. Similarly, if a recombinant's proximal end lacks a particular distal variant, the exchange event must have taken place proximal to all copies of that variant. Thus, proceeding from the centromere, the last exchange lacking a variant from the distal parental rDNA defines the proximal limit of that variant. Once again, a parallel process is used to map the distal limits of variants which are unique to the target chromosome's proximal NO.

Finally, the two sets of exchange point maps are compared. For example, there are two maps of exchanges within the distal parental end: one generated by analysis of the distal end variants remaining in recombinant distal ends and one generated from distal end variants moved to the proximal recombinant ends. Each map has the exchange points in a particular relative order from telomere to centromere. The two maps can be compared to see if the exchange points are in the same relative order. If they are, that indicates that the exchanges were simple single exchanges. Differences in the relative order would indicate that there had been rearrangements during the exchange events.

RESULTS

Verification of the content of the Rex-bearing target chromosome - After construction of the $Dp(1;1)23^{L_{Rex}R}_{car}$ target chromosome, it was essential to show that it did indeed function as a target for Rex activity. Males bearing this target chromosome were mated to Rex females. The results are shown in Table 10. $Dp(1;1)23^{L_{Rex}R}_{car}$ is a target, though a relatively poor one with only 0.14% detachment frequency. This rate is low even when compared with the original $Dp(1;1)w^{m51b,m4}_{m4},23$ chromosome which was used as the source of the distal end (0.40%). It was also important to show that the proximal end of the target had retained normal Rex activity. The ends of the target chromosome were separated and females bearing the proximal NO were mated to males bearing an attached-XY tester chromosome (Table 13, Line 2). Clearly, the proximal NO retained Rex activity since it induced detachments at a high rate. The separated ends of the target chromosome were also tested for the ability to suppress the phenotype of a standard Rex chromosome. The distal end was found to have a Su(Rex) (Table 14, Line 1).

Recovery of recombinant $In(1)w^{m51bL,m4R}_{m4R},car$ chromosomes - The mating scheme (see above and Fig. 9) allowed the daughters to be tested directly for y car recombination without a stocking step. Complete progenies were not counted. Instead, the females were judged non-recombinant, and discarded, whenever there were enough crossover products to indicate that the data were outside the 99% (cumulative binomial) confidence limit for an inversion heterozygote. For each

TABLE 10 - SENSITIVITY OF TARGET CHROMOSOMES

TARGET CHROMOSOME	REGULAR PROGENY		NON-DISJUNCTIONAL PROGENY			DETACHMENT PROGENY		
	female	male	female	male	percent	males	morphs	Percent
<u>Dp(1:1)w^{m51b}md₂₃</u>	1998	1717	1	7	0.4%	4	4	0.45%
<u>Dp(1:1)w^{m51b}Rev⁸</u>	2828	2816	2	5	0.3%	3	2	0.14%

y cv y f Rex/y females were mated to males bearing the indicated target chromosome. putative recombinant, several non-crossover y⁺ males were used to establish isoline stocks.

From each isoline, a male was individually mated to $y \text{ cv } y \text{ f } \underline{\text{Rex}}/\underline{\text{FM7}}$ females. The map distance between y and $\underline{\text{car}}$, markers spanning the X euchromatin, was then measured in the resulting $\underline{\text{In(1)w}}^{\underline{\text{m51bL}}}\underline{\text{w}}^{\underline{\text{m4R}}}\underline{\text{car}}/y \text{ cv } y \text{ f } \underline{\text{Rex}}$ daughters. In addition, for one of the isolines of each recombinant, the crossover frequency in each marked regions was measured. In all cases the isolines were identical (data not shown), and all the recombinants had significantly reduced map distances, reflecting the absence of single crossover products (Table 11). In total, nine hairpin exchange chromosomes were recovered from among 3912 chromosomes screened, a frequency of 0.08%.

Genetic analysis of recombinants - Each hairpin recombinant has two ends that were separated and then tested for $\underline{\text{bb}}$, $\underline{\text{Rex}}$ and $\underline{\text{Su(Rex)}}$ phenotype. For each end, three independent recombinant lines were tested. The results are shown in Tables 12-14, and summarized in Table 15.

Note that when $\underline{\text{Rex}}$ activity was measured in the distal ends, which are y^+ , there may be some background because exchanges between y^+ and $\underline{\text{w}}$ yield one crossover type ($y^+ \underline{\text{w}} \underline{\text{spl}}$ males) which is phenotypically indistinguishable from a detachment male. To estimate the frequency of such events, the map distance between y and $\underline{\text{w}}$ was measured in two crosses: $\underline{\text{Dp(1;1)23}}^{\underline{\text{LRexR}}}\underline{\text{car}}/y \underline{\text{w}} \underline{\text{spl}} \times y \underline{\text{w}} \underline{\text{spl}}/Y$ and $y \underline{\text{w}} \underline{\text{spl}}/\underline{\text{Tp(1;1)NO,Bx}} \times y \underline{\text{w}} \underline{\text{spl}}/Y$. There were 6/15,215 recombinants for the first cross (0.063% crossover frequency) and 15/9455 recombinants for the second cross (0.138% crossover frequency when the regular and recombinant males are doubled to account for the hemizygous lethality of $\underline{\text{Tp(1;1)NO,Bx}}$). A G test indicates that the results of the two tests are homogeneous, and together they yield a map distance of 0.11 map units.

Table 11 - Recombination data for Dp(1:1)w^{51bL}Rex^B and In(1)w^{51bL}FM7 chromosomes

CHROMOSOME	NOD	SINGLE CROSSOVERS				DOUBLE CROSSOVERS						map length
		y-cv	cv-y	y-f	f-car	y-cv	y-cv	y-cv	cv-y	cv-y	y-f	
						cv-y	y-f	f-car	y-f	f-car	f-car	
<u>Dp(1:1).car</u>	245	16	61	115	24	2	7	1	9	6	5	56
<u>In(1)w^{m51bL}.m4R.car</u>												
35	384	0	1	2	0	0	4	2	15	8	5	17
2034	363	1	3	2	1	0	3	1	11	4	7	15
2057	577	0	1	5	0	1	3	0	17	12	5	13
2337	538	0	7	1	0	0	2	1	20	5	4	13
2569	392	0	3	1	0	0	6	0	10	4	4	12
2934	503	2	3	2	0	2	4	3	14	2	2	11
3318	507	2	4	5	0	0	5	4	17	15	3	18
3394	457	1	3	0	1	1	5	3	10	8	2	13
3488	318	0	3	5	1	0	1	0	5	3	2	9

An individual male bearing the indicated chromosome was crossed to y cv y f Rex/FM7 females. Five chromosome/Rex daughters were mated to y² cv y f car males. Apparent single crossovers in the inversion chromosomes are double crossovers with one event outside the marked region, but were counted as single crossovers in calculating map length. Map length is calculated as:

$$(SCO + 2 \times DCO) \times 100 / (NOD + SCO + DCO).$$

To confirm these results, at least two other individual males bearing the indicated chromosome were mated to y cv y f Rex/FM7 females and chromosome/Rex daughters were mated to y² cv y f car males to score the y-car distance was measured (data not shown).

Table 12 - **bb** phenotype of parental and recombinant NO's

X-Chromosome	Chromosome/ <u>sc⁴sc⁸</u>			Chromosome/dl-49
	bb⁺	bb	bb^l	
Parental NO's				
<u>Tp(1;1)NO.23D.Bx</u>	666	1	0%	700
<u>y ac sc f car Rex</u>	125	313	71%	547
Recombinant NO's				
35, distal	726	599	45%	1601
35, proximal	1369	0	0%	1499
2034, distal	265	679	72%	1056
2034, proximal	672	71	10%	829
2057, distal	0	0	bb^l	1231
2057, proximal	953	3	0%	961
2337, distal	727	43	6%	840
2337, proximal	804	8	1%	924
2569, distal	659	4	1%	717
2569, proximal	165	502	75%	873
2934, distal	0	0	bb^l	918
2934, proximal	627	0	0%	724
3318, distal	661	37	5%	732
3318, proximal	617	9	1%	679
3394, distal	11	352	97%	699
3394, proximal	687	2	0%	665
3488, distal	0	0	bb^l	821
3488, proximal	133	2	99%	1032

Males bearing the indicated chromosome were crossed to In(1)dl-49,y ct y B/In(1)sc⁴sc⁸,y females. The data are pooled from three independent recombinant lines for each NO.

Table 13 - Rex activity of parental and recombinant NO's

CHROMOSOME	REGULAR		NON-DISJUNCTIONAL			DETACHMENT PROGENY		
	PROGENY		PROGENY			gynandro-		
	female	male	female	male	percent	males	morphs	Percent
<u>Parental NO's</u>								
<u>Top(1:1)NO.23D.Bx</u>	7649	4132	4	9	0.2	5	0	0.1%
<u>y ac sc f car Rex</u>	1257	1743	3	10	0.8	105	21	9.1%
<u>Recombinant NO's</u>								
35, distal	1877	2257	2	133	6.0	87	42	10.3%
35, proximal	2916	2130	4	203	7.6	0	0	0%
2034, distal	909	833	1	49	5.3	35	12	8.3%
2034, proximal	1860	1221	3	124	7.6	0	0	0%
2057, distal	2237	1089	5	140	6.2	3	2	0.2% ^a
2057, proximal	2630	1682	0	133	5.8	1	0	0.0%
2337, distal	2258	1592	0	102	5.0	0	0	0%
2337, proximal	2723	2087	3	103	4.2	0	0	0%
2569, distal	3292	3647	2	102	2.9	1	1	0.1%
2569, proximal	2248	2433	1	121	4.8	88	33	5.1%
2934, distal	2713	1590	4	130	4.3	1	0	0.0% ^a
2934, proximal	2789	1870	3	96	4.1	1	0	0.0%
3318, distal	3624	3615	2	137	3.7	6	2	0.4%
3318, proximal	1369	1020	0	51	4.1	0	0	0%
3394, distal	762	845	5	39	5.1	31	14	9.1%
3394, proximal	1553	1133	1	50	3.7	1	0	0.1%
3488, distal	1847	1023	3	42	3.0	0	0	0%
3488, proximal	691	524	2	44	6.9	19	7	3.6%

Proximal recombinant NO's are all In(1)w^{51b}Y m f while distal recombinant NO's are all In(1)w^{51b}ct. Males bearing the indicated chromosome were crossed to y w spl/y w spl females and the resulting daughters were mated to the standard y^{Sx}-y^L.In(1)En.y y f B-y⁺ tester males. Since the recombinant NO's are in inverted sequence, the number of patroclinous males reflects both those resulting from non-disjunction and those resulting from four-strand double exchanges in the female. The distal NO's are all marked with y⁺, so only y⁺ w spl males could be scored as whole-body detachments, and the detachment rate is calculated as:

$$\frac{(2 \times y^+ w \text{ spl males}) + \text{gynandromorphs}}{\text{regular females} + (2 \times y^+ w \text{ spl males}) + \text{gynandromorphs}}$$

Note, however, that in the distal NO crosses, crossovers between y⁺ and y occur at a frequency of 0.1% and include one class that is indistinguishable from detachments. This point is discussed in the text. The numbers are pooled data from three independent recombinant lines for each NO.

^aSince these NO's are bb all surviving y⁺ males are scored as detachments.

Table 14 - Su(Rex) activity of parental and recombinant NO's

CHROMOSOME	REGULAR		NON-DISJUNCTIONAL			DETACHMENT PROGENY		
	PROGENY		PROGENY			gynandro-		
	female	male	female	male	percent	males	morphs	Percent
<u>Parental NO's</u>								
<u>Top(1;1)NO.23D.Bx</u>	2917	1267	1	1	0.1%	2	0	0.3%
<u>y ac sc f car Rex</u>	653	799	4	8	1.6%	40	8	6.9%
<u>Recombinant NO's</u>								
35, proximal	1680	1453	0	52	3.2%	1	0	0.1%
2034, proximal	1583	1502	1	50	3.2%	0	0	0%
2057, distal	1023	656	2	28	2.4%	53	9	5.7% ^a
2057, proximal	1866	1524	1	54	3.1%	1	2	0.2%
2337, distal	2110	1570	0	46	2.4%	0	0	0%
2337, proximal	2404	2105	2	87	3.8%	0	0	0%
2569, distal	2879	3136	2	51	1.7%	4	0	0.3%
2934, distal	423	332	0	11	2.7%	27	3	6.6% ^a
2934, proximal	1269	1363	4	62	4.8%	0	0	0%
3318, distal	3393	3064	3	78	2.4%	7	8	0.6%
3318, proximal	1791	1502	1	52	3.1%	0	0	0%
3394, proximal	1676	1338	1	61	3.9%	0	2	0.1%
3488, distal	1866	1010	1	32	2.2%	23	11	3.3% ^a

With the exception of the parental proximal NO, only NO's with no Rex activity were scored for the presence of Su(Rex). Males bearing the indicated chromosome were crossed to y w spl cv y f Rex/FM7 females and NO/Rex daughters were mated to the standard Y^SX^Y^L In(1)En.Y y f B-y⁺ tester males. Detachment rates for distal NO's were calculated as in Table 9B. The recombinant NO's are in inverted sequence, the number of patroclinous males reflects both those resulting from non-disjunction and those resulting from four-strand double exchanges in the female.

^aSince these distal NO's are bp all y⁺ males were scored as detachments.

Table 15 - Summary of results for parental and recombinant NO's

Chromosome	DISTAL NO			PROXIMAL NO		
	% bb	Rex activity	Su(Rex)	% bb	Rex activity	Su(Rex)
Original target <u>Dp(1;1)w^{m51bl}Rex^R</u>	0%	0.1%	yes	71%	9.1%	no
Recombinants						
2569	1%	0.1%	yes	75%	5.1%	no
35	45%	10.3%	no	0%	0%	yes
2034	72%	8.3%	no	10%	0%	yes
3394	97%	9.1%	no	0%	0.1%	yes
2057	bb ^l	0.2%	no	0%	0%	yes
2934	bb ^l	0%	no	0%	0%	yes
2337	6%	0%	yes	1%	0%	yes
3318	5%	0.4%	yes	1%	0%	yes
3488	bb ^l	0%	no	99%	3.6%	no

The recombinants are grouped according to their similarities as discussed in the text. All data were taken from Tables 12-14. Note that the distal NO Rex activities have a low background due to the confusion of a crossover class with actual detachment males.

These crossovers are very infrequent and do not significantly affect the calculation of Rex activity in ends which are clearly Rex. In the parental distal NO, and in the 2934 distal NO, the small number of y^+ w males can be considered crossovers.

There are, however, three cases (2057, 3318 and 2569) where there may be some partial Rex activity in the distal end. For these, at least one gynandromorph patroclinous for only one marker (y^+) was recovered. These gynandromorphs are undoubtedly detachment products and not spontaneous. Although an occasional y^+ male that looks like a detachment is recovered in control experiments where Rex is not present, no gynandromorphs have ever been observed. In one such group of control crosses, among 41,086 daughters there were 4 detachment males but no gynandromorphs. Thus, the maximum rate of spontaneous gynandromorphs is 0.011% (99% binomial confidence interval). Unless 2569-distal carries Rex, even one gynandromorph is highly unlikely. 2057-distal and 3318-distal each gave two gynandromorphs and, surely evince some Rex activity.

The hairpin recombinants recovered are quite diverse, but there are some general patterns. The target chromosome was bb and Rex at the proximal end, and bb⁺ and Su(Rex) at the distal end. One recombinant, 2569 is similar to the target, although there may also be some Rex activity at the distal end (see above). Three others, 35, 2034 and 3394 appear to be the reverse of the target, having Rex and bb at the distal end and Su(Rex) and bb⁺ at the proximal end.

The other recombinants show more dramatic changes. Two recombinants, 2057 and 2934, are now bb¹ at the distal end and bb⁺ and Su(Rex) at the proximal end, with little or no Rex activity at either

end. Presumably, most or all of the Rex activity has remained at the proximal end and is suppressed. Two other recombinants, 2337 and 3318, have lost the bb phenotype altogether and have split Su(Rex).

Interestingly, 3318 appears to have a weak Su(Rex) along with Rex at the distal end, since there is partial Rex activity (0.38%, including two diagnostic gynandromorphs) along with the suppressor phenotype.

Finally, 3488 has lost Su(Rex) completely, and shows a net loss of rDNA expression, with a bb¹ distal NO and a severely bb and Rex proximal NO. It appears that 3488 has a deletion of rDNA as a result of the exchange.

Molecular analysis of recombinants - The first step in the molecular analysis was to prepare genomic DNA from X/O males where the only rDNA was in the end being studied. This precluded analysis of the three bb¹ ends, however. In the case of the distal NO of the parental target chromosome, X/In(1)sc⁴sc⁸ females were used as the source of DNA because the distal NO chromosome bears two euchromatic lethals that are not covered by a Y chromosome, but are covered by In(1)sc⁴sc⁸.

It was important to determine whether the changes in bb phenotype seen in the recombinant ends were due to inactivation of rDNA cistrons by insertion sequences, or to changes in rDNA copy number. DNA from each end was digested with HincII/HindIII and probed with PA56 (materials & methods). The ratio of intensity of the band at 373 bp to all the other bands reflects the proportion of intact to interrupted cistrons. As is clear from Fig. 13, there is no evident change in that ratio, even when comparing the left parental end in lane 1 (bb⁺) to the most severely bb recombinant tested, 3488P, lane 3. The wide range of bb phenotypes in recombinant ends must be due to changes in the number

	35		3318		parents		2057	2934	3488	2569		2337	
	D	P	D	P	P	D	P	P	P	D	P	D	P
bb phenotype:	+/+	+	+	+	-	+	+	+	-	+	-	+	+

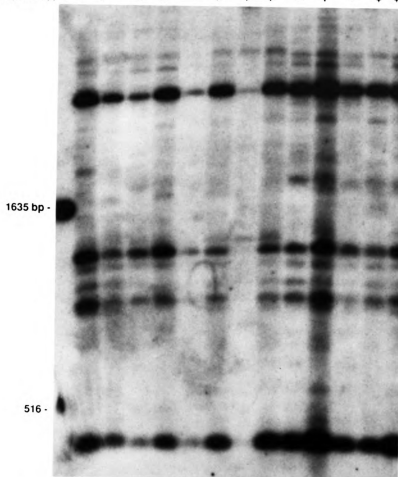


Figure 13 - HincII/HindIII digest of NO's generated by Rex-induced hairpin exchange

Genomic DNA from $X/0$ males bearing the indicated NO was digested with HincII and HindIII, except in the case of the parental distal NO where Tp(1;1)NO,23L/In(1)sc⁴sc⁸ females were the source of DNA. The bb phenotype of each NO is indicated, with a "+" denoting a bb⁺ (<10% bb) NO and a "-" denoting a bb (>50% bb) NO. Note that there was an incomplete digestion of the DNA in lane 7 (2057 proximal).

of repeats and not to an alteration of the proportions of active to inactive copies.

Molecular map of the rDNA - To generate a molecular map of the two rDNA arrays of the target chromosome, genomic DNA from X/0 males with only one specific rDNA array was digested with HaeIII and probed with IGS spacer to reveal the array of IGS length variants (Fig. 14). The parental ends were compared to find informative variants. Each recombinant end was then scored for the presence or absence of these variants in at least two independent digests by two independent observers. The results of that analysis are shown in Table 16. The data in Table 16 were used to construct the map of IGS length variants shown in Figure 15.

The mapping procedure is described in Experimental Design, but it is instructive to follow an example; the distal and proximal limits of variant k will be mapped using the data in Table 16. Note, however, that recombinant 2034 will be considered separately below. The recombinant ends are already ordered in Table 16 according to the numbers of variants. Looking at the recombinants' distal rDNA arrays (part c), 35 is the most distal exchanges within the parental distal NO, since it has retained the fewest variants. Both exchanges 35 and 3394 must have taken place distal to all copies of k because k is absent from their distal NO's. The other exchanges, 2337, 2569 and 3318, must have taken place proximal to at least one copy of k, since they retain k in their distal NO's. Proceeding from the telomere, exchange 3394 defines the distal limit of variant k's distribution, since it is the last exchange removing all of variant k.

Table 16 - IGS length variants in recombinant NO's

Proximal (Rex -bearing) NO Variants								
<u>a) Distal NO's</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>Rex</u>
telomere								
2337	-	-	-	-	-	-	-	Su
2569	+	+	-	+	+	-	+	Su
3394	+	+	-	-	+	+	+	+
3318	+	+	-	+	+	+	+	+a
35	+	+	-	+	+	+	+	+
2034	+	+	-	+	+	+	+	+
centromere								
<u>b) Proximal NO's</u>								
centromere								
2034	-	-	-	+/-	-	-	-	Su
35	-	-	+	+/-	+	-	+	Su
3318	-	-	+	+	+	-	+	Su
3394	-	-	+	+	+	-	+	Su
2569	-	-	+	+	+	+	+	+
2057	-	+	+	+	+	+	+	Su
2337	+	+	+	+	+	+	+	Su
2934	+	+	+	+	+	+	+	Su
3488	+	+	+	+	+	+	+	+
telomere								

Table 16 (cont'd)

Distal (<u>Su(Rex)</u> -bearing) NO variants										
<u>c) Distal Ends</u>	<u>b</u>	<u>c</u>	<u>d</u>	<u>g</u>	<u>j</u>	<u>k</u>	<u>h</u>	<u>l</u>	<u>i</u>	<u>Su(Rex)</u>
telomere										
2034	-	-	+	-	-	-	-	+	-	-
35	-	-	+	-	-	-	+	+	-	-
3394	+	-	+	-	-	-	+	+	+	-
3318	+	+	+	+	+	+	+	+	+	+
2337	+	+	+	+	+	+	+	+	+	+
2569	+	+	+	+	+	+	+	+	+	+
centromere										
<u>d) Proximal Ends</u>										
centromere										
3488	+	-	-	+	-	-	-	-	-	-
2569	+	-	+	+	-	-	+	+	+	-
2337	+	-	+	+	-	-	+	+	+	+
3318	+	+	-	+	-	-	+	+	+	+
2057	+	+	+	+	-	+	+	+	+	+
3394	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+
2934	+	+	+	+	+	+	+	+	+	+
2034	+	+	+	+	-	-	+	+	+	+
telomere										

HaeIII digests of genomic DNA from flies bearing the indicated NO were probed with IGS clone. The autoradiograms were scored for the presence (+) of each parental NO IGS length variant. An "x" indicates that the band was not scorable on the autoradiograms. In addition, using data from Tables 13 and 14, the last column indicates whether Rex or Su(Rex) were present (+). It is not possible to score Rex activity if the NO bears Su(Rex), and that is indicated by the notation "Su".

^aAlthough the distal NO of 3318 did have Su(Rex) (Table 14), it also had Rex activity (Table 13), based on the fact that there were two gynandromorphs produced (see text).

Rex 23
Prox. Dist.

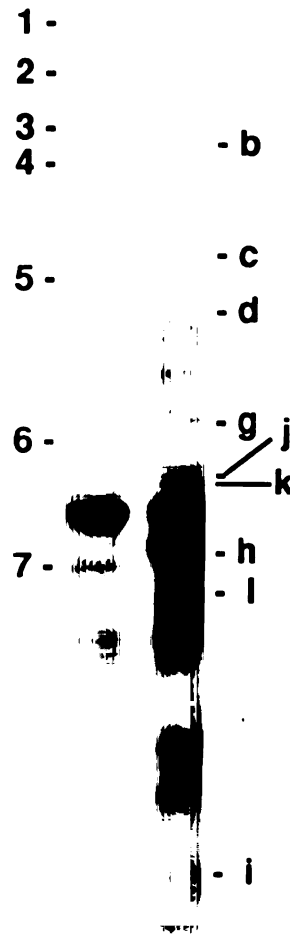


Figure 14 - IGS length variants in target chromosome NO's

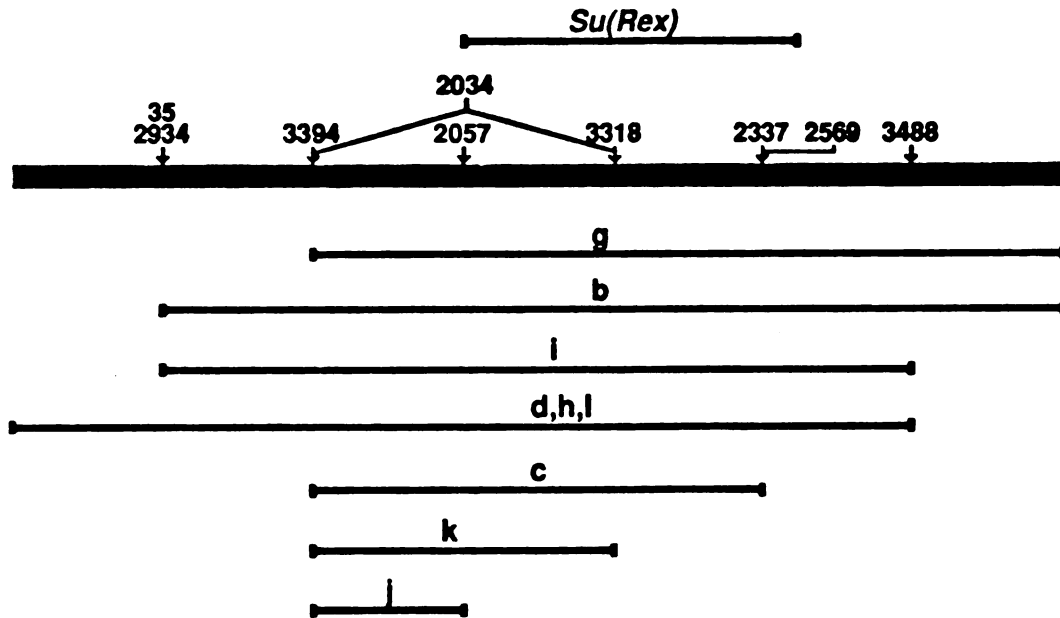
Genomic DNA was prepared from flies bearing the indicated NO and digested with HaeIII. The blot was probed with cloned IGS sequence. IGS length variants unique to one or the other NO are marked, using numbers for the Rex NO and letters for the distal Su(Rex) NO.

Figure 15 - Molecular map of target chromosome NO's

The presence or absence of IGS length variants was scored in the 9 Rex-induced recombinants of the target chromosome shown. Exchange points are indicated by arrows and were determined in 6 cases by using information from both NO's of the recombinant chromosome. In three cases (2057, 2934 and 3488), only the proximal NO was assayed because the distal NO was bb¹. Some exchange points cannot be distinguished on the basis of the scorable IGS length variants, and these are shown as being at the same position. In one case, 2034, there were clearly deletions of all copies of some length variants. The site of 2034 exchange event is shown as a region instead of as a point based on the positions of the deleted variants and the variants which remain.

The proximal and distal limits of each IGS variant are shown beneath the chromosome. The locations of Rex and Su(Rex) are shown above the chromosome. Note that even though 2337 and 2569 have identical distal breakpoints based on the IGS variants, 2569 does not move Su(Rex) to the proximal NO, but 2337 splits Su(Rex). Exchange event 2569 must, therefore, have been proximal to 2337. The proximal limit of Rex is indicated by a dashed line, because we cannot determine if any Rex activity remains in the 3394, 35, 3318 and 2034 proximal NO's because they carry Su(Rex).

DISTAL NO



PROXIMAL NO

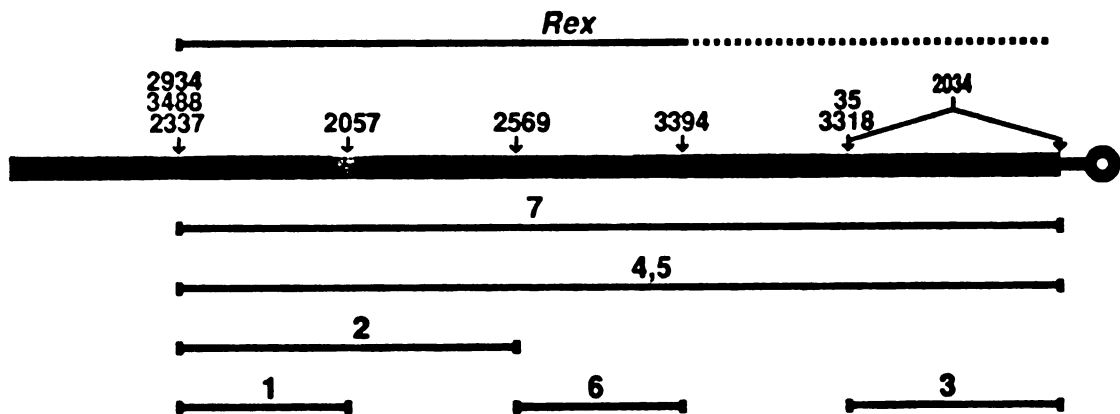


Figure 15

The exchange points in the distal parental NO can also be ordered based on the number of distal-end variants moved to the proximal recombinant rDNA arrays (part d). 3488 is the most proximal event, while 35 and 2934 are the most distal events. Exchanges 3488, 2569, 2337, and 3318 must all have taken place proximal to all copies of variant k, since they did not move variant k to the proximal NO. Exchanges 2057, 3394, 35 and 2934 must have taken place distal to at least one copy of k, since they have moved some copies to the proximal NO. Proceeding from the centromere, the proximal limit of variant k's distribution is defined by 3318, the last exchange that does not move any copies of k.

In the case of 2034, some variants are missing from both ends (j,k, and 3). Variant h is also missing from the distal end. Clearly this exchange event involved a deletion at the site of exchange. In Figure 15 the 2034 breakpoint is shown spanning a region. It is also important to note that there are data from only the proximal NO's for 2057, 2934 and 3348 because the distal end is bb¹. These events may also have involved deletions, but we cannot determine that using data from only one end. The genetic data above clearly indicate, however, that 3488 indeed has a deletion. Su(Rex) is no longer present at either NO of this recombinant. Moreover, 3488 showed a net loss of rDNA expression as measured by bb phenotype (Table 12), compared to the parental NO's.

DISCUSSION

The major result of this work is the map shown in Figure 15. The proximal and distal recombinant NO's were used independently to order the exchange events within each parental rDNA arrays. There was

excellent agreement between the two sets of data. There were 5 exchange points within the distal NO which could be unambiguously mapped relative to each other using data from both recombinant NO's. Both sets of data gave the same order. For example, 35 was the most distal exchange in the parental distal NO based on either the variants remaining in its distal NO (Table 16, part c) or on variants moved to its proximal NO (Table 16, part d). Similarly, the order of these 5 exchanges in the proximal NO was determined using both recombinant NO's and again, there was complete agreement. In other words, these five recombinants appear to have resulted from simple single exchanges.

There was one recombinant (2034) where molecular tetrad analysis showed that, in addition to a single crossover, both NO's contain deletions, and both deletions map to the exchange site. Of course, there was only one set of molecular data for the three recombinants with a bb¹ distal NO, but in one case (3488), the genetic data indicate that the exchange event involved a deletion. In 3488, Su(Rex) is completely missing and there is a net loss of rDNA expression relative to the parental chromosome. The data suggest that Rex causes single exchanges that are sometimes accompanied by deletions at the site of exchange.

When the molecular map is combined with the genetic data for each recombinant end, the two loci, Rex and Su(Rex) can be mapped to positions within the rDNA. For example, the three exchanges (35, 2034, and 3394) which clearly moved Rex to the distal end must have taken place within or proximal to Rex. It is impossible to determine if Rex activity remains in the proximal NO's of these recombinants because Su(Rex) has been moved there, and the proximal limit therefore remains ambiguous. As discussed above, it appears that 3318, 2057 and, perhaps,

2569 have some Rex activity in their distal NO. From its map position, it is clear that 3318 did move Rex activity, since the 3318 exchange is proximal to 3394. Assuming that 2057 and 2569 have, in fact, moved some Rex activity to the distal NO, the distal limit of Rex is defined by exchanges 2934, 2337 and 3488, which did not move any Rex activity to the distal NO. Moreover, if 2569-distal does have Rex, Rex is a repeated and divisible since 2569-proximal has Rex as well.

The distal and proximal limits of Su(Rex) are defined by exchange points 2057 and 2569, respectively. No Su(Rex) activity remains in 2934-distal, and no Su(Rex) activity has been moved to the 2569-proximal. Notice that the 2569 and 2337 exchange points are identical on the basis of molecular variants, but they can be differentiated with respect to Su(Rex). It is clear from recombinants 2337 and 3318 that Su(Rex) is a repeated element, since these two exchange events each gave rise to chromosomes with two Su(Rex) NO's. To unambiguously determine if Rex is a repeated element it would be necessary to start with a target which is free of suppressors, such as 3488. Starting with a target free of suppressors would also permit further molecular localization of Rex.

What might Rex be? Rex, which is located in, suppressed by and affects various NO's, appears to be part of a multi-element system. One model is that Rex encodes a site-specific endonuclease activity causing breaks in the target DNA, and that suppressors encode a repressor. The breaks in the target DNA would be recombinogenic. In that light, it is interesting that at least two of the Rex-induced exchanges involved deletions, one of which was mapped to the site of exchange. Under this model, Rex-induced nicks in the two NO's would yield inter-NO recombination, and additional breaks, or exonucleolytic trimming of

broken ends, would yield deletions that map to the exchange site. It also predicts that we are likely to find deletions among non-recombinant NO's that have been exposed to maternal Rex.

This model of Rex action has some similarities to a system described in the literature. The rDNA in Bombyx mori has two major classes of insertion sequences which interrupt ribosomal repeats and which have a high degree of sequence homology to the D. melanogaster Type I and Type II rDNA insertion sequences (Eickbush & Robins, 1985; Eickbush, personal communication). Recently, it has been shown that both of the B. mori insertion elements have open reading frames characteristic of retrotransposons, and that at least one ORF encodes an endonuclease activity which cuts 28S rDNA site-specifically, in vitro (Burke et al., 1987; Xiong & Eickbush, 1988a and 1988b).

Along these lines, we suggest that the endonucleases encoded by the rDNA insertion sequences are the cause of Rex activity. Clearly, not every insertion sequence in D. melanogaster actually produces a Rex endonuclease. Not every NO is Rex, yet in the literature every X-linked NO examined contains both types of insertion sequences (Glover, 1981; Long & Dawid, 1980). In particular we have checked the rDNA arrays in two lab stocks that are neither Rex nor Su(Rex) using the PA56 probe (Chapter 2; Fig. 13), and they contain both Type I and Type II inserts (data not shown). It is likely that Rex is an alteration that allows the endonuclease to be produced at a different developmental stage or at a higher concentration or as a more active enzyme. All these possibilities are consistent with the finding that Rex is a neomorph. Of course, the Rex endonuclease may not be encoded by a known ORF in one of the inserts. It might be encoded by a completely novel gene.

Further experiments to determine whether Rex is a repeated element and delineate the Rex map position within the rDNA will be needed to elucidate the exact nature of Rex and allow it to be cloned.

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SIGNIFICANCE AND RECOMMENDATIONS

Rex was discovered as a maternal-effect dominant which induced exchange between two NO's on an attached-XY chromosome. It exhibits two fascinating phenotypes. One is that Rex induced a high frequency of exchange in a region characterized by very little exchange. The second is that Rex-induced exchange was a mitotic event which took place at the earliest stages of embryogenesis. For these reasons, I undertook a variety of experiments all designed to increase our understanding of Rex.

The results of the studies answer several important questions about Rex and point the way to a model of Rex activity and to other experiments needed to further characterize Rex. The studies also highlight the utility of Rex as a tool for analyzing a multigene family, and suggest that it has additional significance because it is an X-linked heterochromatic locus, one of only 5 known X-linked heterochromatic loci.

Results of the experiments

The major conclusions from these studies were:

- 1) The Rex locus is a neomorph located at a specific position within the rDNA array.
- 2) There are multiple, dominant, maternal-effect suppressors of Rex

(Su(Rex)). At least one is a neomorph located at a specific position within the rDNA array, and it is a divisible element.

3) Rex-induced exchange is conventional, in that there are no gross rearrangements of the target rDNA. However, some Rex-induced exchange events involve deletions at the site of exchange.

4) When in males, mei-41, a meiotic mutant that also suppresses magnification, induces Rex-like exchanges in a chromosome with two NO's.

Model for Rex action

When all these results are taken together they suggest that Rex encodes an endonuclease activity specific for rDNA which makes recombinogenic breaks. This model accounts for all of the observations. Rex is an extreme hypermorph or a neomorph, producing a novel activity not found in other rDNA. Rex induces both magnification and recombination, two events that require DNA breaks. mei-41 males, known to be defective in post-replication repair, also induce Rex-like exchanges in chromosomes with two NO's. Presumably, both Rex and mei-41 act by creating or failing to repair recombinogenic breaks in the rDNA.

Furthermore, Rex appears to be part of a multi-element system, located in, suppressed by and inducing exchange in various rDNA arrays. In this respect, Rex is reminiscent of transposons. If Rex encodes an endonuclease like that which is part of transposases, then analogous roles can be suggested for the suppressors (repressor of transposition) and the targets (site-specific target for transposition). There are examples of transposon systems with both transposase and repressors of transposase, most notably, the Tn5 transposon of E. coli (Isberg et al., 1982; Johnson et al., 1982).

Rex differs from most other transposons in several respects: its target specificity, time of action and maternal effect. Rex acts specifically on the NO, and it acts very early, before there is any reported transcription of rDNA in the zygote (McKnight & Miller, 1976; Zalokar, 1976). It is reasonable to hypothesize that once rDNA transcription begins, the target DNA becomes inaccessible to the Rex endonuclease, and so the only time of action is in the first few divisions in the embryo. The maternal effect is obviously due to some maternal contribution. Considering the enormous transcription rate of rDNA in the ovary (Mohan & Ritossa, 1970; Mermod et al., 1977) it is possible that a Rex gene product encoded within the rDNA might accumulate to a high concentration in the egg and diminish with each embryonic division.

There is a parallel from the literature for this model of Rex activity. The insertion sequences found within some 28S coding regions belong to a class of elements known as "non-LTR retrotransposons" or "retroposons" (Rogers, 1985; Xiong & Eickbush, 1988a). These elements appear to transpose via an RNA intermediate, like retroviruses, but lack the characteristic long terminal repeats (LTR's) of retroviruses. They are characterized by stretches of polyA at one end, and often have duplications of the flanking DNA at the site of insertion.

In fact, two classes of D. melanogaster rDNA insertion sequences (Type I and Type II) are closely related to the well-characterized R1Bm and R2Bm sequences of Bombyx mori (Eickbush and Robins, 1985; Eickbush, personal communication). Both R1Bm and R2Bm have open reading frames which contain sequences similar to portions of the retroviral pol genes encoding reverse transcriptase (Burke et al., 1987; Xiong & Eickbush,

1988a). Although the ORF lacks a promoter, it has been suggested that it is transcribed from an rDNA promoter (Xiong & Eickbush, 1988a) based on the findings of several groups that there are rare transcripts of insert-containing rDNA repeats in D. melanogaster (Jamrich & Miller, 1984; Kidd & Glover, 1981; Long & Dawid, 1979). Presumably, like other reverse transcriptase genes, these ORFs encode an enzyme which carries out both reverse transcription as well as integration via endonucleolytic cleavage (Xiong & Eickbush, 1988b).

There is also in vivo and in vitro evidence that the R2Bm element functions as a transposon with unusual site-specificity. Firstly, Xiong et al. (1988) have found several R2Bm insertions outside the rDNA, and the flanking DNA shows substantial sequence similarity to the integration site in the 28S gene. Although this finding suggests transposition, these non-rDNA insertions appear to predate the separation of several different geographical B. mori races and do not indicate recent activity. Secondly, the races have widely differing numbers of R2Bm inserts in the rDNA, suggesting that the elements may move more frequently within the rDNA. This might, however, be due to the recombination mechanisms which maintain the integrity of the rDNA array and not to transposition. Thirdly, the fact that the insertions inactivate the repeat into which they are inserted suggests that they would be selected against unless there is a mechanism such as transposition to maintain them. Lastly, Xiong & Eickbush (1988b) have demonstrated that the ORF in R2Bm, when ligated to an inducible promoter and expressed in E. coli, does encode a functional endonuclease that cleaves 28S DNA in a site-specific manner. Presumably, this is the integrase or endonucleolytic function of reverse transcriptase. However,

the authors have not demonstrated any in vivo endonuclease activity in B. mori.

Indeed, the Rex system complements the observations made in B. mori, where there is no direct evidence that the endonucleases which appear to be encoded by R1Bm and R2Bm elements are in fact translated or active in vivo. If Rex activity is the result of a mutation which activates an insertion sequence ORF, it should also be possible to search for similar mutations in B. mori.

There is also another retroposon in D. melanogaster rDNA. The G element is specific to the chromocenter, and most G elements are found at a conserved insertion site within the IGS (DiNocera et al., 1986; DiNocera, 1988). The G element target sequence shows a high degree of sequence similarity to the target sites of Type I and Type II inserts in the 28S gene (DiNocera et al., 1986). G elements also contain an ORF which appears, on the basis of sequence similarity, to encode a reverse transcriptase (DiNocera, 1988).

In light of these results, it is tempting to suggest that Rex activity results simply from the endonuclease encoded by a Type I, Type II or G element insert, particularly in view of its location in the rDNA and extreme site-specificity. However, not all Type I and Type II inserts confer the Rex phenotype, since stocks that contain these elements can be Rex, Su(Rex) or neither (this has not been directly tested in the case of G elements; however, they are found in virtually every D. melanogaster stock (DiNocera et al., 1986)).

Instead, Rex could be an activated endonuclease-producing gene. The activation could be a result of a change in the structural gene, making the enzyme more active, or a change in the promoter or enhancer

allowing ectopic or unusually high levels of expression. It is possible to test this hypothesis by further characterization of Rex, including cloning of Rex.

Further characterization of Rex

a) Defining the Rex target

Throughout the preceding discussion there is the implicit assumption that Rex activity is entirely specific to the rDNA and affects no other targets. This assumption has not been thoroughly tested. Swanson (1984, 1987) and this work have shown that Rex can act on any chromosome with two hb loci. Swanson (1987) has also shown that simple duplications of heterochromatin do not constitute a Rex target. Even In(1)w^{m4} which has type I insertion sequences at both ends of the chromosome, although it probably lacks rDNA repeats at the distal end, (Hilliker & Appels, 1982) is not a Rex target. The question remains as to whether an exactly duplicated block of heterochromatin at either end of the chromosome, or a duplication of a different gene family can act as a Rex target.

I propose two experiments to answer this question. The first is to test In(1)w^{m4L}_{rst}^R as a target, since it is duplicated for a portion of the X heterochromatin. The second is to test a duplication of the 5S ribosomal RNA genes, Dp(2;2)M2 (Nix, 1973). This duplication, a tandem repeat of the locus (at 56F) and surrounding material (spanning 56C-59C), is unstable in long-term stocks, but can be readily isolated as a suppressor of M(2)173. Rex-induced exchange between the separated repeats will cause reappearance of M(2)173.

The experiment in Appendix A was designed to ask about the size of the Rex target, if it is, in fact, rDNA-specific. That experiment, using an X chromosome with a normal NO and a P-element at the tip containing a single rDNA repeat, was inconclusive. The element itself had a background level of instability that made it impossible to determine if Rex induced any exchanges in the chromosome.

Nevertheless, the question remains interesting. Modified versions of the P-element rDNA repeat have been recovered by B. McKee (personal communication), including elements with several rDNA repeats, loss of parts of the P-element vector, and loss of portions of the single rDNA repeat. It would be worthwhile to test these, first for stability, and then, if possible, as Rex targets. If Rex is rDNA-specific, these chromosomes offer the best chance at defining the specific Rex target sequences.

b) Looking for the Rex transcript

The model of Rex activity presented above proposes that Rex activity results from activation or increased production of an endonuclease encoded by one of three classes of rDNA insertions. The Rex variant allows either abnormally high or ectopic expression of the site-specific endonuclease, generating recombinogenic breaks in the rDNA during early embryogenesis. The model predicts that we should be able to find mRNA corresponding to the ORF of the insertion element. In general, these transcripts are very low abundance or are present as portions of longer, low abundance, rDNA transcripts (Jamrich & Miller, 1984; Kidd & Glover, 1981; Long & Dawid, 1979). The most logical stages to examine are ovaries and unfertilized eggs, since it is known that Rex acts as a maternal-effect in early embryogenesis.

I propose examining ovaries and unfertilized eggs from Rex and control mothers for transcripts corresponding to the ORF's of the three insertion classes. Since all three insertions have been sequenced (Eickbush, personal communication; DiNocera, 1988), all three probes are readily available. Presumably, if Rex encodes an endonuclease activity, the transcript will be at a detectable level.

It is important to point out that Rex activity may not result from a structural gene mutation, but, instead, may result from a regulatory mutation. Therefore, a cDNA, with only the coding region, may not reveal the nature of the Rex alteration. It is also not clear if a cDNA would lead to cloning of Rex. Considering the high degree of sequence similarity between all members of a given insert class, it may be impossible to identify which particular element of a class encoded the mRNA.

Nevertheless, this result will test a central prediction of the proposed model, and will allow further experiments to characterize Rex. For example, is the transcript present in eggs of Rex/Su(Rex) mothers? Will a P-element insertion of the cDNA from this message confer a Rex phenotype? Can a P-element insertion of the cDNA be used to manipulate the timing of Rex action by use of an inducible promoter?

This last question is particularly interesting, since it is not clear why Rex acts specifically during early embryogenesis. Is the Rex product only made during oogenesis? Or, alternatively, is the target only accessible during early embryogenesis?

c) Cloning Rex

It may be possible to clone Rex from a cDNA if a transcript is detected, as described above. I am proposing an alternative scheme for

cloning in the event that a transcript is not detected, or if the cDNA has homology to multiple elements, and to provide a way to clone Rex even if it is a regulatory mutation. Before considering this line of research, it is important to bear in mind that specific, as opposed to random, portions of a middle repeated gene family have never been cloned. In fact, the results in Chapter 4 represent the first time genes have been mapped to specific portions of a repeated gene family. Therefore, any attempt to clone Rex will involve adapting existing technology to a special purpose. As such, this scheme for cloning Rex may become a model for cloning specific portions of the rDNA.

The approach that I suggest is to map Rex to as small a group of rDNA repeats as possible (10-30 repeats), subclone that portion of the NO, and then screen all the insertion-containing repeats in the subclones for alterations. If an endonuclease transcript has been detected, the screen can be narrowed to only one class of insertion elements.

The first step of delimiting the Rex region involves converting the genetic map of the Rex-bearing NO into a physical map. It will be at this point that we will know whether more recombinants are needed before we can proceed. The mapping data have localized Rex to a specific portion of the rDNA array, part of which includes the entire region spanned by IGS length variant #6. How many repeats are actually in this span? It is possible to estimate that by measuring the intensity of band #6, by knowing the counts per minute in each band (using a beta-particle counter). Assuming that the weakest band represents one spacer, the relative amounts in other bands can be added up to quantify the total number of repeats in the NO, and in each IGS

class. If the weakest band is actually 2 or 3 repeats it will be clear immediately, since a viable bb locus cannot have less than approximately 150 repeats.

Knowing the total number of repeats in a given bb locus, the number of repeats in each diagnostic IGS length class, and the positions of various breakpoints with respect to those length classes, it is possible to estimate the minimum size of the region where Rex is localized. For example, if a particular region bounded by two exchanges contains 10 copies each of 4 diagnostic IGS length classes, and the diagnostic IGS length classes are 50% of all IGS sequences, then the minimum size of the region is 40 repeats and it is reasonable to assume that the region is approximately 80 repeats long. The region can be also be subdivided by further exchanges with appropriate targets in order to map Rex to as small a region as possible.

The next step is to separate this region from the other repeats. One possibility is to cut the NO into a few fragments using an enzyme which cuts infrequently within the NO, such as NotI (the only sites are in Type II inserts). Those fragments, which still contain many repeats, can be separated by pulsed-field gel electrophoresis and eluted. Subsequently, the individual sub-NO pieces can be digested with HaeIII and probed with IGS clone to determine which portion of the NO they contain, based on how many and which diagnostic IGS length variants they contain. Another possibility is to make yeast artificial chromosomes containing portions of the NO, and identify the origin of each cloned region based on the IGS length variants.

In any case, the goal is to get subclones of the specific region of the NO that contains Rex. Then all repeats from that sub-region that

contain inserts can be examined for restriction variations within the insert or within the surrounding regions. Finally, P-element mediated insertion of putative Rex sequences might allow an in vivo test, currently not available for B. mori.

Significance

The results of these studies point in several important directions.

1) The mapping of Rex and Su(Rex) has placed them within the rDNA, and as such, we have increased the number of mapped X-linked heterochromatic loci by 66%. Previously, only three loci had been identified in the X heterochromatin: ABO, cr and bb. ABO is a heterochromatic element distal to bb which suppresses part of the lethality induced by homozygous abo (a 2nd chromosome locus) in females (Pimpinelli et al., 1985; Sandler, 1970, 1977). cr, as discussed above, is involved in rDNA compensation, and bb is the rDNA itself. By studying Rex and Su(Rex) in more detail, we may learn more about heterochromatic loci.

For example, are these repeated elements? It has been suggested that all functional elements of heterochromatin are repeated, and that the absence of recombination has evolved to avoid a high frequency of unequal crossing-over that would lead to sudden, dramatic changes in copy number (Sandler, 1975). This hypothesis seeks to explain both the large size of the heterochromatic regions and the paucity of mutable functions within the heterochromatin. Su(Rex) and bb surely are repeated elements, and perhaps Rex is also. Recently it was shown that another heterochromatic locus, the Rsp locus of the Segregation Distorter system (chromosome 2), is a repeated element (Lyttle, 1989;

Pimpinelli & Dimitri, 1989). Is this true of the other heterochromatic loci?

2) Rex induces frequent mitotic exchange in a region which exhibits little meiotic exchange. mei-41 in males has the same effect. What are these mutants doing to disrupt the stability of the rDNA? Are there special systems that normally reduce rDNA mitotic exchange? For example, what is the function encoded by Su(Rex), and can Su(Rex) suppress the mei-41 lesion? Alternatively, could an extra copy of mei-41⁺ in a male suppress the Rex maternal effect?

Understanding the mechanism of Rex-induced exchange may also shed light on the mechanisms of rDNA exchange. Many models have been advanced to explain the concerted evolution of the rDNA arrays on the X and Y chromosomes (Dover, 1982). The two rDNA arrays are clearly not evolving independently, yet the rate of spontaneous X-Y exchange when compared to the rate of X-X exchange is not sufficient to explain their similarities (Williams et al., 1989). There must either be conversion events and/or multiple exchanges between the two chromosomes.

Does Rex induce conversion and/or multiple exchanges? It should be possible to examine unselected target chromosomes exposed to Rex for conversion events or double exchanges that alter the number or position of IGS length variants. By further characterizing the nature of Rex exchange, we may learn about rDNA exchange in general.

3) Rex has proven its worth as a tool for mapping within the rDNA. I have used it to map loci within the rDNA for the first time. The major advantage of using Rex-induced exchange is that it is an order of magnitude more frequent than spontaneous exchange, which is a non-trivial consideration when many recombinants have to be recovered.

Although the experimental design is a bit cumbersome, it could be streamlined to allow Rex to be used generally to map with respect to the rDNA.

The most daunting part of using Rex to map Rex in Chapter 4 was that hairpin exchanges had to be recovered. This entailed a time-consuming screen for inversions. The reason for using hairpins was that the spiral exchange product, an X-fragment, would make any male too hyperploid to be fertile, even if the cross was manipulated such that all sons had a Y chromosome.

Using a different target, where the distal NO is closer to the tip (such as Dp(1;1)sc^{S1}sc⁴) would enable the product of spiral exchanges to be recovered as fertile males. Spiral exchanges are readily detected in the first generation of progeny from Rex crosses, unlike hairpins which must be detected by screening the first generation progeny individually.

The Dp(1;1)sc^{S1}sc⁴ target chromosome is non-inverted, with a duplication of the NO at the tip. It is a product of Rex exchange (Swanson, 1987). Any NO, from any X chromosome can be used to replace the proximal NO. Therefore, the superstructure of any NO can be dissected, much as the Rex-bearing NO has been analyzed in the previous experiments.

This kind of analysis may prove useful in studying the distribution of various heterochromatic elements. For example, there is an ongoing debate about whether insertion sequences are (England et al., 1988; Long & Dawid, 1979) or are not (Hawley & Tartof, 1983b) clustered in X-linked NO's. Both Type I and Type II inserts are polymorphic in size. By starting with two NO's with distinct arrays of inserts, Rex-induced exchange would clearly demonstrate whether the inserts are clustered.

Another example where Rex exchange will clarify the position of a heterochromatic element is with G insertion elements. Although G elements are only found in the chromocenter, and are often found within the IGS, it is not known how they are distributed with respect to the rDNA (DiNocera et al., 1986). Once again, by starting with two NO's with distinct G element arrays, Rex-induced exchange can map the G elements.

4) Rex is a locus within a multigene family. As such, the cloning of Rex can be seen as a model for the cloning of specific segments of a multigene family.

Summary

In summary, Rex has been mapped to the rDNA and a specific model for Rex action has been developed. The further study of Rex is likely to provide information about Rex itself and the Rex exchange mechanism, perhaps shedding light on the more general topics of heterochromatic loci, rDNA stability, the molecular arrangement of the rDNA, and, perhaps, methods of cloning specific members of this and other middle repetitive gene families.

APPENDICES

APPENDIX A

MITOTIC INSTABILITY OF A P-ELEMENT RIBOSOMAL CISTRON INSERTION

We investigated whether a single ribosomal cistron could serve as one half of a Rex target by using an X chromosome containing both a normal bb locus and a single rDNA cistron inserted distal to y⁺. The single cistron ([rib7]) had been inserted by P-element transposition using a defective P-element vector that lacks transposase activity (Karpen et al., 1988). In order to be able to score Rex exchanges using [rib7], we constructed a [rib7]Dp(1;1)sc^{VI},[rib7,ry⁺] y⁺ v f car su(f)·y⁺ chromosome. Females who were homozygous y (with or without other markers) were then crossed to [rib7]Dp(1;1)sc^{VI}/Y males. Regular progeny are y⁺ daughters and y sons. Non-disjunctional progeny are y females and y⁺ v car males. Mitotic exchanges that delete the material between [rib7] and the normal bb locus, would generate y⁺ X-chromosome fragments. Such exchanges occurring before first division in the zygote would yield y⁺ car⁺ X/fragment sterile males. If the exchange occurs later, gynandromorphs would result.

The initial cross with Rex/Rex mothers gave significant numbers of the predicted y⁺ car⁺ sterile males (Table 17), suggesting that a single cistron is sufficient to serve as a Rex target. However, a control experiment using y/y mothers, gave the same frequency of y⁺ car⁺ sons

Table 16 - Intra-chromosome exchange frequencies

CROSS	REGULAR		NON-		FRAGMENT-			
	PROGENY		PROGENY		PROGENY			
	female	male	female	male	males	Percent ^a		
<u>y cv y f Rex</u>	<u>[rib7]Dp(1:1)sc^{VI}</u>							
x								
<u>y cv y f Rex</u>		Y	1751	1544	5	11	8	0.45%
<u>y</u>	<u>[rib7]Dp(1:1)sc^{VI}</u>							
-								
x								
<u>y</u>		Y	3460	3343	16	24	12 ^b	0.35%
<u>y cv y f Rex</u>	<u>Dp(1:1)sc^{VI}</u>							
x								
<u>y cv y f Rex</u>		Y	1493	1395	4	9	0 ^c	0% ^c

^aPercent exchange is calculated as exchange males/(regular females + exchange males), since the exchange product can only be formed in X/X zygotes.

^b5 exchange products were recovered from one of the 57 single-female matings scored.

^cOne cv y f male was recovered that was a patchy y⁺ mosaic. This exception is not, however, analogous to Rex-induced events, since Rex-induced exchange events yield gynandromorphs but not mosaic males.

(Table 17). Thus, the [rib7]Dp(1;1)sc^{VI} chromosome itself appears to be unstable.

There are three possible sources of this observed instability: the duplicated ribosomal cistron, the P-element transformation vector, or the original Dp(1;1)sc^{VI},y⁺ y f car su(f)·y⁺ chromosome used to construct our target chromosome. Duplicated ribosomal cistrons on the X are not generally unstable. Many such chromosomes have been used to study Rex, and exchanges are only found when maternal Rex is present. In order to test whether the parent Dp(1;1)sc^{VI} chromosome is itself unstable, Rex/Rex females were crossed with Dp(1;1)sc^{VI} males. No y⁺ car⁺ sons were observed.

The data suggest, therefore, that the exchange events observed with [rib7]Dp(1;1)sc^{VI} are due to the P-element transformation vector. Although it has not been reported previously, a low frequency of mitotic instability may be a characteristic even of defective P-elements. We do not, however, know whether another intact P element might have been inadvertently introduced in the course of the experiment.

It is not known what exchange event is taking place, since the y⁺ car⁺ progeny are all sterile. Although the exchange could be a Rex-like intrachromosomal event, it is also possible that the exchange is interchromosomal, between the Y-chromosome bb locus and [rib7]. The interchromosomal exchange would, of course, have to be a male germline, possibly pre-meiotic, event. This possibility is lent some credence by the recovery of one cluster of y⁺ car⁺ progeny in the control cross.

APPENDIX B

EFFECT OF REX ON X-Y EXCHANGE

One obvious question about Rex is whether it can induce interchromosomal as well as intrachromosomal exchange. Both the X and Y chromosomes have bb loci, so an experiment was designed to measure the Rex effect on X-Y exchange. The experiment had to take into account the fact that Rex acts very early in the zygote to induce intrachromosomal exchange, perhaps even before pronuclear fusion. For this reason, exchange had to be measured between chromosomes contributed by a single gamete.

The X and a Y chromosomes were brought in by a single gamete by using XYY males. In XYY males the two Y chromosomes generally disjoin from one another (Sandler & Braver, 1954), giving rise to approximately 50% XY gametes. By using a Y chromosome with one marker at the end of each arm, $y^+y^S \cdot y^Lp^S$, X-Y exchange could be assayed by looking for loss of linkage between of y^+ and p^S .

The mating scheme for the experiment is shown in Fig. 16. The first part of the scheme developed a stock with a high frequency of X/Y/y⁺p^S sons. First, X/X/y⁺p^S exceptional daughters were recovered from a cross with relatively high male non-disjunction (approximately 0.8%, data not shown). These daughters were mated to y cv y f car/Y males, and phenotypically cv y f car p^S daughters (whose genotype was

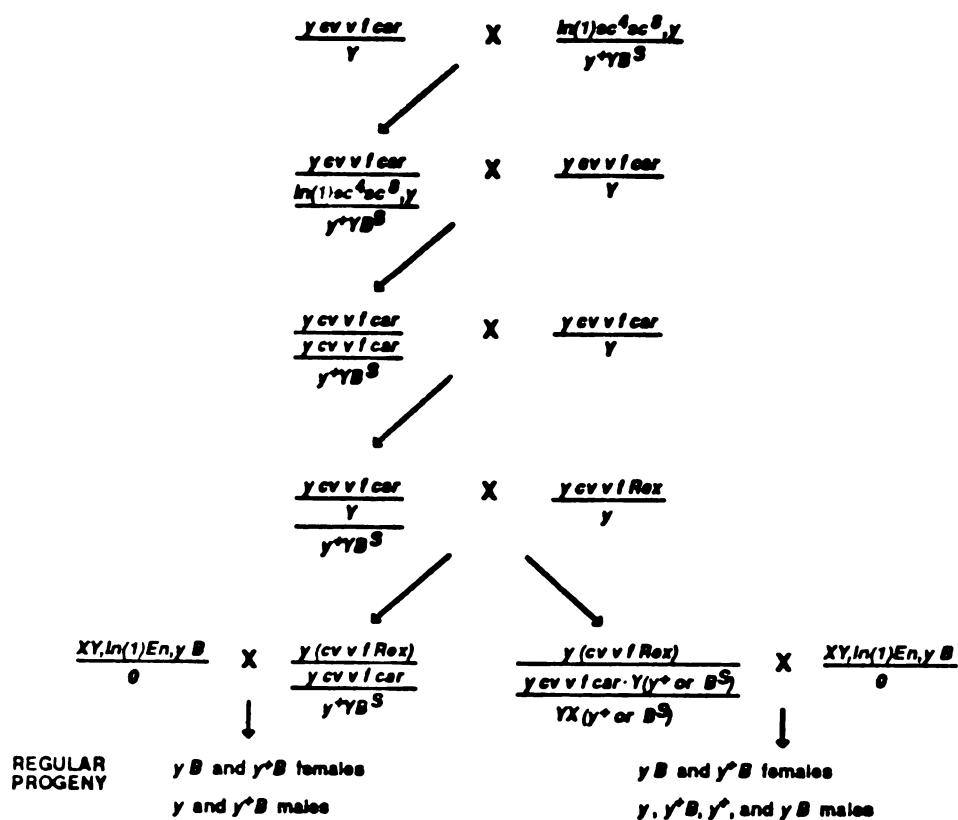


Figure 16 - Mating scheme to recover X-Y exchange products

The first three steps generated a stock to provide a source of $X/Y/y^+YB^8$ males as described in the text. The stock was maintained as shown in step 3, to ensure the presence of both a maternal marked Y chromosome and an unmarked paternal Y chromosome. The $X/Y/y^+YB^8$ males were mated to $y\ cv\ v\ f\ Rex/y$ females. The majority of the y^+B^8 daughters will carry an intact y^+YB^8 chromosome, but a few will carry the two markers on separate chromosomes as a result of an exchange. To distinguish these two genotypically distinct classes, each y^+B^8 daughter is individually mated to $y^+Y/y^+Y, y\ B/0$ males and the progeny were scored for segregation of the markers. Females carrying exchange chromosomes yielded two unique classes of progeny: y^+ and $y\ B$ sons.

$y \text{ cv } y \text{ f } \underline{\text{car}}/y \text{ cv } y \text{ f } \underline{\text{car}}/y^{+}p^{S}$) were selected and backcrossed. The resulting stock was then maintained by selecting females and males of the parental phenotype at each generation. This ensured the presence of the marked and unmarked Y chromosomes (coming from the female and male, respectively) and a high frequency of $X/Y/y^{+}p^{S}$ sons resulting from normal disjunction in the $X/X/y^{+}p^{S}$ female.

There were two technical problems with this stock. One was that $X/Y/y^{+}p^{S}$ males tended to be relatively infertile, so that roughly one-third were completely sterile and the remainder were less fertile (27.5 ± 15.5 progeny/male) than their $X/y^{+}p^{S}$ brothers (42.8 ± 22.4 progeny/male). The second problem was that these two classes of sons were indistinguishable phenotypically. In order to assess the proportion of $X/y^{+}p^{S}$ sons in the stock, individual $y^{+}p^{S}$ males were mated to $y \text{ cv } y \text{ f } \underline{\text{Rex}}/y$ females. $X/Y/y^{+}p^{S}$ males could then be distinguished because there were roughly equal numbers of $y^{+}p^{S}$ and y sons among their progeny. Of the 25 males tested, 20 (80%) were the desired $X/Y/y^{+}p^{S}$ genotype, 4 (16%) were $X/y^{+}p^{S}$, and one (4%) was $X/y^{+}p^{S}/y^{+}p^{S}$.

In the second part of the mating scheme, Rex was tested for induction of X - Y exchange. $X/Y/y^{+}p^{S}$ males from the stock described above were mated to Rex females. Any exchange events which took place in the resulting $X/X/y^{+}p^{S}$ daughters were undetectable in the F1 because both markers (y^{+} and p^{S}) would remain in every cell, even if they had become unlinked by a mitotic exchange. Therefore, each phenotypically $y^{+} p^{S}$ daughter was individually mated to $y^{S}x^{+}y^{L}, y \text{ B/O}$ males and the resulting sons were scored for segregation of y^{+} from p^{S} .

Of 1625 F1 daughters screened, only 3 had undergone interchromosomal exchange, a 0.18% frequency. Each of the six exchange products (two from each event) was tested for the presence of Y fertility factors and bb expression. The results showed that each event was distinct:

1) #133 - XY^LY^S, y v f car:y⁺ and Dp(1;f)133,B^S (apparently an X chromosome fragment with material from B to the centromere, including bb⁺ and some Y heterochromatin).

2) #172 - X-Y^L, y f car:B^S and y⁺Y^S (bb⁺)

3) #1425 - XY^L??, y cv f car:y⁺ and Dp(1;f)1425,B^S (bb)

The structures of the recombinants are inferred, based on the assumption that there is rDNA on both arms of the marked Y chromosome (R.S. Hawley, personal communication) and that exchanges can take place with either rDNA array. It is not clear what event has damaged or eliminated the y^S fertility factors in 1425.

The low rate of exchange suggests that Rex does not induce interchromosomal exchange at anywhere near the frequency with which it induces intrachromosomal exchange. Furthermore, a different experiment indicated that y⁺Y^S has a certain level of intrinsic instability, regardless of the presence of Rex. In this experiment, the same mating scheme was used, except that F1 progeny were scored directly for the presence of both y⁺ and B^S. In crosses with y cv y f Rex/y mothers, 10/1204 progeny showed loss of one or the other marker (0.8%), while in control crosses with y/y mothers 3/1363 progeny showed a loss (0.2%). Although the difference is significant ($\chi^2=5.58$; $0.01 < p < 0.05$), it is not large and it is difficult to evaluate any effects of Rex with such a high background of spontaneous events.

In conclusion, if Rex does, in fact, induce X-Y exchange, it is at a very low frequency. This result raises interesting questions about the mechanism of Rex action. Is there some feature of the Y NO which makes it an ineffective target or is Rex ineffective at inducing interchromosomal events?

In order to truly evaluate the ability of Rex to induce interchromosomal events, the experiment must use two chromosomes with NO's of known Rex sensitivity. One possibility is to use two X chromosomes with sensitive NO's, and use a meiotic mutant in the female to give a high frequency of non-disjunctional XX gametes. This experiment, however, depends on use of maternally-transmitted targets. Swanson (1984) showed that a maternally-transmitted chromosome is sensitive to Rex, but at a very low level (0.14%). However, the chromosome she tested carried a Su(Rex) (data not shown), and it remains possible that other maternally-transmitted targets will be as sensitive as paternally-transmitted targets. Some of the known suppressor-free chromosomes generated in Chapter 4 could be used individually (In(1)3488) or in combination (In(1)2034^L2569^R) in a test of maternally-transmitted targets.

If Rex can induce interchromosomal exchange then it may have an important role in the concerted evolution of the X and Y chromosome rDNA. Therefore, despite this preliminary result which suggests that Rex is ineffective in inducing interchromosomal events, it is still worthwhile to pursue this question using a more stable pair of chromosomes that contain NO's known to be sensitive to Rex.

APPENDIX C

mei-41 IN MALES SHOWS REX-LIKE BEHAVIOR

Magnification and compensation are phenomena which, like Rex, affect rDNA integrity. In particular, magnification seems to be related to Rex action, because Rex is able to induce magnification in females (γ cv y f Rex/In(1)sc⁴sc⁸ females crossed to bb²/Y males yielded phenotypically bb⁺ In(1)sc⁴sc⁸/bb² daughters in 8 of 600 females tested, R.S. Hawley, personal communication). Furthermore, Hawley & Tartof (1985) have suggested that magnification takes place via both pre-meiotic and meiotic exchanges in the rDNA, and, of course, Rex induces rDNA exchange.

In order to further explore the connection between rDNA exchange, magnification and Rex, several experiments were performed with mei-41. The mei-41 mutation, originally discovered as a meiotic mutant affecting recombination frequencies in females (Baker et al., 1976), has several other interesting phenotypes in males. mei-41 suppresses pre-meiotic magnification, but causes high rates of X-Y exchange particularly in the rDNA (Hawley & Tartof, 1983a). The question was whether there is any epistasis between these two loci, since both stimulate rDNA exchange, but have different effects on magnification.

A target attached-XY chromosome that carried mei-41 was constructed. The presence of mei-41 in the male was sufficient to

induce Rex-like detachments (Table 18). Crossing to Rex/y females did not appreciably enhance the detachment rate. However, when the mei-41 target-bearing males were crossed to Rex/Rex females, the detachment rate was significantly higher ($\chi^2=20.56$, $p<0.001$). Heterozygous mei-41 females did not induce detachments, and did not inhibit Rex activity. Homozygous mei-41 females are nearly sterile due to the meiotic lesion, and have not been tested.

The data are very preliminary. At least two other experiments need to be done. One, more fertile mei-41 alleles should be tested in females to see if mei-41 has any effect in females. It is also important to determine if there is a synergistic effect between Rex/Rex females and mei-41 males by measuring the "background" level of detachments in crosses of Rex/y and Rex/Rex females to the original X-Y chromosome that was used to construct the mei-41 X-Y target.

There is no doubt, however, that mei-41 is capable of inducing Rex-like detachments. Hawley et al. (1985) suggested that mei-41 males develop recombinogenic breaks in their rDNA which cannot be repaired and that these breaks are what stimulate X-Y exchange, allow meiotic magnification, but inhibit pre-meiotic magnification. It is possible to speculate that these same breaks induce the detachment events. That, in turn, suggests a mechanism of Rex activity. In some way, Rex may also be generating breaks specifically in the rDNA, leading to detachment and magnification events in the zygote. This is consistent with the model of Rex as a site-specific endonuclease proposed in Significance & Recommendations.

Table 17 - mei-41 effect on detachments

MATERNAL GENO/TYPE	TARGET CHROMOSOME ³	REGULAR		NON-DISJUNCTIONAL			DETACHMENT PROGENY	
		female	PROGENY	male	female	male	percent males	gynandro- months Percent
Y/Y	XY, mei-41	2260	3431	7	4	0.4	13	0 0.6
Y <u>mei-41</u> /Y	XY	1192	1040	1	0	0.1	1	0 0.1
Y l2 f car Rex/Y	XY, mei-41	1325	1391	1	2	0.2	11	0 0.8
Y <u>CV</u> Y f Rex/Y <u>CV</u> Y f Rex	XY, mei-41	583	627	1	6	1.1	15	1 2.7
Y l2 f car Rex/Y mei-41	XY	136	273	0	0	0.0	10	2 8.1

Individual females of the indicated genotype were mated to males bearing the indicated target chromosome.

$a_{XY} = Y^S X \cdot Y^L, In(1) En Y Y f B \cdot Y^t$
 $XY, mei-41 = Y^S X \cdot Y^L, Y Y mei-41 \cdot Y^t$

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