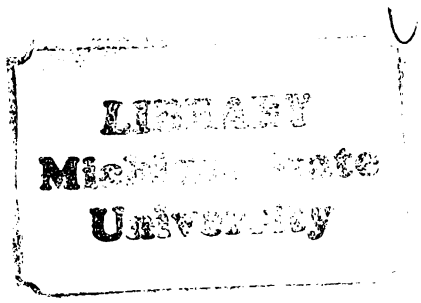

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Parameters Of The *Rex* Phenotype In

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Ellen E. Swanson

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Ph. D. degree in Genetics
Zoology

Leonard A. Robbins

Major professor

Leonard G. Robbins

Date May 4, 1984

PARAMETERS OF THE *REX* PHENOTYPE IN *DROSOPHILA MELANOGASTER*

By

Ellen E. Swanson

A DISSERTATION

Submitted to
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ABSTRACT

PARAMETERS OF THE *REX* PHENOTYPE IN
DROSOPHILA MELANOGASTER

By

Ellen E. Swanson

Rex is a dominant, maternal-effect locus located in the heterochromatin of the *X* chromosome of *Drosophila melanogaster*. It causes an early mitotic exchange between heterochromatic regions of $Y^{S}X.Y^{L}$, *y v f B*.y^{+} in *X/XY* embryos of *Rex* mothers. This exchange leads to the production of males carrying free *Y* chromosomes and gynandromorphs that are part *X/XY* female and part *X/Y* male. The exchange event occurs near or within the *bb* locus, the site of the ribosomal genes.

In this study, crosses and progeny counts were used to uncover and clarify further aspects of the *Rex* phenotype. Data are presented that illuminate several characteristics. 1) The site of *Rex* action in the responding chromosomes is the rDNA; possession of a duplication for the rDNA is necessary and sufficient for a chromosome to be sensitive to *Rex*. 2) Responding chromosomes can pair and exchange in two conformations, a spiral and a hairpin. 3) Some ribosomal cistrons are lost at each exchange event. 4) *Rex* acts on both maternally- and paternally-transmitted chromosomes. 5) *Rex* is a neomorph, and a Rex^{+} allele may not exist.

These results suggest avenues for further investigation of the *Rex* effect, including the use of *Rex* to synthesize unusual chromosomes, the further delineation of the criteria for responsive chromosomes, and the search for *Rex* alleles in other stocks and natural populations. In addition, the relationship of *Rex* to known phenomena is examined. *Rex* is a heterochromatic gene which affects mitotic chromosome behavior at the *bb* cistrons. The phenotype of *Rex* implies the potential involvement of this locus in controlling chromosome stability or the regulation of ribosomal cistron copy number. The study of *Rex* may also present an opportunity to better understand the action of heterochromatic genes in general.

To Lenny

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INTRODUCTION

In 1981, Robbins reported the discovery of a genetic element with a number of interesting properties. This element (technically a variant, as opposed to a mutant, since it was found in a pre-existing stock) is located in the *X* heterochromatin, and it has a dominant maternal effect. It seems to cause a mitotic exchange in an otherwise stable attached-*XY*, producing free *Y* chromosomes. Because this exchange event occurs predominantly between the ribosomal cistrons of the *X* and *Y*, the element was named *Rex*, for "dominant inducer of ribosomal exchange".

The attached-*XY* chromosome in which the *Rex*-induced detachment occurs is $Y^{S}X.Y^{L}$ (Lindsley and Novitski, 1959). The structure and origin of this chromosome is shown in Figure 1. In this and all subsequent figures, broad lines designate heterochromatin and narrow lines designate euchromatin. *In(1)EN* is a complete inversion of the *X* euchromatin. The short arm of the *Y* (Y^{S}) is attached to the distal *X* euchromatin, and the long arm of the *Y* (Y^{L}) is attached to the centromere. Y^{L} is marked with a small transposition of the *X* euchromatin carrying the *X* marker, y^{+}.

The detachment event is seen in crosses of *y/y cv v f Rex* females and $Y^{S}X.Y^{L}$, *y v f B*.y^{+}/*O* males. Along with the regular and non-disjunctional progeny of this cross, y^{+} male offspring are produced. These males carry an *X* chromosome from their mothers and a

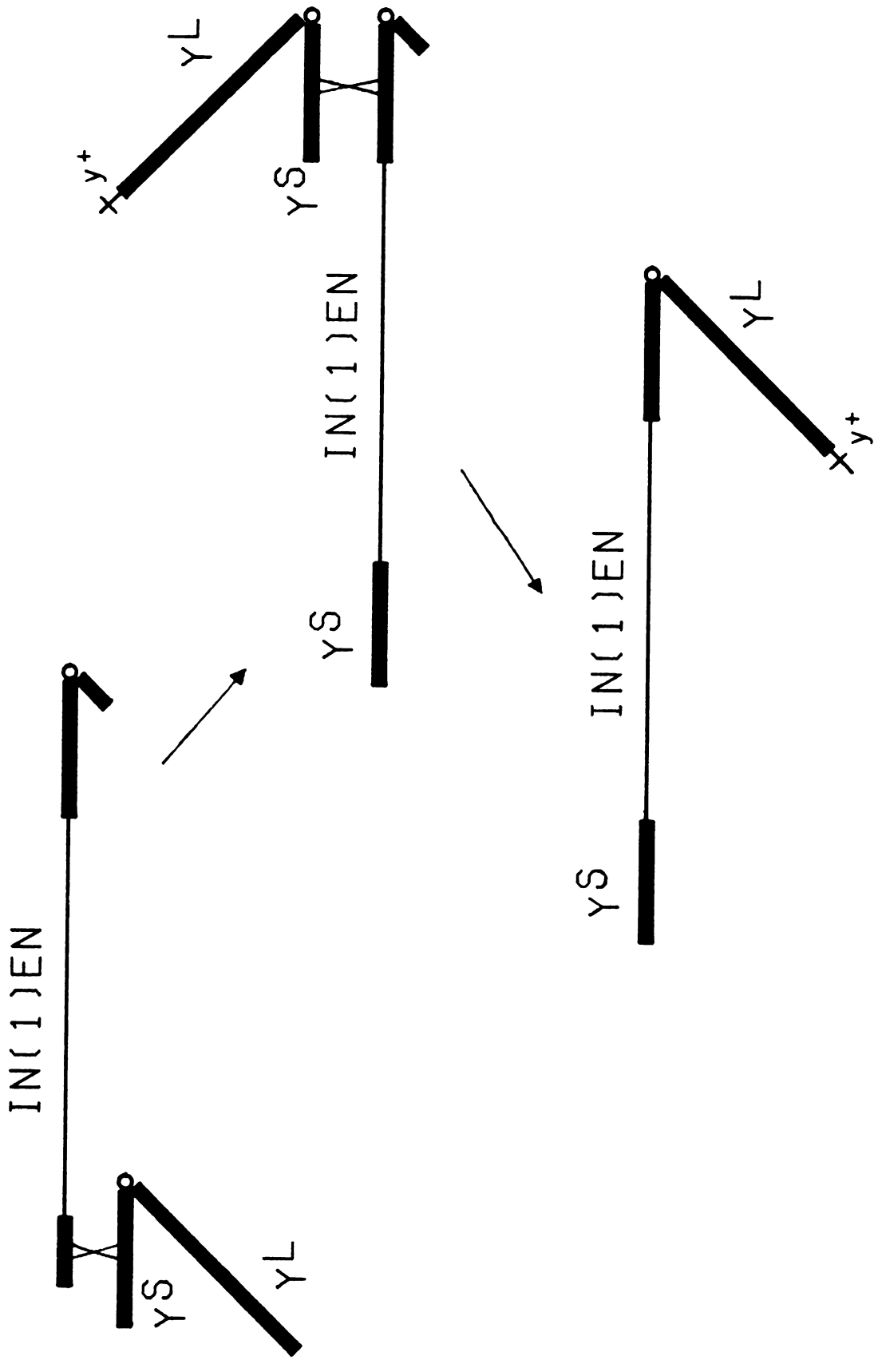


Figure 1. Synthesis of $y^{S}{}_{X}.y^{L}$.

free *Y* chromosome marked with a y^+ transposition of the *X* chromosome (y^+Y). Gynandromorphs, flies which are part male and part female, are also produced. The male parts of the gynandromorphs have phenotypes which indicate that these tissues also carry a maternal *X* and a y^+Y. The y^+Y's can only have been derived from the attached-*XY*. Since the attached-*XY* breakdown event is seen in the progeny of *Rex* mothers, it must occur post-fertilization in what would have been *X/XY* zygotes. Thus, *Rex* causes a maternal effect and is dominant. *Rex* segregates from the *X* chromosome balancer *FM7* (Merriam, 1968), and meiotic recombination mapping places *Rex* proximal to carnation, in or near the centric heterochromatin of the *X*.

Robbins' (1981) hypothesis for the mode of action of *Rex* is shown in Figure 2. The *Y* detachment is envisioned as an exchange-like event between the *X* centric heterochromatin and Y^S, resulting in loss of the *X* euchromatin in an acentric ring. Since the event occurs during post-fertilization mitoses, early events will produce males which have detachment *Y* chromosomes in all their body tissues ("whole-body" detachment males), whereas later events will produce gynandromorphs. Figure 2 shows detachment at a two-strand stage (*i.e.*, in G1 of the cell cycle). If the detachment occurs at the first mitotic division, a whole-body y^+ male will result. A second division detachment will produce a half *X/XY* (female), half X/y^+Y (male) gynandromorph ($X/XY:X/y^+Y$). Exchange at a four-strand stage produces only gynandromorphs, either $X/XY:X/y^+Y$ or $X/XX:X/y^+Y$, depending on which two strands exchange. Since whole-body males occur more frequently than gynandromorphs, it is unlikely that four-strand events occur. Any

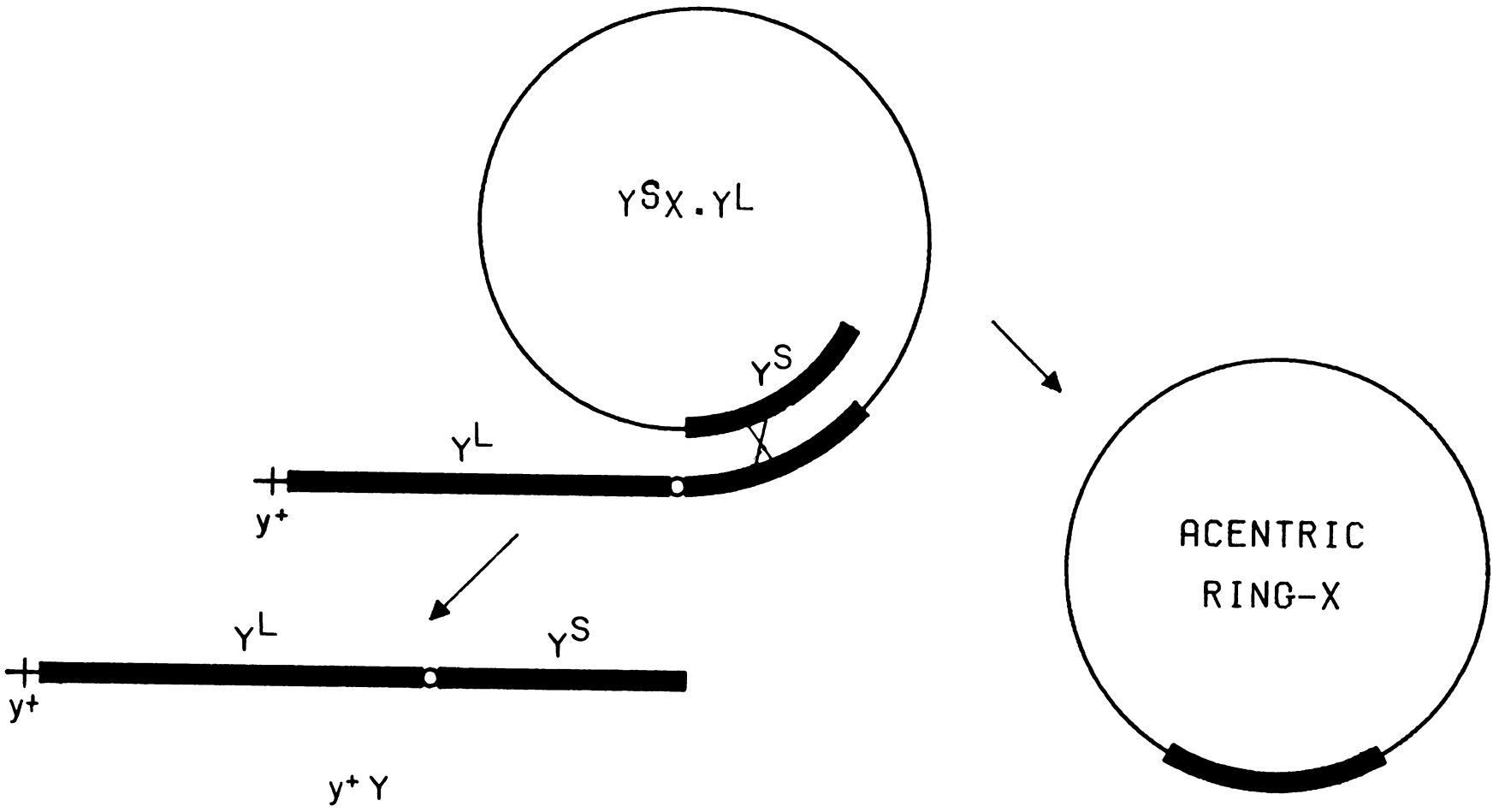


Figure 2. Spiral exchange in $Y^{S}X.Y^{L}$.

event occurring later than second division will produce a gynandromorph with predominantly female tissue. Robbins (1981) scored five body parts in a sample of gynandromorphs and found that each structure was male about half the time. Those data support the notion that the *Rex* event occurs during G1 of (usually) the first or (sometimes) the second mitotic division. Meiotic recombination in the euchromatin and heterochromatin was also examined, and was found to be unaffected by *Rex* (Robbins, 1981).

The site of action of *Rex* was studied by analyzing phenotypes of males bearing the $y^{+}Y$ chromosomes produced. The most obvious region of homology between the *X* heterochromatin and Y^{S} is the bobbed (*bb*) locus. The phenotype of homozygous *bb* flies is short, thin thoracic bristles, delayed development, and, in some backgrounds, etched abdominal cuticle (Lindsley and Grell, 1968). The *bb* locus is the site of the 18S and 28S ribosomal RNA genes in Drosophila and also corresponds with a cytological marker, the nucleolus organizer region (*NOR*) (Ritossa *et al.*, 1966). The terms "bobbed locus", "rDNA", "ribosomal genes" and "nucleolus organizer region" are used interchangeably in the literature and will be so used here. The genes are clustered together, and there are 130-300 copies/locus (Ritossa *et al.*, 1971). Two observations from the progeny of *Rex* mothers make it likely that the detachment event occurs in or near the *bb* locus. First, there are no ring-*X* chromosomes recovered. These would be expected if Y^{S} ever paired and exchanged with Y^{L}. Second, the $y^{+}Y$ chromosomes carry all the Y^{S} fertility factors. Thus the exchanges do not occur distal to these, even though the first Y^{S} factor is just distal to *bb* (Kennison, 1981). However,

Robbins' (1981) investigation does not rule out the possibility that some of the events may occur in the heterochromatin surrounding *bb*.

Robbins (1981) examined the *bb* constitution of 172 y^+Y chromosomes. Males bearing these chromosomes showed wide variation in *bb* phenotype. This was not due to pre-existing *bb* variation in the attached-*XY*, and it could be concluded that *Rex* causes exchange at many points within the ribosomal cluster. However, the phenotypic variation seen in the y^+Y's does not equally represent the full range of exchange possibilities. If the two regions overlapped at random, at least 50% of the products should be bb^+, because they received a normal or greater than normal number of rRNA genes. Actually this number should be a little greater than 50%, since some small deletions should also have a bb^+ phenotype. Fewer than 10% of the detachment chromosomes tested, however, were bb^+.

The paucity of bb^+ chromosomes can be explained by three different models. First, one of the two ribosomal complexes in the attached-*XY* may be much smaller than the other, so that even when the overlap occurs randomly, deficiencies might be much more common than duplications. Second, overlap may not be random, and exchange may occur at preferred sites. This might result in the majority of the rDNA going to the acentric ring-*X*, and the remainder, not enough to produce a bb^+ phenotype, going to the detachment *Y*. Third, though the products seem to indicate an exchange, it might not be a reciprocal exchange event, and ribosomal cistrons may always be lost in the process.

Rex is a Mendelian variant located in the *X* heterochromatin, and it has a dominant maternal effect. It seems to cause mitotic exchange in the ribosomal genes of certain chromosomes very early in development The experiments that follow were designed to further explore the nature of the *Rex* locus and its action. They are designed to answer the following questions: What is the specific chromosomal site that responds to *Rex* action? What conformation(s) do the responding chromosomes assume, and what happens to the genes at the site of the exchange event? What is the functional nature of the *Rex* locus? The experiments show that: (1) the *Rex*-induced event does not depend on the presence of *Y* chromosome material; (2) the sensitive site in the chromosome is the *bb* locus and only the *bb* locus; (3) responding chromosomes can pair and exchange in two conformations, a spiral and a hairpin; (4) some ribosomal cistrons are lost at each exchange event; (5) *Rex* can act on maternally- as well as paternally-transmitted chromosomes; and (6) *Rex* acts as a neomorph, and a Rex^{+} allele may not exist.

Methods and Materials

All flies for stocks and crosses were raised on a standard Drosophila medium of cornmeal, molasses and brewer's yeast. Mass matings were done in polyethelene bottles, 10-15 males with 10-15 females. The matings for counting offspring were done in glass shell vials, one female with three males or one male with three females. All matings were done at 25^{o}.

Unless otherwise noted, a description of all mutants and chromosomes used can be found in Lindsley and Grell (1968). This reference also provides a description of Drosophila nomenclature for those not familiar with its conventions. Except for *Rex* and *bb*, all loci mentioned here serve only to mark regions of chromosomes and their phenotypes *per se* are of no importance.

A note on bobbed nomenclature: The bobbed (*bb*) locus was first described by Sturtevant in 1920, but it was not recognized as a redundant gene until 1966. This situation, the large number of workers involved with the locus, and the lack of a good system for naming genes present on both the *X* and *Y* chromosomes, have combined to produce a seemingly unending list of descriptors added as superscripts to the designation "*bb*". The following is not a complete list, but is designed to help the reader with those *bb* mutants mentioned in this work. The reader should keep in mind that all *bb* mutants are ribosomal cistron deletions of various sizes.

(1) bb^{0}, bb^{NO-}, NO^{-}, bb^{-}: These designations indicate a complete deletion (as well as can be measured) of the ribosomal cistrons.

(2) bb^{1}: These mutants are lethal when homozygous or in combination with a complete deletion; unlike the mutants in (1), they are additive with other bb^{1}'s or *bb*'s to produce less severe *bb* phenotypes or bb^{+}.

(3) *bb*: When homozygous or in combination with a deletion, these mutants have a *bb* phenotype; with other *bb* mutants they may be more or less *bb*, or bb^{+}.

Each of the above can also be used as a superscript to the *X* or *Y* chromosome symbol (as: X^{NO-}, Y^{bb-}) to designate which chromosome

carries the mutant.

CHAPTER 1

THE RESPONDING SITE TO *Rex* ACTION

The hypothesis which has been developed to explain the chromosomes generated by *Rex* (see Introduction) postulates an exchange-like event between heterochromatic segments during early somatic divisions. This exchange is believed to occur between the *X* and *Y* copies of the ribosomal genes. A number of questions can be asked about the pairing and exchange of chromosomes in response to *Rex*. Is this response a peculiarity of the attached-*XY* chromosome in which it was first discovered ($Y^{S}X.Y^{L}$, *y v f B*.y^{+}), or does it occur in any similarly constructed chromosome? Is the response to *Rex* dependent on the presence of *Y* heterochromatin, or will chromosomes containing only *X* heterochromatin also respond? Is the orientation of the heterochromatin and/or of the ribosomal cistrons important? Is a duplication of the ribosomal cistrons alone enough to produce a response, or are other heterochromatic elements necessary?

Rex was first discovered because of its effect on $Y^{S}X.Y^{L}$, *y v f B*.y^{+}. A number of similar attached-*XY* chromosomes are available. A collection of these was tested to determine whether the response to *Rex* is a general characteristic of chromosomes with this structure. All of these chromosomes are derived from the original $Y^{S}X.Y^{L}$, *In(1)EN* synthesized by Lindsley and Novitski (1959). This chromosome was made by two consecutive exchanges between an inverted *X* chromosome, *In(1)EN*, and a *Y* chromosome (Figure 1). All the attached-*XY* chromosomes tested with *Rex* have the same structure with

regard to the *X* and *Y* segments. Most have the *X* euchromatin in inverted order, as it was in the original Lindsley and Novitski chromosome, though the chromosome with which *Rex* was discovered has the euchromatin in normal order. (This is believed to have been a spontaneous reinversion.) They do not all have the same *X* chromosome markers, and one does not have the y^+ transposition at the tip of the Y^L arm.

Whether the rDNA of the attached-*XY* chromosomes is derived from the *X*, the *Y*, or both is not evident. There are other chromosomes that have duplications of the ribosomal cistrons but do not involve the *Y* chromosome. One such chromosome is $In(1)sc^{S1L}sc^{4R}$ ($=sc^{S1}sc^{4}$). The scute inversions of the *X* chromosome have left breakpoints near the euchromatic locus scute (*sc*) and right breakpoints somewhere in the heterochromatin (Figure 3). They all invert most of the *X* euchromatin. $sc^{S1}sc^{4}$ was synthesized by an exchange between two scute inversions with different right and left breakpoints. It has a duplication, near the tip, of a large part of the centric heterochromatin, including all the ribosomal cistrons (Baker, 1971; Hilliker and Appels, 1982). Figure 4 depicts the pairing of $sc^{S1}sc^{4}$ and the products expected if it responds as does the attached-*XY*. Another chromosome of interest is $In(1)sc^{V2}$ ($=sc^{V2}$) (Figure 3). Its right breakpoint is in the middle of the ribosomal cistrons and it moves the distal heterochromatin and half the rDNA to the tip. (Lindsley *et al.*, 1982). Thus, it has ribosomal cistrons at both ends, but there is no duplication of the rDNA region.

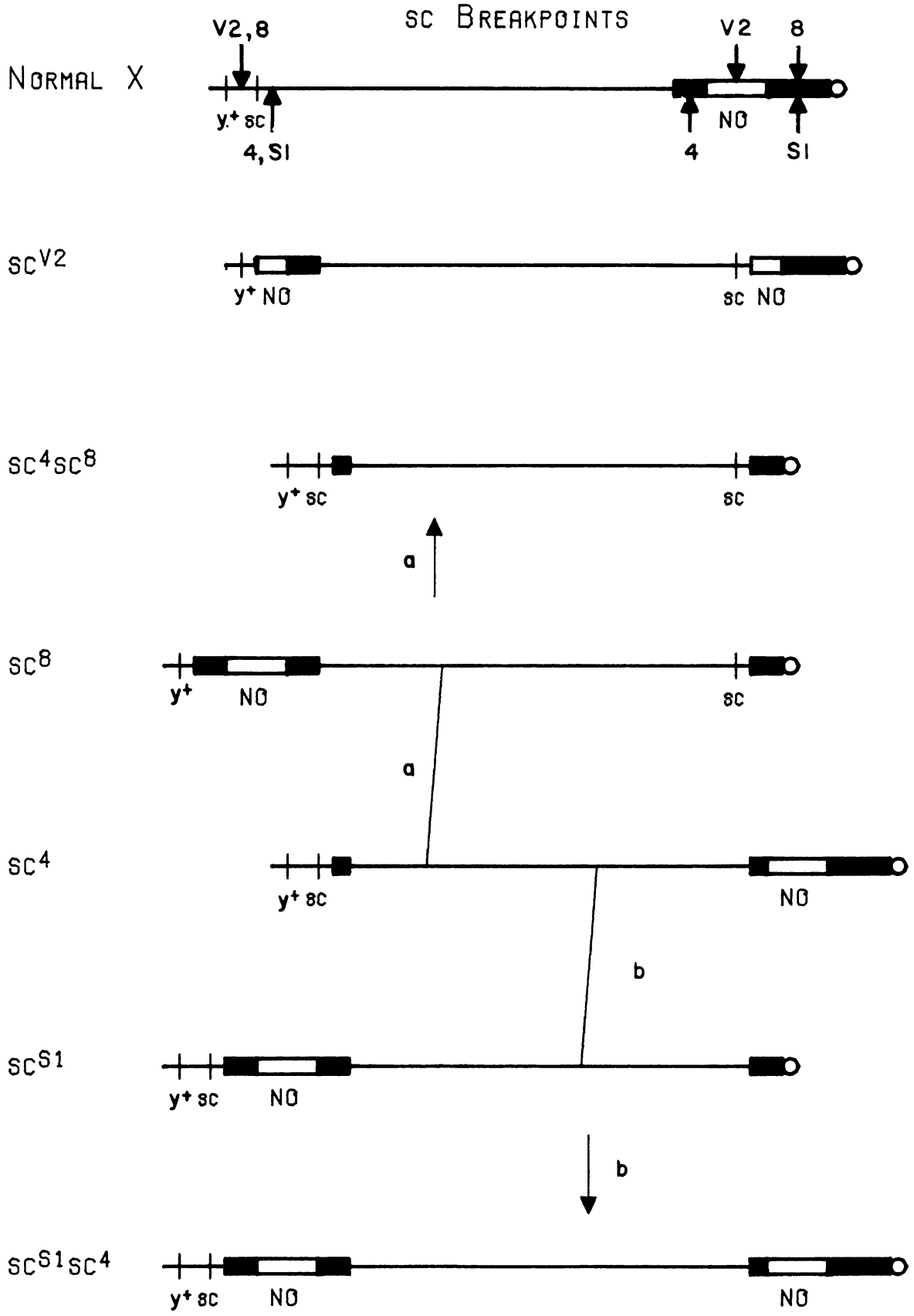


Figure 3. *sc* inversions of the *X* chromosome.

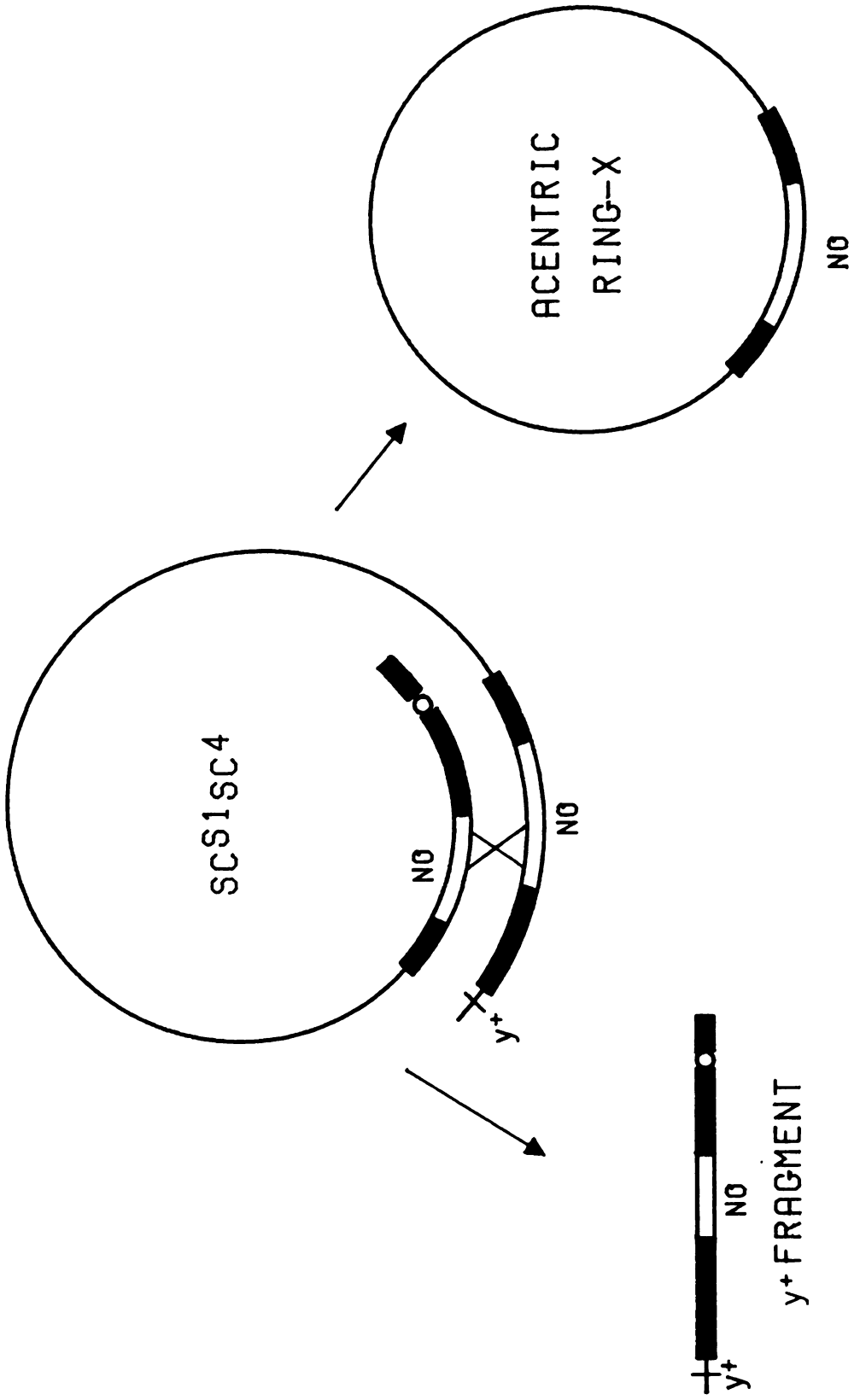


Figure 4. Spiral exchange in $sc^{S1}sc^{4}$.

Three other chromosomes were also tested: *In(1)w*^{m4} (=w^{m4}), *In(1)w*^{m51b} (=w^{m51b}), and *In(1)w*^{m51bL}*w*^{m4R} (=$w^{m51b}w^{m4}$). As shown in Figure 5, the breakpoints of w^{m4} and w^{m51b} define the proximal and distal ends of the *X* ribosomal region (Hilliker and Appels, 1982). *In(1)w*^{m4} moves the heterochromatin distal to the rDNA to a point near the *w* locus at the tip of the euchromatin; it leaves the bulk of the ribosomal cistrons near the centromere. *In(1)w*^{m51b} moves most of the rDNA to the tip but leaves a very small portion near the centromere. *In(1)w*^{m51bL}*w*^{m4R} is produced by an exchange between these two chromosomes and is duplicated for only the rDNA (Figure 5).

It should be noted that all the chromosomes used to test the *Rex* response have been kept in stocks for years with no sign of breakdown. I have observed each of these stocks for at least twelve generations and have never seen any products of spontaneous breakdown. For example, in one experiment $Y^{S}X.Y^{L}$, *y v f B.*y^{+} males were crossed to *FM7/y* females and no male progeny bearing $y^{+}Y$ chromosomes were seen in 10,000 total offspring. Similar results have been obtained with *In(1)sc*^{S1L}*sc*^{4R} and *In(1)w*^{m4}. All the chromosomes which respond to *Rex* are generally regarded as extremely stable elements when *Rex* is not present.

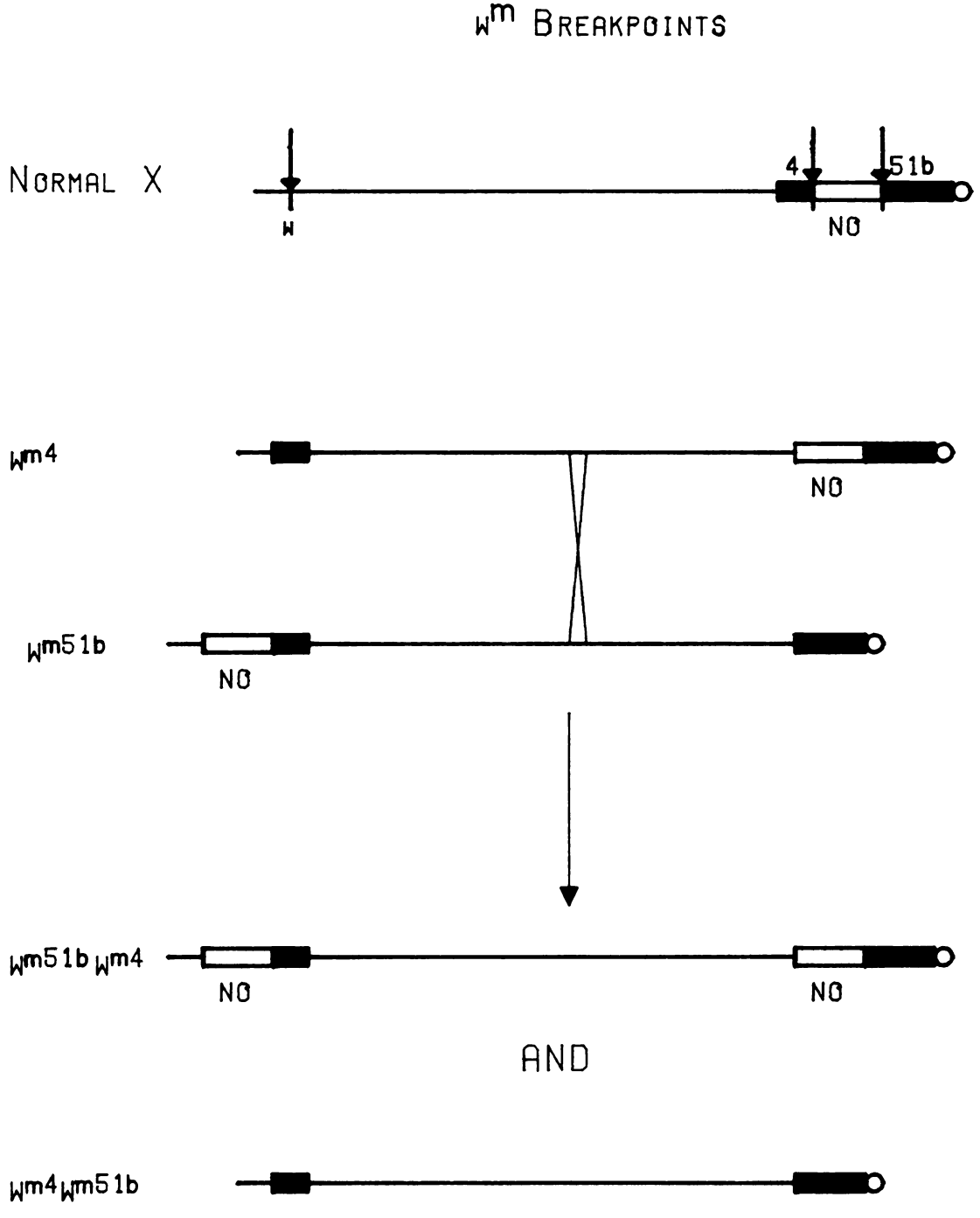


Figure 5. w^m inversions of the *X* chromosome.

CROSSES AND RESULTS

Response of attached-*XY* chromosomes to *Rex*

Four attached-*XY*'s were obtained to test for response to the *Rex* chromosome. These were all described as $Y^{S}X.Y^{L}$, *In(1)EN*. They had four different marker combinations: *y v f B*.y^{+}, *y*.y^{+}, *y v f*.y^{+}, and *y B* (no y^{+} transposition). All were tested in females with an uninverted *X* homolog for the production of single crossover progeny. No single crossovers were produced, so all the attached-*XY*'s still have the *In(1)EN* inversion. They were then tested for the order of the *Y* arms by separating the two arms by a single exchange with another inverted *X* ($In(1)sc^{4L}sc^{8R}$). The recombinants were tested for the ability to confer fertility on males that had received a Y^{S} or a Y^{L} arm from their mothers. All were found to have the *Y* arms in the order described.

Males carrying each of these four attached-*XY*'s and the original *y v f B*.y^{+} attached-*XY* were mated to homozygous *y w car Rex* females. The results are shown in Table 1. Detachment offspring bearing a maternal *X* chromosome and a free *Y* from the attached-*XY* were recovered as y^{+} *w* males or gynandromorphs in all crosses except for those with *y B* fathers. In the latter case, both regular-disjunction males and detachment males were *y w*; however, detachment males had a free *Y* and were fertile, whereas regular-disjunction males were *X/O* and sterile. To determine the number of detachment males produced in this cross, all *y w* males were collected and mated to test for fertility. It can be seen that detachments were recovered from all the attached-*XY*'s. Sensitivity to *Rex* is thus not a property inherent solely in the first

Table 1. A comparison of *Rex* sensitivities of five attached-*XY*'s.

Attached-*XY* chromosome	Regular-disjunction offspring females	Regular-disjunction offspring males	*X*-nondisjunction offspring females	*X*-nondisjunction offspring males	Detachment males	Gynandromorphs	Percent detachment*
y v f B·y+	3115	3484	0	5	13	3	0.51
In(1)EN, y v f B·y+	2888	3464	0	6	17	4	0.72
In(1)EN, y·y+	1805	2070	0	3	18	1	1.04
In(1)EN, y v f·y+	1711	1979	2	8	9	1	0.58
In(1)EN, y B	1844	2535	0	0	5**	---	0.27

y w car Rex/y w car Rex females were crossed to males carrying the indicated attached-*XY*'s.

* % detachment = $\frac{\text{detachment males + gynandromorphs}}{\text{regular females + numerator}}$ X 100.

** Detected as fertile males. Regular males are *X/O*.

attached-*XY* tested.

Two points need to be noted about the data presented in Table 1. First, the frequency of detachment in these experiments is variable but lower than expected. The original attached-*XY* ($Y^{S}X.Y^{L}$, *y v f B*.y^{+}) normally detaches at a rate of 1 to 3%; 2% is common and the rate has been as high as nearly 10% (Robbins, 1981). The data in Table 1 are significantly different from a rate of 2%. (A typical previous experiment had 1520 regular-disjunction females and 32 detachment products; in a test of homogeneity, $X^2 = 43.94$ with 5 d.f., $p < 0.005$.) At the time these experiments were done, the *y w car Rex* chromosome was being kept in a homozygous stock; that is, *y w car Rex/y w car Rex* females crossed to *y w car Rex/Y* males. A stock with a maternal effect variant present in the females is likely to accumulate modifiers by natural selection. Indeed, eventually the *y w car Rex* stock lost all *Rex* phenotype. (Suppressors of *Rex* have been found in a number of stocks; see Appendix. All *Rex* chromosomes are now stocked only in males, balanced by crossing to attached-*X*-bearing females. *Rex* chromosomes from these stocks induce the higher (1 to 3%) rate of detachment.) Despite the lower frequency of detachment seen in Table 1, the reduced *Rex* activity has no effect on the conclusion drawn from this experiment (*i.e.*, that all these attached-*XY*'s are sensitive to *Rex* action). Since a contemporaneous experiment was run using the original attached-*XY* (first line of Table 1), all frequencies can be compared to this standard.

Second, the frequency of detachment of $Y^{S}X.Y^{L}$, *In(1)EN, y B* is quite low compared to the others. The set of data in Table 1 is not homogeneous (χ^2 = 10.05 with 4 d.f., p = 0.041), but becomes so when the *y B* results are removed (χ^2 = 5.11 with 3 d.f., p = 0.22). This chromosome has no y^+ marker on the Y^L arm, making the detection of detachment males difficult, and the detection of gynandromorphs impossible. Detachment males were detected by collecting all *y w* males and testing them for fertility. Single *y w* males were put with two females in each well of a plastic titer plate. The tops of the plates were filled with Drosophila medium and the wells were inverted onto them. This method was not completely satisfactory, since some of the males got stuck to or trapped under parts of the titer wells and died. It is therefore quite possible that not all fertile males were detected, and of course this method would also fail to detect detachment males that were sterile, an event that occurs about 15% of the time (Robbins, 1981).

Response of *sc* inversions to *Rex*

In the next set of experiments, *Rex* females were crossed to *In(1)*$sc^{S1L}sc^{4R}$, *cv v B/Y* males or to *In(1)*sc^{V2}, sc^{V2}/Y males. As Figure 4 shows, the exchange event should produce heterochromatic fragments marked with y^+. Though this event is not really a detachment, since nothing was originally "attached", for continuity the measure of the event will still be called a "frequency of detachment". Fragment-bearing males in these experiments were y^+ and had none of their fathers' *X* chromosome markers. The data are shown in Table 2.

Table 2. *Rex* sensitivities of two *sc* inversions.

Paternal *X* chromosome		Regular-disjunction offspring females	Regular-disjunction offspring males	*X*-nondisjunction offspring females	*X*-nondisjunction offspring males	Fragment bearing males	Gynandromorphs	Percent detachment
In(1)sc^{S1}sc^{4}, cv v B	(A)	2098	1837	2	1	40	16	2.60
	(B)	1068	1022	0	8	13	5	1.65
In(1)sc^{V2}, sc^{V2}	(C)	1190	1285	0	30	42	5	3.8-6.1*

y cv v f Rex/y (experiment A) or *y cv v f Rex/y cv v f Rex* (experiments B and C) females were crossed to males with the indicated *X* chromosomes and free *Y*'s. % detachment was calculated as in Table 1. Experiments A and B are statistically homogeneous.

* See text for explanation.

The frequency of detachment of the sc^{V2} chromosome is difficult to calculate because the chromosome is not well marked. sc^{V2} itself is a poor marker, variable and weak in expression. Therefore, some of the wild-type males classified as *X*-nondisjunction products are probably y/y^{+} fragment males which are either non-crossover, or crossover distal to *cv* or proximal to *f*. The lower of the two frequencies of detachment shown for sc^{V2} has been calculated with only the known fragment-bearing males (y^{+} and/or *cv* and/or *v* and/or *f*) and the gynandromorphs; the upper limit has all the + males (classified as nondisjunction in Table 2) added in. The true frequency is probably somewhere between these two. In any case, both $sc^{S1}sc^{4}$ and sc^{V2} respond well to *Rex* action.

Response of w^{m} inversions to *Rex*

The last set of experiments was done by crossing *Rex* females to $In(1)w^{m4}$, w^{m4}/Y males, $In(1)w^{m51b}$, w^{m51b}/Y males or $In(1)w^{m51bL}w^{m4R}$, *cv*/*Y* males. The results are shown in Table 3. Both w^{m4}, which leaves the rDNA at the distal end, and w^{m51b}, which moves the rDNA to the proximal end, yield few detachments. Only $w^{m51b}w^{m4}$, with a full duplication of the ribosomal region, responds to *Rex*. In all cases, therefore, it is necessary that a chromosome have ribosomal cistrons at both ends for it to respond to *Rex*.

Table 3. *Rex* sensitivities of three w^m inversions.

Paternal *X* chromosome	Regular-disjunction offspring females	Regular-disjunction offspring males	*X*-nondisjunction offspring females	*X*-nondisjunction offspring males	Fragment bearing males	Gynandromorphs	Percent detachment
In(1)w^{m4}, w^{m4}	1255	1942	0	5	0	1	0.08
In(1)w^{m51b}, w^{m51b}	2053	2173	1	6	0	0	0
In(1)$w^{m51b}w^{m4}$, *cv*	2567	2624	3	6	15	4	0.73

y cv v f Rex/y females were crossed to males with the indicated *X* chromosomes. % detachment was calculated as in Table 1.

DISCUSSION

The effect of *Rex* is not due to some peculiarity present in the $Y^{S}X.Y^{L}$, *y v f B*.y^{+} chromosome. Other similar attached-*XY*'s respond to *Rex* as well as the original. It is also apparent that the orientation and markers in the euchromatin do not affect the response, nor does the euchromatic transposition used to mark Y^{L}.

Because of the nature of their construction, these attached-*XY* chromosomes are not good candidates for a detailed investigation of the responding site. *In(1)EN* inverts the entire *X* chromosome; its right breakpoint is in the rDNA and it carries a *bb* allele at either end (Lindsley and Grell, 1968). Thus, either of the two ribosomal regions in the attached-*XY* might be wholly or partially derived from *X* or *Y* cistrons (see Figure 1). This is important because the *X* and *Y* rDNA cistrons have a number of differences (Tartof and Dawid, 1976). It is also known that the ribosomal region in Y^{S} of the original attached-*XY* is partially deficient (Robbins, 1981). For these reasons other less complex chromosomes were used to examine the responding site.

In(1)$sc^{S1L}sc^{4R}$ was chosen because much of the *X* heterochromatin is duplicated in this chromosome. A number of inferences can be made from its positive response to *Rex*. First, elements of the *Y* chromosome need not be present for detachment to occur. Second, the orientation of the two ribosomal regions does not seem to be important. If the attached-*XY* has been made as in Figure 1, both ribosomal regions should be in their normal distal to proximal order. When the attached-*XY* chromosome pairs as in Figure 2, the regions will pair distal with

distal and proximal with proximal. In $sc^{S1}sc^{4}$, however, the distal ribosomal region has been inverted. When this chromosome pairs in a spiral, the two ribosomal regions are in opposite order to each other. This apparently does not affect their ability to "exchange". It may be that the ribosomal cistrons are not all in tandem, but are oriented in both directions. (For further evidence that homologous order is not important, see Chapter 2.)

The experiment done with sc^{V2} indicates that a duplication, in the strict sense, is not even necessary for the *Rex* event. Nothing has been duplicated in sc^{V2}; the ribosomal region has simply been divided and half has been inverted and moved distally. The responsive region (the rDNA) is therefore divisible, and both halves of the rDNA are as competent as a full region would be. In fact, considering the high frequency of detachment, they may be more competent. The only identical regions proximally and distally in this chromosome are the rDNA genes. However, it is possible that *Rex* could work non-specifically on heterochromatin. In fact, one of the molecular characteristics of heterochromatin is that it contains much repetitive material (Kram *et al.*, 1972; Brutlag *et al.*, 1977); similar repeats could be dispersed on either side of the *bb* locus so that sc^{V2} actually does have regions of homology at either end. *Rex*, then, could work on these "duplicated" regions.

If this were the case, then it should be possible to recover y^{+} fragments from $In(1)w^{m4}$ or $In(1)w^{m51b}$. Like sc^{V2}, these chromosomes do not have any duplicated material. Some of the heterochromatin, including (w^{m51b}) or excluding (w^{m4}) the *bb* locus, has simply been

moved to a distal location. Therefore, they should be functionally identical to sc^{V2} except that they have the rDNA in only one place. As Table 3 shows, however, these chromosomes do not respond to *Rex*. On the other hand, $In(1)w^{m51bL}{}_{w}{}^{m4R}$ is responsive to *Rex*. $w^{m51b}{}_{w}{}^{m4}$ is a single crossover product made from w^{m4} and w^{m51b}, and contains the same heterochromatin blocks along with an rDNA duplication (Figure 5). Hence, possession of two sets of ribosomal cistrons is both necessary and sufficient in a chromosome to provide a site of response to *Rex*.

CHAPTER 2

THE *Rex* EXCHANGE EVENT

By analyzing the products of the *Rex* event, Robbins (1981) made a number of conclusions about the event itself. Figure 2 illustrates the current hypothesis for the *Rex*-induced event. When the responding chromosome is an attached-*XY*, this event leads to the production of a male carrying a $y^{+}Y$ or a gynandromorph which is $X/XY:X/y^{+}Y$. From the variation in *bb* phenotype of the $y^{+}Y$ males, Robbins determined that the event is not site specific within the ribosomal cistrons. Analysis of the gynandromorphs set the time of occurrence of the event at or before G1 of the second mitotic division (see Introduction). Comparison of the structures of the chromosomes produced by *Rex* with the structures of the parental chromosomes should allow inferences to be drawn about the mechanism involved. To make this comparison, it is necessary to know the structure of the chromosomes before the event occurred. As was discussed above, there are ambiguities surrounding the formation of the attached-*XY* which preclude a full knowledge of its structure. Therefore, the $sc^{S1}sc^{4}$ chromosome, whose structure is well-defined, was used in the present investigation.

It was the purpose of the experiments described below to answer four questions about the *Rex* event. The first two questions were: (1) Are there alternative pairing conformations (other than the spiral illustrated in Figure 2) in which the *Rex* exchange can take place?; and (2) Can *Rex* act on a chromosome transmitted from the mother? Figure 4 shows $sc^{S1}sc^{4}$ in the spiral configuration. From this kind of pairing

and exchange two offspring classes are possible: males carrying a y^+ heterochromatic fragment and $X/X:X/y^+$ fragment gynandromorphs. Both of these products have been observed. Another possible pairing configuration is shown in Figure 6. An exchange in this case would result in a chromosome in which the euchromatin has been reinverted. The possibility of this exchange was investigated by mating $sc^{S1}sc^4$ males to *Rex* females, collecting heterozygous female progeny, and looking at their offspring for crossover classes from recombination between the putative reinverted $sc^{S1}sc^4$ and the normal-sequence, *Rex*-bearing X. If there were also males carrying the y^+ fragment among the progeny of these $sc^{S1}sc^4/Rex$ females, this would show that *Rex* could act on a responsive chromosome when it is transmitted to the embryo by the mother.

The third and fourth questions were answered by examining the products of the hairpin and spiral types of exchange. (3) Does the spiral pairing and exchange of $sc^{S1}sc^4$ generate the same preponderance of deleted (bb) chromosomes from $sc^{S1}sc^4$ as were produced from the attached-XY? Robbins' (1981) investigation of the y^+Y chromosomes resulting from detachment of the attached-XY showed that fewer than 10% of the y^+Y chromosomes were bb^+. This indicated that the two ribosomal regions did not overlap and exchange at random; if they had, at least 50% of the products should have been bb^+. The excess of deletions could have occurred if the two rDNA regions in the attached-XY were so unequal in size that a duplication was infrequent. The discovery that the Y^S portion of the attached-XY is bb does not seem to indicate an extreme enough difference in size to make this explanation likely. To

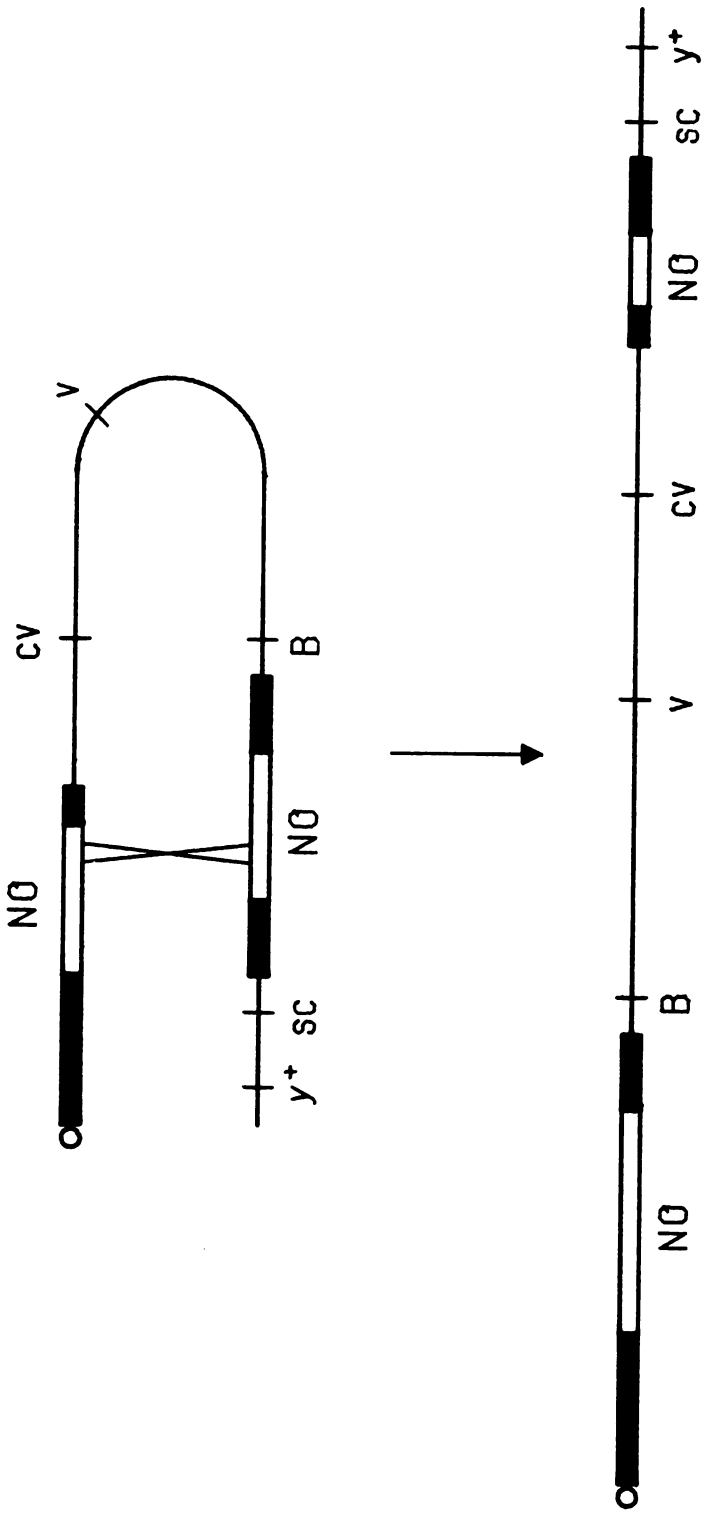


Figure 6. Hairpin exchange in $sc^{S1}sc^{4}$.

resolve this question, males bearing y^+ fragments from the $sc^{S1}sc^4$ pairing and exchange were collected and used to determine the *bb* constitution of these fragments. Since $sc^{S1}sc^4$ has two complete rDNA regions, examination of the y^+ fragments should test whether the production of over 50% *bb* chromosomes is a feature of all *Rex* exchange or is due to a peculiarity of the rDNA of the attached-*XY*.

If the $sc^{S1}sc^4$ does produce an overabundance of *bb* chromosomes, then (4) Why does *Rex*-induced recombination result in an excess of deletions? There are two possible explanations. Pairing may not be at random and exchange, though reciprocal, may occur at preferred sites. Perhaps only the recoverable products from spiral exchange (y^+ fragments or y^+Y's) are always deleted, and the duplication products are in the acentric rings. Alternatively, the exchange may not be reciprocal, so that cistrons are always lost. The products of hairpin pairing and exchange were examined to test these hypotheses. Both products of the exchange were recovered by separating the two ends of the reinverted $sc^{S1}sc^4$. The ends were tested for *bb* phenotype to examine the reciprocity of the *Rex* "exchange" event. If the event is reciprocal, when one end is a deletion, (*bb* or bb^1), the other end should be a duplication. If there are cistrons lost in the process, both ends may carry deletions.

CROSSES AND RESULTS

Test for hairpin pairing

The mating scheme to test for hairpin-type pairing is shown in Figure 7. Homozygous *Rex* females were mated to $In(1)sc^{S1L}sc^{4R}$, *cv v B* males in bottles (generation (1)). If hairpin pairing and exchange can occur, some of the female offspring of this cross should carry the *Rex* chromosome and a homolog with a heterochromatic duplication and the euchromatin in normal, rather than inverted, sequence. Henceforth this new chromosome will be designated $Dp(1;1)sc^{S1}sc^{4}$. One thousand virgin female offspring were collected and mated singly with *y* males (generation (2)). The Drosophila *X* chromosome is over 60 cM long. If the $sc^{S1}sc^{4}$ chromosome has re-inverted, at least 50% of the offspring of a heterozygous female in generation (2) should be single crossovers. If the $sc^{S1}sc^{4}$ has not re-inverted, there should be only a few offspring that look like single crossovers, and those will actually be double crossovers with one crossover outside the marked regions. Offspring were scored as y^{+} *B*, *y* B^{+} (non-crossovers), *y B*, and y^{+} B^{+} (crossovers). Six generation (2) females were found to have $Dp(1;1)sc^{S1}sc^{4}$ chromosomes (frequency of reinversion = 0.6%). Table 4 shows a sample of the results of the progeny test, including the six reinversions and six chromosomes which remained inverted.

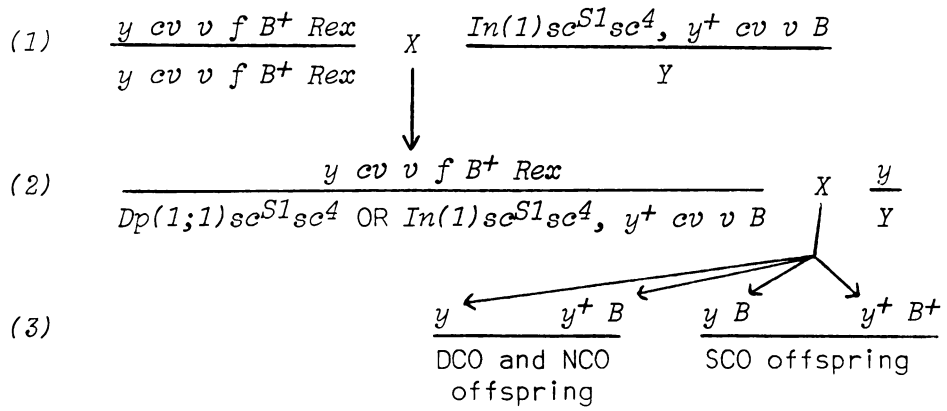


Figure 7. Mating scheme to test for hairpin exchange.

Table 4. Test for hairpin pairing

Maternal *X* chromosome	Line #	NCO and DCO		SCO	
		y	*y⁺ B*	*y B*	*y⁺ B⁺*
Dp(1;1)sc^{S1}sc^{4}	53A	32	17	28	16
	108A	38	35	28	16
	128A	46	27	42	7
	230	38	26	28	15
	708	6	4	5	6
	744	9	11	11	10
In(1)sc^{S1}sc^{4}	107	25	27	1	1
	325	27	26	1	0
	462	31	25	0	1
	619	18	21	4	0
	726	15	9	0	1
	918	32	16	0	0

Heterozygous females of *y cv v f Rex* and the chromosomes indicated were crossed to *y/Y* males. Apparent SCO's in the inversion cromosomes are DCO's with one event outside the marked regions.

Effect of *Rex* on maternally-transmitted chromosomes

Males carrying a y^+ fragment were also found among the progeny in generation (3) of Figure 7; they are the only y^+ males that are not also *cv*. These males arose from spiral exchange of $sc^{S1}sc^4$ in the embryos of the *Rex*-bearing mothers of generation (2). Thus, they indicate that *Rex* can act on chromosomes transmitted to the embryo from the mother as well as from the father. An accurate estimate of the frequency of the y^+ *males is difficult because it is so low. The progeny of 345* $sc^{S1}sc^4/y$ *cv v f Rex* females was summed to 21,314. Since the offspring were not scored with regard to sex, this number was halved to estimate the number of daughters. There were 13 fragment-bearing males and 2 gynandromorphs, a frequency of detachment of 15/10672 X 100 = 0.141%. Apparently, maternally-transmitted chromosomes are sensitive to *Rex*, though they may be less sensitive than paternally-transmitted chromosomes. The low frequency of detachment is considered further in the Discussion section below.

Production of *bb* deletions from $sc^{S1}sc^4$

The production of 90% *bb* deleted chromosomes from $Y^SX.Y^L$, *y v f B.*y^+ (Robbins, 1981) may be due to a peculiarity of this attached-*XY*. To determine whether $sc^{S1}sc^4$ spiral exchange also produces a high frequency of *bb* deletions, y^+ fragments were examined. The usual mating which produces y^+ fragment-bearing males is *y/y cv v f Rex* females X *In(1)*$sc^{S1L}sc^{4R}$, *cv v B/Y* males. These y^+ fragments cannot be studied further, however, because the males which bear them lack a *Y* chromosome and are sterile. Figure 8 shows the mating scheme used to

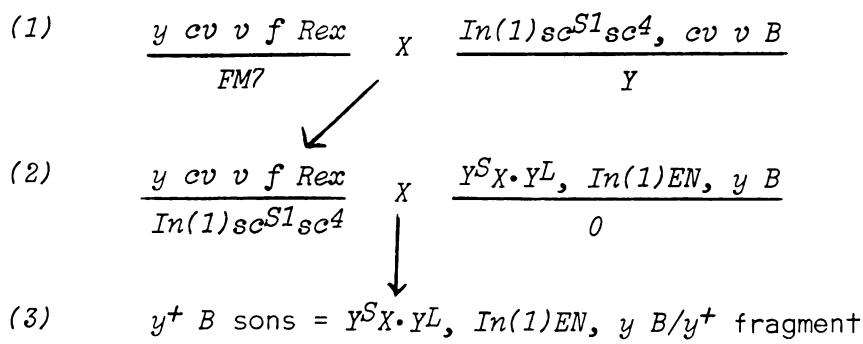


Figure 8. Mating scheme to collect y^+ fragments.

collect y^+ fragments in fertile males. This scheme takes advantage of the result from the previous experiment, that *Rex* can act on maternally-transmitted chromosomes. Since the frequency of this event is so low, generation (2) was done in bottle matings of 15 females with 15 males. From these bottles, approximately 25 y^+ *B* males were collected. Each of these males was mated to three *C(1)RM, y v/O* females, and y^+ progeny were selected to set up a stock. *C(1)RM is a* bb^+ attached-*X*. Only eight of these males were fertile. Male progeny from these eight stocks were then crossed to *C(1)DX, y f/Y* females to test for *bb* penetrance. *C(1)DX* is an attached-*X* totally lacking the rDNA. Progeny of this cross were scored for the presence and *bb* phenotypes of y^+ *f* females. The results are shown in Table 5. The phenotypes range from bb^1 to *bb*. As was the case with the y^+Y chromosomes recovered from the attached-*XY* (Robbins, 1981), there are no duplications recovered from spiral exchange in $sc^{S1}sc^4$.

Reciprocity of the *Rex* event

The products of hairpin pairing and exchange were also examined to determine the reason for the high frequency of *bb* deletions produced. Three types of males were collected from among the progeny of the six females carrying *Dp(1;1)*$sc^{S1}sc^4$ chromosomes. The three phenotypes were y^+ *cv v B*, the non-crossover progeny; *y B*, single crossover progeny bearing the proximal end of *Dp(1;1)*$sc^{S1}sc^4$; and y^+ *cv v f* B^+, single crossover progeny bearing the distal end of *Dp(1;1)*$sc^{S1}sc^4$. Each male was used to set up a separate stock by mating him with three *C(1)DX, y f* females. Only the *y B* males from line #744 were fertile,

Table 5. Bobbed variability of y^+ fragments

Fragment #	*y B* Males	*f* Females	*f bb* Females	Female recovery	*bb* penetrance
1	287	0	40	0.139	1.0 (strong *bb*)
2	306	188	232	1.373	0.55 (*bb*)
3	343	1*	0	0.003	1.0 (lethal)
4	657	2*	0	0.009	1.0 (lethal)
5	229	30	146	0.769	0.86 (*bb*)
6	510	1*	0	0.002	1.0 (lethal)
7	391	0	0	0.000	1.0 (lethal)
8	335	231	141	1.110	0.40 (*bb*)

Males bearing a *y B* attached-*XY* and the indicated y^+ fragments were crossed to *C(1)DX, y f/Y* females.

Female recovery = females/males

bb penetrance = $\frac{bb \text{ females} + \text{lethal females}}{\text{total females} + \text{lethal females}}$; lethal females = males - females

*These females are probably the result of maternal nondisjunction, and have a *C(1)DX/Y/y*$^+$ fragment genotype.

so no further testing could be done on this line. For the remaining five *Dp(1;1)sc*^{S1}*sc*^{4} lines, one of each of the stocks (that is, the progeny from one of each class of males recovered in generation (3) of Figure 7) was used for testing.

The non-crossover chromosomes from each of the five lines were tested to make sure these chromosomes were actually in normal sequence. Heterozygous *y*/*y*$^{+}$ *cv v B* females were crossed to *y* males. The results are shown in Table 6. All of the five lines carry normal sequence chromosomes.

Meiotic exchange in generation (2) of Figure 7 separates the two ends of the reinverted *X* chromosome. Thus, each class of single crossover males collected carries one of the two products of hairpin pairing and exchange. Both ends can be examined for their *bb* constitutions to determine if the exchange event is reciprocal. The *y B* males, carrying the proximal product from each of the five lines, were tested for *bb* penetrance by crossing them to *C(1)RM, y v*/*O*. Sons from this mating were *X*/*O* and their only source of rDNA was from the *y B* chromosome. The results are shown in Table 7. The phenotypes for this experiment range from *bb*^{1} to *bb*.

Testing the distal end, carried by the *y*$^{+}$ *B*$^{+}$ males, required more manipulation. Before this end could be tested, the proximal rDNA which had come from the *y* chromosome had to be removed. The mating scheme to accomplish this is shown in Figure 9. The proximal rDNA from the *y* chromosome was replaced with the rDNA of *y bb*^{1452}. This chromosome had been previously tested and shown to be *bb*^{1} and to have no other *X*-linked lethals. (A cross of *y bb*^{1452}/*FM7* X

Table 6. Retest of "reinversions" (*Dp(1;1)sc^{S1}sc^{4}*).

	NCO and DCO		SCO	
Line #	*y*	*y^{+} B*	*y B*	*y^{+} B^{+}*
53A	225	101	143	89
108A	218	111	151	81
128A	394	230	256	143
230	133	58	91	46
708	109	53	96	62

y/y^{+} cv v B females from the putative reinversion lines were crossed to *y/Y* males and the indicated phenotypes of their offspring were scored.

Table 7. Bobbed variability of proximal end of reinverted chromosomes.

Line #	*y v* Females	*y B* Males	*y B bb* Males	Male recovery	*bb* penetrance
53A	414	1*	1	0.005	1.0 (lethal)
108A	522	119	257	0.720	0.77 (*bb*)
128A	461	89	55	0.312	0.81 (strong *bb*)
230	510	1*	2	0.006	1.0 (lethal)
708	199	0	238	1.196	1.0 (strong *bb*)

Recombinant *y B/Y* males from the indicated reinversion lines were crossed to *C(1)RM, y v/0* females.

Male recovery = males/females

bb penetrance = $\frac{bb \text{ males} + \text{lethal males}}{\text{total males} + \text{lethal males}}$; lethal males = females - males

* These males probably result from paternal nondisjunction and have a *y B/Y/0* genotype.

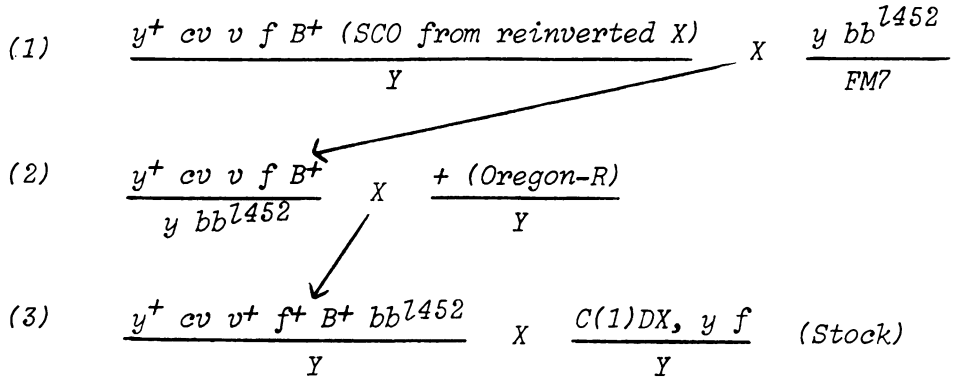


Figure 9. Mating scheme for distal end of reinversions.

In(1)sc^{4L}*sc*^{8R}, *y*/*y*$^{+}$*YB*^{S} produced no *y B*$^{+}$ females and many non-*FM7* males.) Males with single crossover *y*$^{+}$ *cv v*$^{+}$ *f*$^{+}$ *B*$^{+}$ *bb*^{1452} chromosomes were mated to females carrying *In(1)w*^{m4L}*w*^{m51bR}, which is deficient for rDNA (Hilliker and Appels, 1982; see Figure 5). The results are shown in Table 8. Again, a range from *bb*^{1} to *bb* is present. The data for the two ends is summarized in Table 9. It appears that the exchange event is not reciprocal, since in no case did one end receive a duplication of ribosomal cistrons when the other end received a deletion.

Table 8. Bobbed variability of distal end of reinverted chromosomes.

Line #	y^2 *B* Males	w^m Males	*B* Females	B^+ Females	B^+ *bb* Females	Female recovery	*bb* penetrance
53A	68	269	272	3*	253	0.952	0.99 (strong *bb*)
108A	88	238	214	0	0	0.000	1.0 (lethal)
128A	77	232	246	0	230	0.991	1.0 (strong *bb*)
230	131	250	251	0	264	1.06	1.0 (strong *bb*)
708	175	603	516	4*	0	0.007	0.99 (lethal)

Recombinant *(bb?) cv* bb^{l542}/Y males from the indicated reinversion lines were crossed to $w^{m4}w^{m51b}$, *y f* $bb^-/FM7$, y^2 *B* females.

Female recovery = B^+ females/B^+ males

bb penetrance = $\frac{B^+ \text{ bb females + lethal females}}{\text{total } B^+ \text{ females + lethal females}}$

* These females probably result from paternal nondisjunction and have a $w^{m4}w^{m51b}/(bb?)$ *cv* bb^{l452}/Y genotype.

Table 9. Comparison of *bb* variability of two ends of reinverted chromosomes.

	Recovery		Penetrance		Phenotype	
Line #	Distal	Proximal	Distal	Proximal	Distal	Proximal
53A	0.952	0.005	0.99	1.0	Strong *bb*	Lethal
108A	0.000	0.720	1.0	0.77	Lethal	*bb*
128A	0.991	0.312	1.0	0.81	Strong *bb*	Strong *bb*
230	1.06	0.006	1.0	1.0	Strong *bb*	Lethal
708	0.007	1.196	0.99	1.0	Lethal	Strong *bb*

Data are summarized from Tables 7 and 8.

DISCUSSION

Results from the experiments above show that hairpin pairing and exchange can occur. In this configuration, the two ribosomal regions are paired in identical register (proximal to proximal and distal to distal) because the distal heterochromatic block has been inverted. This does not, however, increase the frequency of exchange; in fact, it would appear from the frequency of reinversion that the spiral configuration is favored: fragments are produced more frequently than reinversions. Apparently strict chromosome homology is not necessary for the *Rex* event. This is contrary to the conclusions from the evidence presented by Palumbo *et al.* (1973), who found that the rRNA genes had a definite polarity for exchange. However, these data support the conclusions of Maddern (1981), whose experiments showed that exchange could occur with the rDNA in either orientation. This certainly raises the possibility that, on the molecular level, the cistrons are in alternating direction. Laird and Chooi (1976) have reported a polarity of rRNA synthesis and a tandem gene arrangement of the ribosomal cistrons. Their evidence is based on electron micrographs of nascent rRNA fibers being synthesized from ribosomal DNA transcription units. However, even their longest DNA strands show only a few rDNA genes at a time, while each locus contains 130-300 genes (Ritossa *et al.*, 1971). From the present results, it seems likely that the genes are arranged in blocks, with the genes within each block being in tandem, but with adjacent blocks being in reverse order to each other.

The experiment which shows hairpin pairing also shows that *Rex* can act on chromosomes transmitted maternally. The frequency at which this occurs appears to be very low (0.141%); however, there are reasons why the number calculated may not be a completely accurate estimate. The way the experiment was conducted provides two of these reasons. (1) The offspring were not scored with regard to sex, and patroclinous (*y*) males, produced by *X*-nondisjunction or four-strand double exchanges with inverted chromosomes, were included with the other progeny. Thus, half the total progeny is an inflated estimate of the number of regular females. (2) Many of these matings were only counted once and discarded, since very few flies are needed to establish a 50% single crossover rate. Gynandromorphs are likely to be late hatching; detachment males may also have this problem. Thus, counting only once may have deflated the number of detachment products. However, another experiment done earlier did not have either of these flaws and still showed a very low frequency of detachment. The experiment was essentially the same except that it used the $Y^{S}X.Y^{L}$, *y v f B.*y^{+} chromosome instead of *In(1)*$sc^{S1L}sc^{4R}$. Progeny were scored with regard to sex and patroclinous males were scored separately. The frequency of detachment was 0.062% (1/1+1614 X 100).

Another possible explanation for the low frequency is genetic. The *In(1)*$sc^{S1L}sc^{4R}$ stock may, like many other stocks, carry a suppressor of *Rex* (see Appendix). Alternatively, the actual frequency of detachment of maternally-derived chromosomes may be low. This is the more interesting explanation, since it implies that the transcribed and/or translated product(s) of the *Rex* gene in the egg cytoplasm may

distinguish between maternally- and paternally-derived chromosomes. An experiment with the maternal and paternal nuclei carrying differently-marked responsive chromosomes could discriminate between these alternatives. In that case, both chromosomes would be subject to the same effects of any suppressor, and differences in their response would necessarily reflect a distinction between maternally- and paternally-transmitted chromosomes.

The range of *bb* phenotypes among the y^+ fragments is comparable to the range of *bb* seen in the y^+Y chromosomes collected by Robbins. Eight y^+ fragments is a small sample and the low fertility of the y^+ fragment-bearing males (8/25) is perplexing. Effects of chromosomal content on male fertility are known (Lifschytz and Lindsley, 1972), but sterility in those studies was caused by either a loss or translocation of part of the *X*. This situation is believed to keep the *X* from condensing early in spermatogenesis as it should. It is possible that, in the present case, the y^+ fragment either fails to condense or causes the *X* to fail to condense. This particular kind of sterility effect, caused by a heterochromatic duplication, has not been previously reported.

There is enough variation in this sample, small as it is, to say that exchange does not occur at only one site. There also is a lack of *bb* duplications, if their expected frequency is 50%. However, because of the unexplained sterility of nearly 70% of the males, there is doubt about whether these eight are a representative sample of the fragments produced. Larger fragments containing *bb* duplications may have been produced, but they may have been those which caused sterility.

The products of reinversion are more revealing, since both exchange products can be studied. A comparison of the two ends of the reinversion chromosomes (Table 9) shows a striking result. Though *In(1)sc*^{S1L}*sc*^{4R} carries two complete *bb* loci, in all cases reinversion results in both regions being deleted for rRNA cistrons. It must be emphasized that each line of *Dp(1;1)sc*^{S1}*sc*^{4} chromosomes was derived from a single female, so that the two chromosome ends represent the two products of a single event. It is obvious that the reinversion does not involve a reciprocal exchange; some portion of the rDNA repeats is always deleted. There also appears to be a trend toward loss of the same size portion each time, since a *bb* lethal at one end is often accompanied by a *bb* at the other. Neither the number of observations nor the precision of measurement of phenotype is sufficient to push this idea too far, but the event caused by *Rex* is obviously more complex than simply pairing and exchange. Perhaps the rDNA regions pair in a loop, and when exchange occurs, the looped-out section is lost. If so, there may be times when the looped sections are excised but the breaks are not resolved as a crossover; this should result in a chromosome which remains inverted but loses rDNA at both ends. This hypothesis can be tested with an experiment like that shown in Figure 7, but in this case separating and testing the ends of a sample of *Rex*-exposed but still-inverted *sc*^{S1}*sc*^{4}'s would be needed.

CHAPTER 3

THE NATURE OF THE *Rex* LOCUS

One way to get an understanding of the function or action of a locus is to study its phenotype while varying the dose of the locus. The wild-type allele of a gene expresses a normal function. According to a classification scheme first proposed by Muller (1932), a mutant can differ in expression from the normal state in five ways. The mutant can be an amorph, which has no expression of the normal function; a hypomorph, whose expression is less than normal; a hypermorph, whose expression is greater than normal; a neomorph, which expresses a function not present in the wild-type state; or an antimorph, which expresses a function exactly the opposite of normal.

Of these five, an antimorph is the most difficult to demonstrate conclusively. The proof requires the ability to add copies of the mutant allele and the normal allele to the genome, to show that the effects of one cancel the effects of the other. In Drosophila, this requires construction of small free duplications, since flies will not tolerate extensive aneuploidy. These duplications must contain the gene in question, must be adequately marked and must contain as little extraneous material as possible. They are difficult to produce and maintain, especially in multiple copies. With current techniques for transferring plasmid-like elements into the genome, testing for antimorphs may soon become more routine in Drosophila. Though this study does not directly test *Rex* for antimorphy, the other tests that are done nevertheless rule out antimorphy as a possibility.

Many dominant mutants are hypermorphs or neomorphs; however, a dominant could also be a hypomorph or amorph if there is a threshold level of expression necessary for the normal phenotype. These four types of expression can be distinguished by a series of dosage tests. To perform these tests, it would be ideal to have an inactive deficiency and a fully active duplication for the region where the mutant is located. If the mutant were a hypomorph or amorph, mutant individuals would have a phenotype similar to individuals carrying the deficiency. For a hypomorph, increasing the number of copies (the dose) of the mutant allele would bring the phenotype closer to the wild-type state; a true amorph would not show this response. The difference between a severe hypomorph and an amorph can be difficult to demonstrate. If the mutant were a hypermorph, individuals carrying it would be similar in phenotype to those carrying a duplication. The phenotype of individuals carrying a hypermorphic mutant would approach the normal phenotype when the mutant is heterozygous with a deficiency. If the mutant is a neomorph, there would be no clear dose response; neither the phenotype of the duplication nor that of the deficiency would resemble the mutant phenotype, nor would the duplication nor the deficiency suppress the mutant. The presence or absence of the mutant phenotype would depend only on the presence or absence of the mutant.

The chromosome which carries *Rex* (*y cv v f Rex*) is also *bb*, which raises the possibility that *Rex* is caused by a loss of function in the ribosomal cistrons. Since *Rex* maps to the centric *X* heterochromatin (Robbins, 1981), duplications and deletions of this region were used to construct a dose series. Two chromosomes are of use here,

In(1)sc^{4L}*sc*^{8R} (=*sc*^{4}*sc*^{8}) and *In(1)sc*^{S1L}*sc*^{4R} (=*sc*^{S1}*sc*^{4}). The former is a deletion of most of the heterochromatin, and the latter is a duplication. Each was constructed from two scute inversions (Figure 3). The mating schemes used to construct the relevant females to test for the *Rex* phenotype are shown in Figures 10 and 11. These schemes produced females with a series of different doses of the *X* heterochromatin. The females were mated to *In(1)sc*^{S1L}*sc*^{4R}, *cv v B* males and scored for the production of y^{+} B^{+} sons and gynandromorphs. If the *Rex* allele is an amorph or hypomorph, it will be mimicked by *sc*^{4}*sc*^{8} and suppressed by *sc*^{S1}*sc*^{4}; experiments 1, 2, 3 and 4 will give similar positive results, and experiments 5, 6 and 7 will show no *Rex* activity. Conversely, the *Rex* allele is a hypermorph, the duplication will mimic and the deficiency suppress the response; experiments 1, 4, 5, 6 and 7 will show positive results while 2 and 3 will show no activity. If the *Rex* allele is a neomorph, only those experiments where the female parent carries *Rex*, *i.e.*, 1, 3, 4 and 6, will show any activity, and those genotypes lacking *Rex*, *i.e.*, 2, 5 and 7, will be negative.

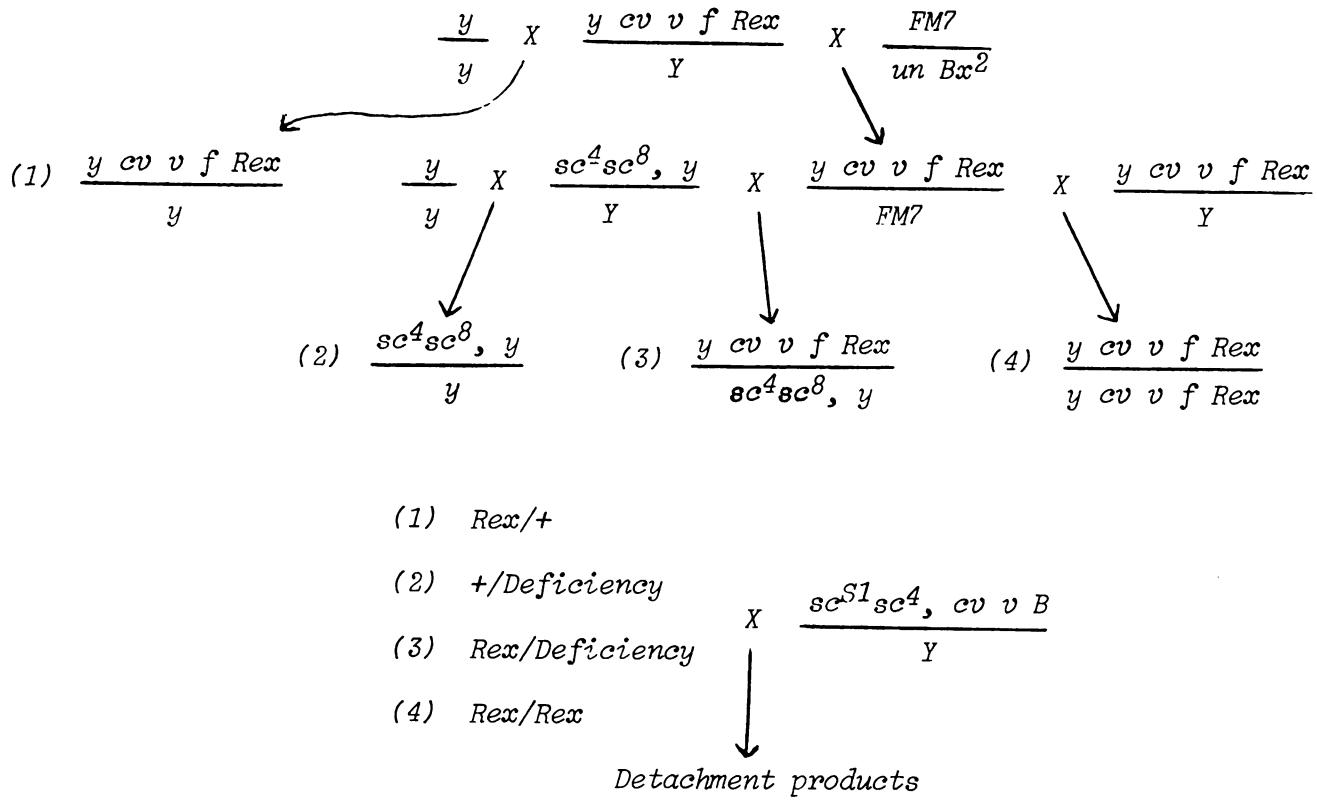
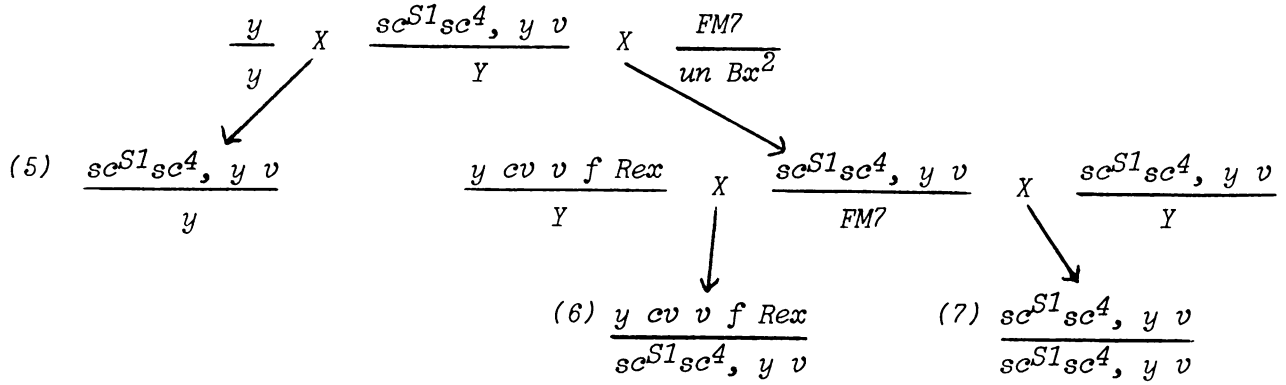


Figure 10. Mating scheme for control and reduced-dose tests.



(5) Duplication/+

(6) Duplication/Rex X $\frac{sc^{S1}sc^4,\ cv\ v\ B}{Y}$

(7) Duplication/Duplication

Figure 11. Mating scheme for increased-dose tests.

RESULTS

Table 10 summarizes the results of all seven experiments. Detachment frequencies in experiment 3 required special calculation. sc^4sc^8/fragment males will not survive unless the y^+ heterochromatic fragment generated has enough ribosomal cistrons to cover their complete absence in the *X*. The first of the two detachment frequencies shown for experiment 3 is calculated on the assumption that all sc^4sc^8-bearing detachment males survive. For the second frequency, the number of detachment males and gynandromorphs is doubled in the numerator and the denominator on the assumption that no sc^4sc^8 detachments survive. Given that a minority of the y^+ fragments probably are bb^+ (Robbins, 1981 and Chapter 2 of this work), the true frequency should be an intermediate value, somewhat closer to the second figure.

Table 10 shows that neither $sc^{S1}sc^4$, the duplication, nor sc^4sc^8, the deficiency, mimics nor eliminates the effect of *Rex*. Experiments 1, 3, 4 and 6, where *Rex* is present in the mothers, all produce *Rex*-induced detachments and gynandromorphs among the progeny. (The somewhat low activity seen in experiment 6 may indicate the presence of a suppressor in the $In(1)sc^{S1L}sc^{4R}$, *y v* stock; see Appendix) Experiments 2, 5 and 7, where *Rex* is not present in the mothers, show no detachment activity. These results are consistent with the hypothesis that *Rex* is a neomorph; that is, the *Rex* allele is expressing a new function.

Table 10. Results of dosage experiments.

Dose of parental females	Regular offspring		*X*-nondisjunction		Detachment males	Gynan-dromorphs	Percent detachment
	females	males	females	males			
(1) *Rex/+*	2098	2129	2	1	40	16	2.6
(2) *+/Df*	3569	3338	0	87	0	0	0.0
(3) *Rex/Df*	2477	2395	1	116	18	4	0.88-1.7*
(4) *Rex/Rex*	1068	1022	0	8	13	5	1.7
(5) *Dp/+*	2177	1671	1	23	0	0	0.0
(6) *Dp/Rex*	3311	2876	2	123	21	2	0.66
(7) *Dp/Dp*	1246	1024	0	1	0	1	0.08

For matings, see Figures 10 and 11. % detachment calculated as in Table 1.

* See text for explanation.

DISCUSSION

The results in Table 10 show clearly that *Rex* is most likely a neomorph. There are two assumptions made in the design of this experiment which, if incorrect, could alter this conclusion. First, it is assumed that since *Rex* maps proximal to *car* (Robbins, 1981), it is located in the heterochromatin. *car* is, however, about 3 cM from the locus generally used as the most proximal euchromatic marker, suppressor of forked (*su(f)*; Lindsley and Grell, 1968). *Rex* might be located in these 3 cM of euchromatin. Second, it is assumed that *Rex* is located in the portion of the heterochromatin deficient in sc^4sc^8 and duplicated in $sc^{S1}sc^4$. As Figure 3 shows, the heterochromatic breakpoints of these two chromosomes are not at the very boundaries of the heterochromatin. A small amount of heterochromatin is present in sc^4sc^8, and is in only one copy in $sc^{S1}sc^4$. *Rex* might be located in this area of the heterochromatin. The only experimental evidence bearing on this point comes from the original mapping of *Rex*: 75 of 76 crossovers between *car* and the centromere also separate *car* and *Rex*. This puts *Rex* 75/76 of the way from *car* to the centromere, and is fairly good evidence that *Rex* is in the heterochromatin. A stronger case will have to await more precise mapping.

A neomorph presents a difficult problem in analysis. Mutants are often used to deduce normal gene function; by studying the phenotype and dose response of a mutant one can make deductions about the wild-type gene product. The assumptions necessary for thes deductions

may not be valid with a neomorph. *Rex* could be a lesion in a wild-type gene which causes it to function in a totally new and different way. For example, perhaps Rex^+ is a gene which works at a particular time in development, and its functioning at a different time produces a wholly different effect. On the other hand, there may be no entity which corresponds to a Rex^+ allele. The *Rex* phenotype may be caused by a wholly new element introduced into the genome. As such, it could work in one of two ways; it could either control the normal cellular "equipment" for a new purpose, or it could bring in its own "equipment". The former would imply that there are other genes that make transcription and/or translation products capable of performing at least some of the sequence of events induced by *Rex* (G1 pairing between two *bb* loci in the *X* and subsequent exchange). The latter would imply that the *Rex* element is large and complex enough to produce all the transcription and/or translation products necessary to perform these events. More information on the structure of *Rex*, most likely on a molecular level, is needed to choose between these possibilities.

The chromosome which carries *Rex* (*y cv v f Rex*) also carries a *bb* mutation. This raised the possibility that *Rex* was caused by a loss of function in the ribosomal cistrons. The experiments with sc^4sc^8 above clearly show that this is not the case. That chromosome is completely devoid of rDNA, but does not have any *Rex*-like activity. However, it may be that the *Rex* element is an insertion into the rDNA that causes a loss of function of some genes. This opens the possibility that *Rex* can be cloned, a normally impossible task with a heterochromatic gene having an unknown product because of the repeated nature of

heterochromatin.

CHAPTER 4

SIGNIFICANCE AND RECOMMENDATIONS

Rex is a dominant, neomorphic, maternal-effect variant causing a pairing and exchange-like event in the ribosomal cistrons which is always accompanied by a loss of some of these cistrons. This event occurs in G1 of the first or second mitotic division of the offspring of *Rex* mothers. The pairing can occur in two configurations, resulting in either the loss of the *X* euchromatin between the two rDNA regions or its inversion. Both maternally- and paternally-transmitted chromosomes are affected, though there may be some preference for paternally-transmitted chromosomes.

All of the information to date indicates that *Rex* is an intriguing locus which has a rather bizarre effect on early mitotic chromosome behavior. It is interesting enough to warrant study on its own merits. The studies presented here have suggested some lines of future research. *Rex* can act on a number of different chromosomes; the only criterion so far discovered is that the chromosome must carry two rDNA regions. The range of chromosomes on which *Rex* can act has not been fully explored. Many of the problems are technical: chromosomes and/or genotypes must be devised to enable the detection of the product of the *Rex* event. For example, one might like to ask how far apart in the chromosome the two rDNA regions need to be for *Rex* to act on them. This will probably be difficult to answer. Because of Drosophila's sex determination system, mitotic loss of a whole *X* (which *Rex* causes) simply converts female cells to male cells. If the rDNA were moved to

the center of the euchromatin instead of the distal end, though, *Rex* would cause loss of only half an *X*. This would result in cells that were either extremely hypoploid female or extremely hyperploid male, and neither is likely to survive. It might be possible to answer this question using the hairpin-type exchange, if a chromosome with an rDNA duplication in the center of the euchromatin can be constructed.

An easier question to answer about the range of *Rex* is whether *Rex* can cause interchromosomal events. Since most *Rex*-induced events occur at first G1 before pronuclear fusion, it will be necessary to transmit both chromosomes through one gamete. One of the ways to test this would be to set up a cross of *y/y cv v f Rex* females to $y/Y/y^{+}YB^{S}$ males. One of these *Y*'s has its short arm marked with y^{+} and its long arm marked with B^{S}. Since the two *Y*'s of an *X/Y/Y* male tend to separate at meiosis I (Lindsley and Grell, 1968), half the sperm will be $y/y^{+}YB^{S}$. If an interchromosomal exchange between the *X* and Y^{S} occurs in this pronucleus, the result will be an *X* with $Y^{L}B^{S}$ attached and a free $y^{+}Y^{S}$. These $y/X.Y^{L}B^{S}/y^{+}Y^{S}$ females will look like their $y/y/y^{+}YB^{S}$ sisters, and progeny tests will be required to determine if *Rex* has caused an interchromosomal exchange. If it has, the females with the $y/X.Y^{L}B^{S}/y^{+}Y^{S}$ genotype will produce B^{S} daughters and y^{+} sons among their progeny, whereas the $y/y/y^{+}YB^{S}$ females will produce y^{+} B^{S} sons.

Another possible interchromosomal event would be the production of attached-*X* chromosomes. This could be accomplished by having an exchange event between the rDNA of a normal sequence *X* and an *X* with the rDNA moved distally, like $In(1)sc^{S1}$ (Figure 3). This event would

form a compound reverse acrocentric chromosome and a free y^+ fragment. Again, since this is a mitotic event, a progeny test would be required to find those females that produced only *y* matroclinous daughters, indicating that they were carrying an attached-*X* which had lost the y^+ marker.

The normal-sequence *X* in the original cross of the above experiment would have to carry *Rex*. This raises another question which would have to be answered before that experiment could be done. Can *Rex* act on the chromosome it is in? Since there is some suspicion that the site of the *Rex* locus may be the *bb* cistrons, it may be that these cistrons are altered so that they can't participate in the *Rex* event. A test of this possibility is straightforward. *Rex* could be put into $Dp(1;1)sc^{S1}sc^4$ by meiotic recombination, and the resulting $Dp(1;1)sc^{S1}sc^4$, *cv v B Rex* chromosome could be tested for detachment production.

The results in Chapter 2 have shown that *Rex* can act on maternally-transmitted chromosomes, albeit at a lower frequency than on paternally-transmitted chromosomes. This raises the possibility that *Rex* has a preference for paternally-transmitted chromosomes, and that some part of the *Rex* system can distinguish between paternal and maternal chromosomes in the embryo. An experiment with differentially-marked responsive chromosomes brought into the embryo simultaneously in the maternal and paternal nuclei could test this hypothesis. In this case any suppressing background effects on *Rex* should be equal for the two sensitive chromosomes, since they would both be exposed to egg cytoplasm from the same mother. A reciprocal

experiment, which reversed which chromosome came from which parent, would correct for any possible difference in sensitivity of the two chromosomes.

The suggestion was made in Chapter 3 that *Rex* may be located in the *bb* locus. Because of the repetitive nature of heterochromatin, it is impossible to "walk" into this region to clone a gene located there. The ability to clone heterochromatic genes generally depends on having a known gene product, which is lacking for *Rex*. The ribosomal cistrons, however, have been cloned, and if *Rex* is an element inserted into these cistrons, it should be possible to find it by looking for differences in the restriction fragments of the ribosomal cistrons from the *Rex* chromosome. If *Rex* can be cloned, the molecular structure of the *Rex* locus could provide some insight into its function. In particular, it would be interesting to know just how large the *Rex* element is. If *Rex* is large, it might be producing all the transcription and/or translation product(s) necessary to express its phenotype; if it is small and simple, it is more likely to be changing or controlling normal cellular products to its own ends. A clone of *Rex* could also be used to search for *Rex* loci in other stocks or in natural populations. Since *Rex* was not discovered as the direct result of a mutagenesis, but was found in a pre-existing stock, it is possible that its occurrence is widespread. This would have implications for the function of *Rex* and its evolutionary significance.

There are also a number of aspects of the *Rex* phenotype that suggest it may be connected to other phenomena which have been studied. The potential role of *Rex* in three of these will be examined below.

The first is the control of gene copy number at the *bb* locus, the site of action for *Rex*. The second is the functioning of genes in the heterochromatin, where *Rex* is located. The third is early mitotic chromosome stability, which *Rex* affects and which Rex^+, if a Rex^+ exists, may control.

The *bb* locus

Since the site of action of *Rex* is the *bb* locus, it is worthwhile to review the work that has been done on these genes. The *bb* locus has been the subject of many studies, both genetic and molecular, and it has a number of interesting properties. This locus and its regulators are the only *X*-linked genes known in Drosophila which have homologous genes on the *Y*. They are also the only essential genes known in the *X* heterochromatin or on the *Y* (Lindsley *et al.*, 1960). The phenotype of homozygous *bb* flies is short, thin thoracic bristles, delayed development and, in some backgrounds, etched abdominal cuticle (Lindsley and Grell, 1968). Ritossa *et al.* (1966) first showed that the *bb* locus was the site of the DNA coding for ribosomal RNA. It is now believed through many sources of evidence (Ritossa and Spiegelman, 1965; and for reviews see Atwood, 1969; Birnstiel *et al.*, 1971; Long and Dawid, 1980) that this locus also exactly corresponds to the cytological locus of the nucleolus organizer region (*NOR*).

The *bb* locus consists of a series of repeats of the genes for the 18S and 28S rRNA's (Wellauer and Dawid, 1977; White and Hogness, 1977; Glover and Hogness, 1977). Each repeat consists of an external transcribed spacer, the 18S gene, a short internal transcribed spacer

which probably contains the 5.8S and 2S rRNA genes (Pellegrini *et al.*, 1977), and the 28S gene (Long and Dawid, 1980). The foregoing is transcribed into one long rRNA precursor, which is then processed. There follows on the DNA a non-transcribed spacer region of variable length (4-20 kb; Indik and Tartof, 1980). The genes on the *X* and *Y* both follow this basic pattern. RNA fingerprinting techniques have shown that the rRNA from the *X* and *Y* are identical. However, there are a number of differences in the DNA. The 18S and 28S and the internal and external spacers of the *X* are homologous to the same regions of the *Y*. The non-transcribed spacer varies in size and has internal repeats (Wellauer and Dawid, 1978; Long and Dawid, 1979b); the distribution of size classes of this spacer is not the same in the *X* and *Y*. There are also insertion sequences in some of the 28S genes. The first to be described were the type 1 sequences; these are not found in the *Y* (Tartof and Dawid, 1976; Dawid *et al.*, 1978). Most type 1 insertions have a length of 5 kb; there are also some of 1.0 and 0.5 kb. The smaller insertions are homologous to parts of the 5 kb insertion (Dawid *et al.*, 1978). As well as being dispersed throughout the 28S genes of the *X* chromosome (Tartof and Dawid, 1976), these sequences are found in the heterochromatin of all four arms of the second and third chromosomes and at a euchromatic site on the fourth chromosome (Peacock *et al.*, 1981). From 49% (Wellauer *et al.*, 1978) to 60% (Kidd and Glover, 1981) of the 28S genes on the *X* contain type 1 inserts. Another class of inserts has also been described; these type 2 inserts have no homology to type 1 (Dawid *et al.*, 1978). They have lengths of 1.5 to 4 kb (Wellauer and Dawid, 1978; Wellauer *et al.*, 1978) and

insert at a slightly different place in the 28S gene (Dawid and Rebbert, 1981; Kidd and Glover, 1981). They are found in the rDNA of both the *X* and *Y* chromosomes (Wellauer *et al.*, 1978), and 16% of the 28S genes of both have type 2 inserts (Wellauer and Dawid, 1978; Kidd and Glover, 1981).

It is thought that the genes with 28S inserts do not contribute to the active cellular rRNA (Long and Dawid, 1980). Transcripts with type 1 inserts are extremely rare (Long and Dawid, 1979a; Long *et al.*, 1981). Type 2 transcripts are found at somewhat higher levels, but they are not long enough to contain a complete 28S gene (Kidd and Glover, 1981). If insertion-containing genes are truly inactive and the distribution figures for these sequences quoted above are accurate, then 65% to 75% of the *X* rDNA sequences are nonfunctional.

The number of total copies of the rDNA repeat varies within and among strains, and a wild-type locus contains 130-300 genes (Ritossa *et al.*, 1971). If the total number of copies in a fly is below 130, the *bb* phenotype is expressed.

Regulation of the *bb* locus is complicated and not completely understood. An in-depth study of the literature uncovers much conflict, due not only to the complexity of this locus but also the personal disagreements of the investigators. There are two major phenomena involved. These are compensation and magnification/reduction. Both are changes in the copy number of the rDNA cistrons. (Selective amplification, another type of copy number change seen in the oocytes of some species, in which extra copies of the rDNA are produced extrachromosomally in a cascade fashion, has not

been found in Drosophila. Birnstiel *et al.*, 1971; Long and Dawid, 1980).

Compensation was first described by Tartof in 1971. Usually, the amount of rDNA is directly proportional to the number of *NOR*'s in the genome (Ritossa and Spiegelman, 1965). However, in *X/O* males and X/X^{NO-} females there are on average 150 more rRNA genes per *X* as compared to normal *X/X* females or *X/Y* males (Tartof, 1971). This increase in rDNA is somatic and is not inherited; *X/X* daughters of X/X^{NO-} mothers do not get the extra 150 genes. Copy number in all experiments was determined by RNA/DNA saturation hybridization.

The *X* in X/Y^{bb-} males also compensates (Tartof, 1973a). There has been much controversy about the nature of the Y^{bb-} chromosome. Ritossa (1968a) reported several stocks with Y^{bb-} chromosomes which had nearly wild-type amounts of rDNA (about 100 genes). However, X^{bb}/Y^{bb-} flies have a *bb* phenotype and X^{NO-}/Y^{bb-} and $X^{NO-}/Y^{bb-}/Y^{bb-}$ are inviable. Ritossa explained this apparent contradiction by concluding that these Y^{bb-} chromosomes contained nonfunctional ribosomal genes. Tartof (1973a) disputed this conclusion, and reported that the Y^{bb-} chromosome carried only about 41 genes; the phenotypes of flies with this chromosome can be explained by assuming deletion of ribosomal genes, therefore, and not a loss of function. The "error" in Ritossa's results, Tartof explained, was that Ritossa measured Y^{bb-} in X/Y^{bb-} flies, where the *X* is compensating. However, a later report (Henderson and Ritossa, 1970) confirms Ritossa's earlier finding, in this case in attached-XY/Y^{bb-} flies, where there is no compensation. Endow (1982b) attempted to resolve this controversy by measuring rDNA

content in a different way. She ran Southern transfer blots of Y^{bb-} DNA and probed it with ^{32}P-labelled cloned rDNA. The Y^{bb-} DNA did hybridize with the cloned DNA, indicating that this *Y* carries some ribosomal genes. Endow then probed the *Y* DNA with cloned type 2 insert. She concluded that the insert does hybridize, the Y^{bb-} does carry the type 2 insert, and therefore its genes are largely nonfunctional (Long and Dawid, 1980; Franz and Kunz, 1981). It is difficult to evaluate these differing results. Endow's measurements are not quantitative. The high error inherent in saturation hybridization determinations (discussed below) hinders a direct comparison of Ritossa's and Tartof's data. A simple resolution to the conflict has been proposed (R. S. Hawley, personal communication). It is possible that Tartof and Ritossa have been examining Y^{bb-} chromosomes of different origin. If this is the case, it may be that Tartof's Y^{bb-} is a simple deletion, whereas Ritossa's carries nonfunctional genes.

Two mechanisms for the regulation of compensation have been proposed. In 1973, Spear and Gall published a report comparing rDNA levels in diploid and polytene tissues of *X/O* and *X/X* flies. They found that, in diploid tissues, the number of rRNA genes is proportional to the number of *NOR*'s present (*i.e.*, one in *X/O* and two in *X/X*). However, in polytene tissues, the number of rRNA genes is constant regardless of the number of *NOR*'s. In *D. melanogaster* different regions of the chromosome show independent polytenization (Tartof, 1975). Euchromatic regions go through about ten rounds of DNA replication with no cell division. Centric heterochromatic regions,

along with most of the *Y* chromosome, replicate little, if at all. The rDNA is intermediate, going through seven or eight rounds of replication (Spear and Gall, 1973). Spear and Gall explained compensation with the hypothesis that the higher level of rDNA per *X* chromosome in *X/O*, X/Y^{bb-}, and X/X^{NO-} comes from looking at a mixture of diploid and polytene tissues in adult flies. The consequence of looking at diploid tissue, with its ribosomal gene ratio of 2:1 in *X/X vs. X/O*, and polytene tissue, with its higher copy number but a ratio of 1:1, is that overall *X/O* flies appear to have more than half the rDNA of *X/X* flies, and the *X* seems to be compensating. There are a number of problems with this hypothesis. The proportion of adult tissue that is polytene and the extent of its polytenization is unknown. It is also not known whether all polytene tissues underreplicate the rDNA; this has only been examined in salivary glands. Spear and Gall's hypothesis does not account for the observation that diploid tissues can show compensation (Spear, 1974). The 5S RNA genes, which are wholly euchromatic, also show compensation (Tartof, 1975; Long and Dawid, 1980); they have never been shown to undergo independent polytenization. On the other hand, the *Y* chromosome rDNA does undergo independent polytenization, but it does not show compensation.

Endow (1982a) later offered an extension and further explanation of Spear and Gall's hypothesis. She showed that the EcoR1 rDNA restriction patterns of six different chromosomes, the *X*'s and *Y*'s from Canton-S, Oregon-R and OK-1 strains, are all unique. Combination of any two of these chromosomes in diploid tissues gives a restriction

pattern which looks like a mixture of the two chromosomes. In polytene tissues, however, one pattern predominates. She was able to construct a dominance hierarchy among the six chromosomes. She believes that only one *NOR* is active in polytene tissues. This would explain Spear and Gall's results, since both *X/O* and *X/X* flies would only have one active *NOR* in polytene tissues. Compensation, then, would not be the result of turning up the rDNA copy number in *X/O* flies but of turning it down in *X/X*. Though more detailed, Endow's hypothesis suffers from the same faults as that of Spear and Gall (see above). In addition, restriction patterns on her gels are not always unambiguous. She makes no attempt to map the locus responsible for inactivating one *NOR*. Autosomal background effects were not controlled, and inasmuch as they may be a factor, the dominance hierarchy constructed may be altered.

A different sort of explanation for compensation has been offered by Procunier and Tartof (1977). Working under the hypothesis that the observations of compensation imply the existence of a locus which senses a deficiency in its homolog and directs compensation, they set out to find it. Since it is logical that this locus would be near the rDNA, they used a set of heterochromatic deficiencies and duplications to define the locus. They found such a locus, which they named compensatory response (*cr*) in the distal penultimate one-eighth of the *X* heterochromatin. This gene has two functions. It works in *trans* to sense the presence or absence of its homolog, and in *cis* to control the disproportionate replication of the rDNA if its homolog is absent. The *cis* function is dependent on contiguity of cr^+ with the rDNA; chromosomes with inversions which move cr^+ away from the rDNA can be

sensed by their homologs as cr^+ (*i.e.*, they do not induce compensation in their homologs) but do not themselves compensate when their homologs are cr^-. Procunier and Tartof posit that the *Y* carries a cr^+ locus too far away from its rDNA to control disproportionate replication, because *Y* chromosomes behave like *X* inversions. However, they did not attempt to map *cr* in the *Y*, and it seems equally likely that the *Y cr* may simply have lost its *cis* function. There is a later report (Krider *et al.*, 1979) that the Canton-S *X* chromosome also fails to compensate. Tartof's model would attribute this to loss of cr^+. However, in all these studies, no attempt has been made test or control for autosomal effects on compensation. Would the Canton-S *X* compensate in an Oregon-R background, where *cr* was originally found? What happens to an Oregon-R cr^+ *X* in a Canton-S background? The explanations of Procunier and Tartof, and Endow and Spear and Gall are not mutually exclusive, and it is possible some melding of the models, perhaps with *cr* controlling independent polytenization of the rDNA, more accurately describes the mechanism of compensation. However, since all these studies fail to control for background effects on the system, it is likely that the actual control of compensation is either much different or much more complex than what has been envisioned.

The other major phenomenon associated with the rDNA genes in Drosophila is magnification/reduction. When *bb* mutations are kept in homozygous stocks, so that the flies are phenotypically *bb*, the *bb* phenotype has a tendency to disappear. This was generally ascribed to the accumulation of modifiers (Lindsley and Grell, 1968). In addition, *bb* mutations of unknown origin recur in many laboratory stocks.

Normally one would not expect to see high rates of mutation in a repeated gene, since the effect of any single point mutation will be swamped out. In order to see a mutant phenotype, one would need many simultaneous point mutations or a large deletion. However, *bb* mutants are frequently discovered in stocks.

In 1968, Ritossa explained both these occurences as increases and decreases in the number of rRNA cistrons. His experiments showed that an X^{bb} carried in a stock over a Y^{bb-} has a bb^{+} phenotype after three generations. If this *X* is now put into *X/O* males (with half the autosomes having been replaced) those males are still bb^{+}. If the new X^{bb+} is now carried over Y^{bb+} or X^{bb+}, it can revert once again to *bb*. Ritossa called the process of increasing the rDNA from *bb* to bb^{+} "magnification".

Early reports on magnification, especially those involving measurement of rDNA levels by saturation hybridization, are confusing and at times invalid. Since compensation was not discovered until 1971, flies with genotypes likely to be compensating were often used to measure rDNA levels. In those experiments, it is impossible to separate compensation from magnification effects (for example see Ritossa *et al.*, 1971). There is also the difficulty, described above, of assigning the correct number of genes to Y^{bb-} when this chromosome is used as a reference. Furthermore, Spear (1974) has shown that great variation can occur between separate saturation hybridization experiments, even among those done under identical conditions in the same laboratory. An analysis of variance shows these to be true differences in hybridization. Unless two experiments are run

simultaneously in the same rRNA solution, a comparison of their results may be meaningless.

Despite these problems, most of the observations of magnification are clear. In addition to the original observations described above (Ritossa, 1968b; Ritossa and Scala, 1969) it was shown (Boncinelli *et al.*, 1972) that Y^{bb} chromosomes can also magnify when they occur in a male with either an X^{bbl} or an X^{NO-}.

Two very diferent theories have been offered to account for magnification. Ritossa (1972) has proposed that magnification is due to extrachromosomal rDNA synthesis into small circles followed by integration. The synthesis occurs in all cells of the male, but integration can only occur in the germ line. (There have been no reports of magnification in the female.) These extrachromosomal circles are transcribed, but they do not contribute to the cell's mature rRNA pool (Ritossa *et al.*, 1971, 1973). The integration occurs during meiosis (Ritossa, 1973; Ritossa *et al.*, 1973). In experiments designed to detect exchange between the *X* and *Y*, Ritossa (1973) found a 20- to 30-fold increase in this exchange among flies undergoing magnification. This would be expected if, during integration, it were optional which rDNA region the extrachromosomal genes will be integrated into. Simultaneous integration of a large circle into both the *X* and the *Y* would sometimes lead to an exchange between them. The production of extrachromosomal circles could explain the observations that magnification occurs as a stepwise phenomenon and that newly magnified *bb* chromosomes are unstable. A number of studies have shown that it takes about 3 generations for a *bb* chromosome to become

completely bb^+ (Ritossa, 1968b; Ritossa and Scala, 1969; Henderson and Ritossa, 1970; Ritossa *et al.*, 1971; Boncinelli *et al.*, 1972). There is also evidence that after these chromosomes have been under magnifying conditions for about four generations, there is less tendency to revert to bb than in earlier generations (Ritossa, 1968b; Ritossa and Scala, 1969; Henderson and Ritossa, 1970; Boncinelli *et al.*, 1972). Details of the mechanism with which Ritossa *et al.* (1973) propose to connect this instability and the production of extrachromosomal copies of rDNA are quite vague.

Tartof (1973b, 1974) has presented evidence that magnification occurs by a wholly different mechanism. He proposes that unequal sister chromatid exchange (SCE) during mitosis in the male germ line is responsible. This idea was originally proposed by Atwood (1969). Tartof presents a number of lines of evidence to support this model. A fluctuation test shows that magnified bb^+ chromosomes tend to be produced in clusters. This indicates a premeiotic origin for these chromosomes. These new bb^+ chromosomes are stably inherited. A loss of ribosomal cistrons occurs simultaneously, so that some of the progeny are bb^1 while others are bb^+. Tartof has named this process of loss "reduction", and it would be expected to occur under his hypothesis since one chromatid will receive more rDNA and its sister less. These reduced chromosomes can be re-magnified in further generations. Somatic mosaics are also produced, and X^{bb}/Y^{bb-} males with mosaic cells show a 4- to 5-fold increase in magnification over unselected males. No magnification is seen in ring-X^{bb} chromosomes. This would also be expected from the proposed mechanism, since SCE

would lead to dicentric bridges and interlocked rings, which might be broken or lost in later mitotic or meiotic divisions.

Deciding between these two conflicting models is quite difficult. Ritossa's hypothesis of synthesis of extrachromosomal rDNA has a parallel in the process of amplification, which is not known for Drosophila ribosomal cistrons but does occur in Drosophila histone genes (Long and Dawid, 1980). There are some reports of extrachromosomal circles seen in male germ cells undergoing magnification (Long and Dawid, 1980) and of unintegrated ribosomal genes in some tissues (Zuchowski and Harford, 1976a, 1976b). The extrachromosomal synthesis mechanism would explain the observed increase in *X-Y* exchange. Double exchange that might occur could explain how the *X* and *Y* have maintained extremely homologous rRNA genes through evolution (Maden and Tartof, 1974; Long and Dawid, 1979). However, there is recent evidence (Tartof and Dawid, 1976; Long and Dawid, 1980; Peacock *et al.*, 1981) that the *X* and *Y* have a number of sequence differences in the ribosomal cistrons. If *X-Y* exchanges have occurred with any frequency, those differences should have been eliminated. Ritossa's evidence for meiotic timing of synthesis and integration (Ritossa *et al.*, 1973) is very weak. It is based on the notion that the *NOR* of the *Y* is inverted with respect to the *NOR* of the *X* (Palumbo *et al.*, 1973). That idea was later refuted by Maddern (1981) who showed that exchange between the two regions can occur in either order. The results presented in Chapter 2 of the present work support Maddern's conclusion. The extrachromosomal synthesis hypothesis is very complex, requires a meiotic exchange-like event in

male *D. melanogaster* where meiotic exchange does not usually occur, and requires a cellular mechanism which can differentiate between rRNA transcribed from extrachromosomal rDNA and rRNA transcribed from chromosomal rDNA. It can explain the rectification of *bb* mutants after they occur, but does not explain their high rate of formation.

Tartof's hypothesis of sister chromatid exchange would appear to be a simpler and more complete model. It can explain both the production and loss of *bb* mutations. The two phenomena are seen as the results of a regular occurrence that keeps the number of rRNA cistrons in flux around some mean, with large deletions and duplications being selectively eliminated. There is evidence that unequal SCE in the rDNA occurs during mitotic divisions in yeast (Szostak and Wu, 1980). In addition, regularly occurring SCE could account for the maintenance of intraspecies homogeneity by "horizontal" evolution of this tandem gene array. Computer simulation studies (Smith, 1973; Tartof, 1973b) have shown that reasonable levels of SCE can lead to crossover fixation of a single sequence in a short time. However, Tartof's arguments are not completely convincing. Like male meiotic recombination, SCE does not generally occur in *D. melanogaster*, either meiotically (Novitski, 1952) or mitotically (Gatti *et al.*, 1979). Thus magnification would require a special mechanism for the ribosomal cistrons to overcome this absence. The SCE hypothesis offers no explanation for early instability of magnified chromosomes as seen by Ritossa. Tartof tried to refute Ritossa's evidence of instability, but the test he used was inappropriate. Ritossa maintained the magnified *bb* chromosomes over a bb^{+}, then tested for production of *bb*. Tartof maintained a magnified

bb over bb^0 (sc^4sc^8) thus selecting for maintenance of bb^+ and guaranteeing stability. There is always the problem of a difference in stocks, and it is quite possible that the instability that Ritossa has seen is not a general phenomenon. These stock and background differences could also account for the more gradual accumulation of rRNA cistrons seen by Ritossa (three to four generations to bb^+) as opposed to Tartof's observations (usually only one generation to bb^+). Tartof's evidence for somatic mosaics is also questionable. Small spots of a variably expressed mutant like *bb* must be extremely difficult to unambiguously identify. These spots could also be explained by somatic nondisjunction, especially since they occur with equal frequency in magnifying and nonmagnifying males (Tartof, 1973b, 1974). In addition, one of Tartof's major arguments for SCE is that a ring-X^{bb} does not magnify. However, Y^{bb-} also fails to magnify although, even by Tartof's measurements (Tartof 1973a), Y^{bb-} is not a complete deletion and should be capable of SCE. Other chromosomes with *bb* mutations which fail to magnify have also been discovered (R. S. Hawley, personal communication).

The factors influencing the change in copy number of ribosomal genes are far from completely understood. Since the site of action of *Rex* is the ribosomal cistrons, it is possible that *Rex* may be involved in the regulation of this process. Some preliminary studies on how *Rex* affects magnification have been done (R. S. Hawley, personal communication). Unlike many other *bb* mutants, the *bb* mutant present on the *Rex* chromosome (Chapter 3) does not magnify. However, *Rex* can induce magnification in other *bb* mutants, and this magnification can

occur in females, a heretofore unreported event. *Rex* also shows a maternal effect on the magnification of paternally-transmitted chromosomes. Some of the X^{NO-}/bb daughters from a cross of Rex/X^{NO-} X bb/Y have a bb^{+} phenotype. Further testing has shown that the *bb* chromosome has magnified to bb^{+}. This appears to be magnification in early mitoses of female embryos. The meaning of these results is not yet clear. No work has been done to discover if *Rex* affects compensation. A number of lines of enquiry can be suggested. Does the *Rex* chromosome have an active cr^{+} locus? Does *Rex* have a maternal effect on compensation? If so, is it the maternally- or paternally-transmitted chromsome which is affected? The problem with proceeding with any of these experiments is the uncertainty about the validity of the studies and the merits of the conclusions on magnification and compensation to date. The mechanism of magnification is in doubt; however, the existence of the phenomenon of magnification has not been questioned. Studies of how *Rex* affects that phenomenon are therefore possible, and may eventually shed some light on its mechanism. In the case of compensation, however, it is possible that the phenomenon itself may be simply an artifact of the technique used to measure gene copy number. Even if compensation does exist, there remain many questions about background effects. Any test of *Rex*'s effect on compensation would introduce yet another set of background effects from the *Rex* stock which would be impossible to separate from the consequences of *Rex* itself. Until these doubts about compensation are answered, it seems better to leave the *Rex* experiments undone.

Heterochromatin

Rex is probably located in the centric *X* heterochromatin. Though the heterochromatin of Drosophila has been extensively studied, only a little is understood about its structure and even ,less is understood about its function. It is known to be condensed when the euchromatin is not, chiefly during mitotic interphase (Heitz, 1928; Shah *et al.*, 1973). It is therefore believed to be inactive during much of the cell cycle. The heterochromatin is located around the centromeres in Drosophila, and much of it is composed of repeated sequences (Kram *et al.*, 1972; Brutlag *et al.*, 1977). These areas lack exchange (Baker, 1959), and conventional mutation analysis has uncovered very few genes in them, considering their physical size. Some of the difficulty in finding genes in the heterochromatin may stem from a difficulty in defining the exact boundaries of these regions. There is some evidence (Schalet and Lefevre, 1973; Lifschytz, 1978) that there is no actual junction of euchromatin and heterochomatin, but there is an alternation of euchromatic and heterochromatic elements, gradually becoming more euchromatic distally and more heterochromatic proximally.

Though not exactly "genes", in the commom understanding of the word, the elements controlling a number of phenomena have been shown to reside in the heterochromatin. The pairing sites for the *X* and *Y* in males are located in the heterochromatin of these chromosomes (Lindsley and Sandler, 1958; Cooper, 1964). Heterochromatin is also responsible for position-effect variegation, a phenomenon in which euchromatic genes moved in or near broken heterochromatin fail to function uniformly. There are also apparently some factors in centric

heterochromatin responsible for the kinetic strength of centromeres during division (Lindsley and Novitski, 1958). In addition, there are a few more ordinary genes which have been mapped to the heterochromatin. These genes do, however, have some unusual properties which distinguish them from euchromatic genes.

Most of the systematic studies of heterochromatin have focussed on the *X* and *Y*. Nevertheless, there are a few genes known in the autosomal heterochromatin. Hilliker (1976) analyzed the heterochromatin of the second chromosome, and uncovered nine complementation groups of lethal alleles. They were mapped by deficiencies, are late larval and pupal lethals and are apparently non-repetitive. This is the first indication of lethal genes located in Drosophila heterochromatin, and it shatters the notion that this material is largely dispensible.

The other genes known to be in the autosomal heterochromatin are associated with the segregation distortion (*SD*) system (Ganetsky, 1977). *SD* causes meiotic drive: SD/SD^{+} fails to produce SD^{+} sperm. This is due to a gametic failure causing SD^{+} sperm to "suicide", possibly because of a failure of the SD^{+} chromosomes to condense normally during spermatogenesis. Three components of the *SD* system have been identified. The *Sd* locus itself is euchromatic, located on the second chromosome. The responder (*Rsp*) locus is in the heterochromatin of *2R*. Chromosomes containing Sd^{+} Rsp^{sens} suicide in the presence of an Sd Rsp^{ins} homolog. A third part of the system is an enhancer locus (*E(Sd)*) which is in the heterochromatin of *2L*.

The *Y* chromosome, which is wholly heterochromatic, has six male fertility genes (Kennison, 1981). Four of these are located on Y^L and two are on Y^S. All of them function during spermatogenesis (Williamson, 1976) and all are necessary for the production of motile sperm. The *Y* chromosome also contains a *bb* locus. This is the only locus shared by both the *X* and the *Y* and the only essential heterochromatic locus known for either of these chromosomes (Lindsley *et al.*, 1960). There is also a partially active *cr* locus on the *Y*, (Procunier and Tartof, 1977) as was discussed above.

A few genes have also been discovered in the *X* heterochromatin. In addition to a *bb* locus and a fully active *cr* locus, there are also apparently a number of loci located distally which affect early development and which interact with a group of closely linked genes on the second chromosome. These second chromosome genes are daughterless (*da*; Bell, 1954), abnormal oocyte (*abo*; Sandler *et al.*, 1968), *da-abo*-like, wavoid-like, and hold-up (*dal*, *wdl*, and *hup*; Sandler, 1977). These five genes are euchromatic and are located within 2.5 cM of each other on the left arm of the second chromosome. They are all recessive, hypomorphic, maternal-effect mutants which cause unequal recovery of the sexes in their progeny. All produce zygotic mortality before egg hatch, and all interact with the sex-chromosome heterochromatin. In most cases, an increase in *X* or *Y* heterochromatin in either the mothers or their offspring serves to rescue all or part of this mortality. In the case of *abo*, the region of this rescue effect has been mapped to the penultimate one-eighth of the *X* heterochromatin (Parry and Sandler, 1974). Sandler (1977) has

postulated that the other second chromosome loci also have corresponding, though different, sites of interaction in the heterochromatin. There is already some evidence of this for *da* (Sandler, 1972, 1977). These heterochromatic loci can apparently substitute for the euchromatic loci early in development, if the egg is deficient for the maternally-packaged *da* or *abo* products. It does not seem unreasonable, then, to assume that these are actually heterochromatic copies of the euchromatic genes, and that they function early in development before the euchromatic genes are active.

Except for the ribosomal cistrons, these heterochromatic genes all have one thing in common. They are all active at times when the rest of the genome is not. For *SD* and the *Y* fertility factors, this is during spermatogenesis, when the rest of the chromatin is condensed. The second chromosome genes which affect development function in the very early embryo, which normally depends on maternal gene products (Robbins, 1980, 1983). It is possible that heterochromatin's function is to contain certain genes meant to be "on" at times when the euchromatic genes are "off". *Rex* also functions at one of these times -- in the early embryo before the pronuclei have even fused (Sonnenblick, 1950; Davring and Sunner, 1973).

A number of experiments could clarify the possible developmental role of *Rex* and its interaction with the rest of the genome. A mutagenesis experiment to look for enhancers of *Rex* is currently underway. The *Rex* phenotype is difficult to use for precise mapping; at this time only one to two percent of what were originally female embryos show the effects of *Rex* when their mothers carry *Rex*. Boosting

this frequency to ten to twenty percent would drastically reduce the numbers of progeny needed to do a more precise mapping experiment -- to map *Rex* between *su(f)* and *bb*, for example. Meiotic-recombination and deletion mapping would assure that *Rex* was indeed located within the heterochromatin. Assuming that the heterochromatic location were confirmed, experiments could then proceed to see if *Rex* has any interaction with *abo* or the other developmental genes near it. If the cloning of *Rex* (mentioned above) could be accomplished, *in situ* hybridization of the cloned *Rex* DNA to Drosophila chromosomes would show if *Rex* has a euchromatic sister locus. (Interestingly enough, *abo* also has an interaction with the rDNA (Krider and Levine, 1975; Krider *et al.*, 1979) lending support to the notion that *Rex* may be related to *abo* and its nearby loci.)

Mitotic Chromosome Instability

Genetic elements which affect mitotic chromosome behavior have been previously identified. Some of these have effects similar to *Rex*, though all have differences. It is intriguing to speculate that *Rex* is part of a system, with these elements, that controls early chromosome behavior. An examination of the effects and actions of these elements is therefore worthwhile.

One of the first described instances of mitotic chromosome instability was the loss of ring-*X* chromosomes (reviewed by Leigh, 1976). These rings tend to be lost in early cleavage divisions, producing gynandromorphs. *X/0* males are also produced; these were assumed to come from meiotic loss of rings, but there is no evidence

that these are not losses very early in mitosis. The loss of rings has several similarities to *Rex* action. The instability disappears over several generations in culture, which might be due to the accumulation of modifiers (see Chapter 1 and Appendix). There is evidence of factors in the oocyte which influence the stability of rings and can cause stable rings to become unstable. This suggests that there might be a maternal effect involved. Finally, the locus causing ring instability was mapped to the centric *X* heterochromatin. Taken together these facts suggest that there might be a factor similar to *Rex* in unstable ring systems.

Rex causes a mitotic recombination event. Mitotic recombination has been studied extensively both for itself and for its use in determining developmental pathways. Mitotic exchange occurs spontaneously in *Drosophila melanogaster* at a low rate (Garcia-Bellido, 1972). Unlike the meiotic process, mitotic recombination is proportional to physical chromosome length; that is, the heterochromatin exchanges as readily as the euchromatin. Mitotic recombination requires homology (Ripoll and Garcia-Bellido, 1978), and it increases in frequency when cells are treated with X-rays. Therefore, its spontaneous occurrence may be due to chromosome breaks. A number of meiotic recombination-defective loci which also have mitotic effects have been identified (Baker *et al.*, 1978). In addition to increasing mitotic recombination, these loci cause breakage and loss. They are believed to be defective in cellular DNA repair functions. One of these, *mei-41*, has recently been shown to induce interchanges between the *X* and the *Y* which involve the *X* rDNA (Hawley

and Tartof, 1983). *Rex* has a similar effect, and might also be involved in this recombination-repair system.

There are three other mutations affecting mitotic chromosome behavior. The first is mitotic induced loss (*mit*; Gelbart, 1974). *mit* is a recessive, maternal-effect mutant which causes loss of the *X* or fourth chromosomes in *mit* or mit^+ progeny. The *X* or *4* lost can be of maternal or paternal origin. The loss occurs during the third or fourth mitotic division. Loss of the second and third chromosomes was not tested, but *mit* does not cause loss of a *Y* or an attached-*XY*. *mit* maps in the proximal *X* euchromatin. Modifiers tend to accumulate in *mit* stocks, but these modifiers are readily removed by outcrossing.

The second mutant of interest is paternal induced loss (*pal*; Baker, 1975). This mutant is located on the left arm of *2*. When the progeny of homozygous *pal* males are examined, there are many that have lost one or more paternal chromosomes. Baker assumes that this is meiotic loss; however, there is no way to decide if an embryo never received a chromosome or if it was lost during G1 of the first embryonic division. Moreover, there is a high frequency of gynandromorphs and mosaics in the progeny as well. It seems simpler to attribute both of these results to early mitotic chromosome loss. The effect is seen equally in *pal* and pal^+ progeny of both sexes, but in all cases only the paternally-derived chromosomes are ever lost. Offspring missing one chromosome are more likely to be missing two than would be expected if the two events were independent. There is also an interaction between *pal* and the *X* heterochromatin: some *X* chromosomes are less sensitive to loss induced by *pal* and this insensitivity .maps

to the *X* heterochromatin.

The third mutant is claret-nondisjunctional (ca^{nd}; Davis, 1969). It causes meiosis I nondisjunction and maternal chromosome loss; again the loss probably occurs at early mitotic divisions in the zygote. Exchange is normal in meiosis, so the nondisjunction is not due to a failure of pairing or a disruption of recombination. The loss is a maternal effect.

All of these mutants are similar in causing chromosome loss during mitotic divisions. The last two are similar to *Rex* in that they may be active as early as G1 of the first mitotic division. A meiotic nondisjunction phenotype similar to ca^{nd} has been seen in *Rex* crosses; Tables 1-3 show an excess of exceptional male offspring. In other experiments involving *Rex* (S. Keller and L. Robbins, personal communication), exceptional female offspring, many of which are recombinant and homozygous for distal markers, have been seen. These observations taken together resemble the products from meiosis I nondisjunction (Bridges, 1916; Merriam and Frost, 1964): *X*-diplo exceptions homozygous for distal markers and, at a higher frequency, *X*-nullo exceptions. Some of the excess of patroclinous males may also come from early mitotic chromosome loss.

The similarities in phenotype of *mit*, *pal*, ca^{nd}, and *Rex* suggest that *Rex* may be part of a group of genes controlling early chromosome behavior. It would be interesting to study their interactions. For example, does the *Rex* locus correspond to the *X* heterochromatic locus which is insensitive to *pal*? If so, other *Rex* alleles should be present in either the *pal*-sensitive or the *pal*-insensitive stocks Baker

(1975) identified. In addition, the enhancer now being sought would provide a frequency of *Rex* products high enough that a cytological examination of the early mitotic divisions of *Rex*-affected embryos might be feasible, following the technique of Zalokar, *et al.* (1980). This examination would provide further insight into the *Rex* event. The enhancer would also enable further investigation of some of the rarer products associated with *Rex*. These include the putative meiosis I nondisjunction products mentioned above. In addition, occasionally flies are produced from *Rex* crosses which can only be explained by postulating that an early mitotic exchange followed by a meiosis I (reductional)-like segregation has occurred. These products are recovered at frequencies an order of magnitude lower than are detachment products. To date it has not been clear that these are definitely products of *Rex*; an increase in their frequency with an enhancer would back the notion that they are, make feasible experiments to map them unequivocally to *Rex*, and allow a more complete study of them.

APPENDIX

APPENDIX

Suppressor of *Rex*

In the course of experiments done to investigate *Rex*, another element of the system has been uncovered. This element acts as a suppressor of the *Rex* effect, and has been designated *Su(Rex)*. Because of the suppressor's detrimental effect on some experiments and its possible utility in others, a number of experiments have been done to discover something about its mode of action and location. Although many aspects of this element or elements remain unclear, the following has been shown: the suppressor acts as a dominant; it is not a maternally-transmitted cytoplasmic element; and it is present in different chromosomal locations in different stocks. It may be present in more than one chromosome location in some stocks.

Figure 12 shows a general mating scheme similar to the one that originally uncovered the suppression and which has been used since to test stocks for the suppressor. The suppressor acts in the heterozygote and is therefore a dominant. Table 11 presents the results of tests of a number of *X* chromosome stocks for the presence of a suppressor.

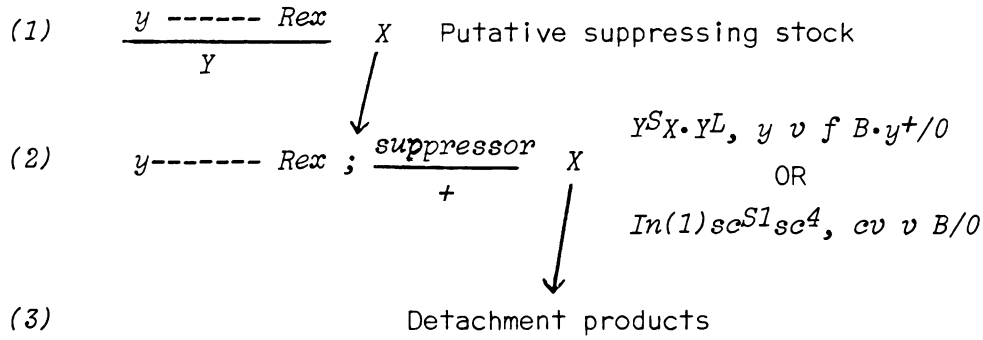


Figure 12. Mating scheme for detection of suppressor(s).

Table 11. Suppressing stocks.

Sex chromosomes of stock	Genotype of females tested	Regular females	Detachment products	Percent detachment
Homozygous *y cv v f car*	$\frac{y\ cv\ v\ f\ car}{Df(1)w^{rJ1},\ y^2\ sn\ Rex}$	16,933	6	0.071
$\frac{FM7}{un\ Bx^2}\ X\ \frac{un\ Bx^2}{Y}$	$\frac{FM7}{y\ cv\ v\ f\ Rex}$	4,106	5	0.122
$\frac{FM7}{y}\ X\ \frac{y}{y^+Y}$	$\frac{FM7}{y\ cv\ v\ f\ Rex}$	1,342	3	0.223
Homozygous $y^2\ cv\ v\ f\ car$	$\frac{y^2\ cv\ v\ f\ car}{y\ cv\ v\ f\ Rex}$	598	3	0.502

For matings, see Figure 12. % detachment calculated as in Table 1.

An attempt was made to map the location of the suppressor found in the *y cv v f car* stock. From previous experiments (Robbins, unpublished data) it was known that the suppressor did not segregate with the *y cv v f car* *X* chromosome or any of the recombinant *X* chromosomes from a *y cv v f car/y* heterozygote (for mating scheme see Figure 13). The stock *y ; T(2;3)e/SM1;TM2 ; spa*^{pol} was used to replace the autosomes of the *y cv v f car* stock both singly and in combination. *SM1* and *TM2* are, respectively, second and third chromosome balancers. The mating scheme for this experiment is shown in Figure 14, and the data are presented in Table 12. Eight different genotypes are produced, and each was tested for *Rex* activity with $Y^{S}X.Y^{L}$, *y v f B.y*$^{+}$. All the data are statistically homogeneous, and each of the classes shows evidence of suppression. These data can be explained by at least three possibilities. The autosome balancer stock itself might carry a suppressor, or the suppressor might be a non-chromosomal element which is transmitted through maternal cytoplasm, or there might be multiple suppressing elements in the *y cv v f car* stock. To test the first of these, mapping experiments were set up using the autosome-balancer stock as the source of the suppressor, as shown in Figure 15. The data are presented in Table 13.

Once again, the data show no particular trends. Most of the classes (1, 2, 3, 5, and 6) have detachment frequencies that do not indicate suppression. A contingency test shows that all classes are homogeneous. Of tests of the relevant combinations of genotypes, *spa*^{pol} *vs.* *spa*$^{pol+}$, *SM1 vs.* *SM1*$^{+}$, *TM2 vs.* *TM2*$^{+}$, only the last was significant (X^2 = 7.309 with 1 d.f., 0.01>p>0.005) and this is in the

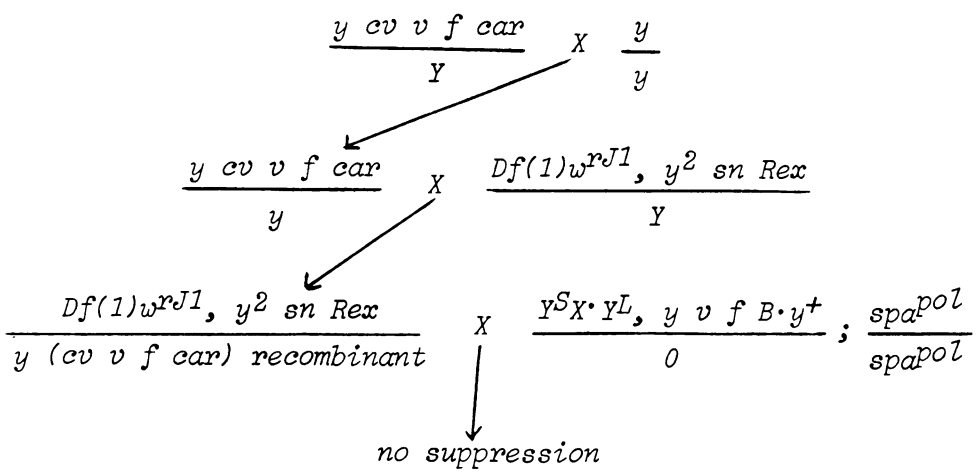


Figure 13. Loss of *Su(Rex)* effect.

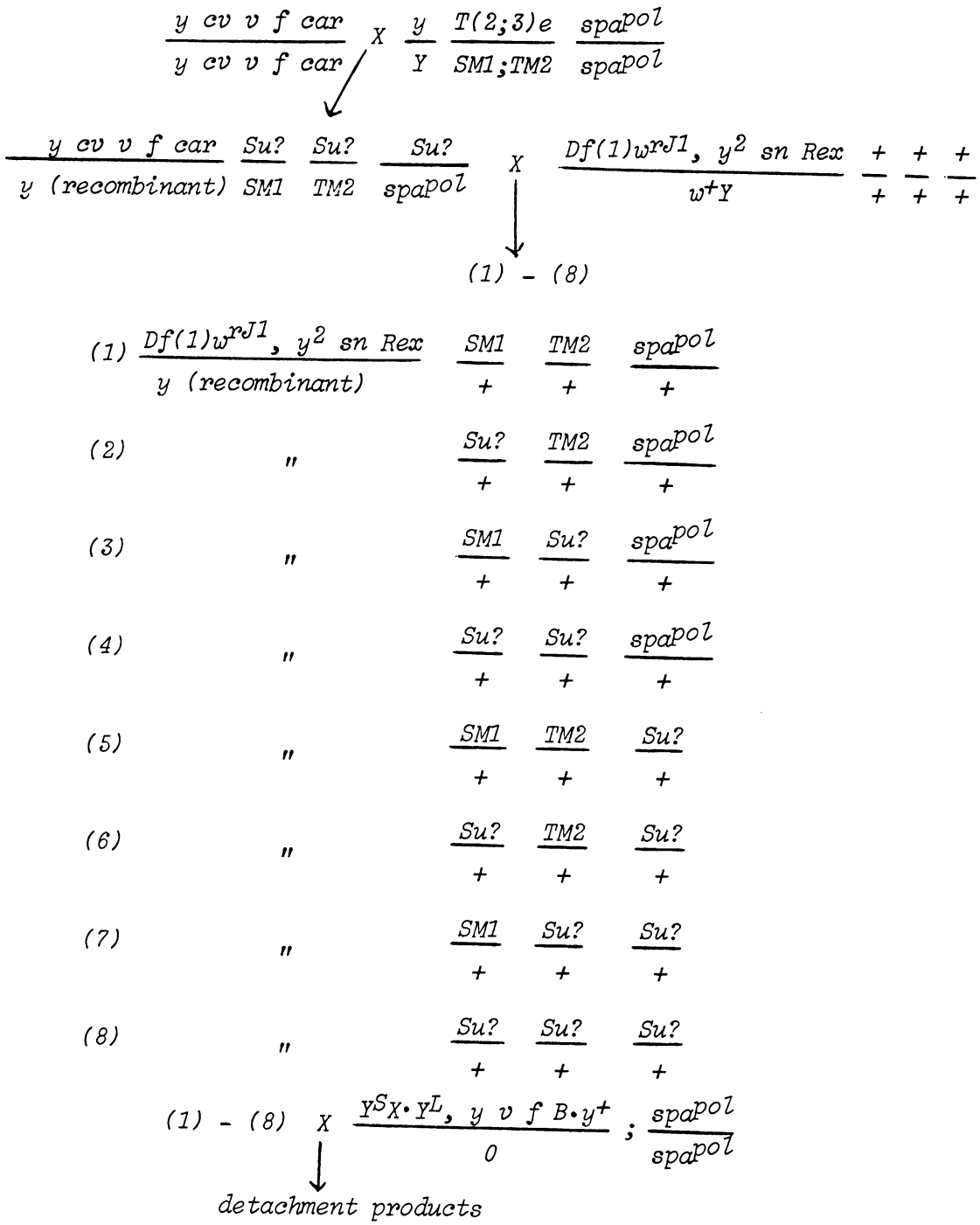


Figure 14. Mapping *Su(Rex)* from *y cv v f car* stock: mating scheme.

Table 12. Mapping *Su(Rex)* from *y cv v f car* stock: results.

Genotype	Regular females	Detachment products	Percent detachment
(1) *SM1 TM2 pol*	430	0	0
(2) *Su? TM2 pol*	1063	1	0.188
(3) *SM1 Su? pol*	791	0	0
(4) *Su? Su? pol*	1185	0	0
(5) *SM1 TM2 Su?*	397	1	0.504
(6) *Su? TM2 Su?*	1661	1	0.120
(7) *SM1 Su? Su?*	1037	3	0.579
(8) *Su? Su? Su?*	1693	1	0.118
Totals	8257	7	0.170

$Y^{S}X \cdot Y^{L}$, *y v f B*$\cdot y^{+}/0$; *spa*^{pol}/*spa*^{pol} males were mated to females with the indicated genotypes (for mating see Figure 14). % detachment calculated as in Table 1.

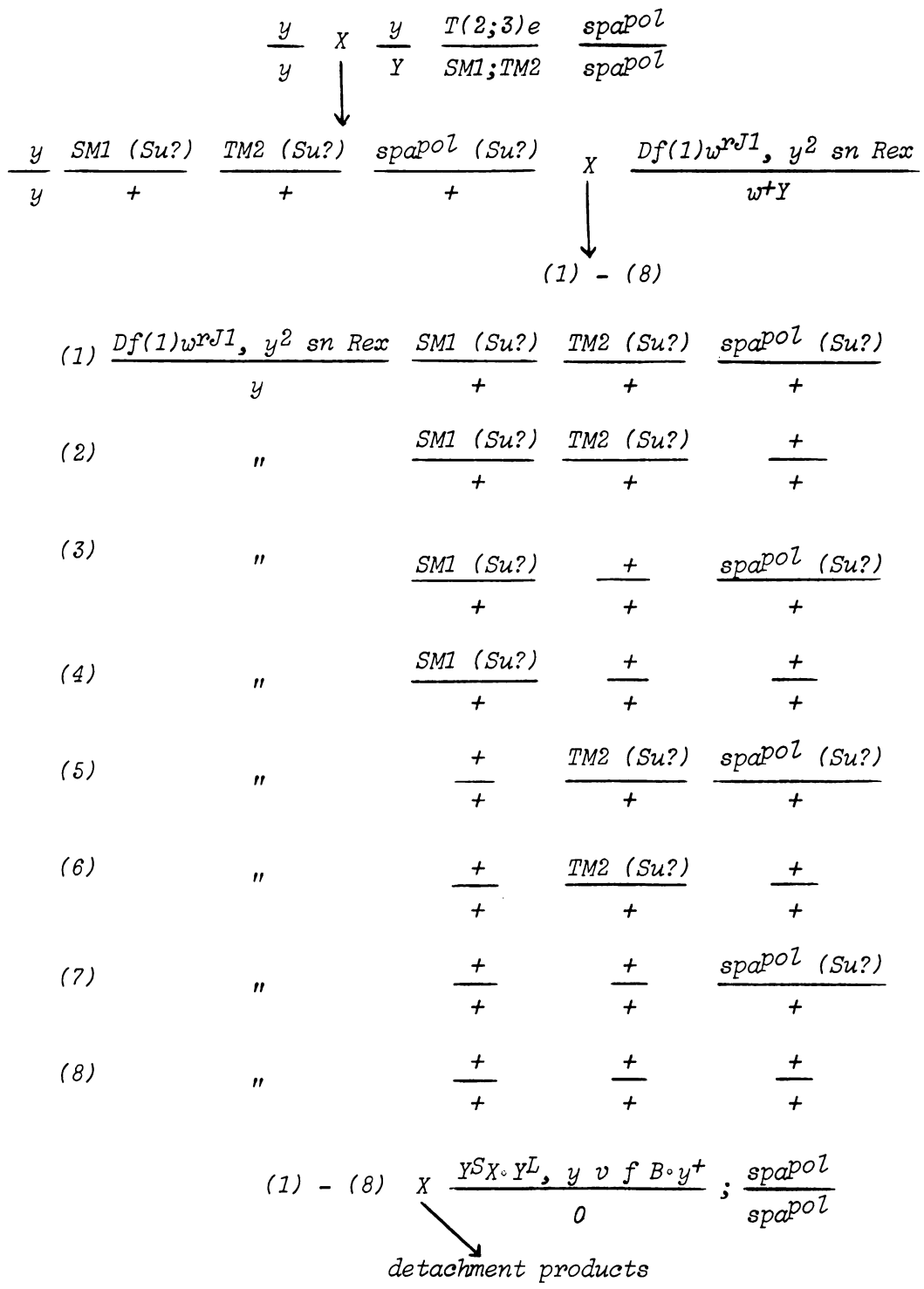


Figure 15. Mapping *Su(Rex)* from $y ; \frac{T(2;3)e}{SM1;TM2} ; \frac{spa^{pol}}{spa^{pol}}$ stock: mating scheme.

Table 13. Mapping *Su(Rex)* from *y* ; $\frac{T(2;3)e}{SM1;TM2}$; $\frac{spa^{pol}}{spa^{pol}}$ stock: results

Genotype	Regular females	Detachment products	Percent detachment
(1) *SM1 TM2 pol*	469	5	2.09
(2) *SM1 TM2 +*	187	2	2.09
(3) *SM1 + pol*	547	4	1.44
(4) *SM1 + +*	905	3	0.65
(5) *+ TM2 pol*	374	3	1.58
(6) *+ TM2 +*	742	7	1.85
(7) *+ + pol*	821	2	0.48
(8) *+ + +*	1087	3	0.55
Totals	5132	29	1.12

$Y^{S}X.Y^{L}$, *y v f B.y+/0* ; spa^{pol}/spa^{pol} males were mated to females with the indicated genotypes (for mating see Figure 15). % detachment calculated as in Table 1.

wrong direction: *TM2* showed less suppression than $TM2^{+}$. These data do not support the hypothesis that the autosome-balancer stock carries a suppressor.

The second possibility, that *Su(Rex)* is a maternally-transmitted cytoplasmic element in the *y cv v f car* stock, was tested using the mating scheme shown in Figure 16. Genotypically identical F_1 females are derived from reciprocal crosses. If *Su(Rex)* is maternally transmitted, the F_1 females from cross (1) should produce fewer detachment products than those from cross (2). However, this result was not obtained. Females from cross (1) produced 2860 regular daughters and 7 detachment offspring, while females from cross (2) produced 2142 regular daughters and 9 detachment offspring. The data are homogeneous and both detachment frequencies (0.17% and 0.42% respectively) show evidence of suppression. Thus, neither a suppressor carried by the autosomal balancer stock nor a maternally-transmitted cytoplasmic element can explain the results of the mapping experiment in Table 12.

This leaves the possibility of multiple suppressing elements. A contrary result, however, is obtained in experiments with stocks of the *X* chromosome balancer *FM7* (Merriam, 1968). As was noted above, previous experiments had shown that all crossover and noncrossover daughters from *y cv v f car/y* mothers and *Rex* fathers no longer show any suppression (Figure 13). However, a very different result is obtained when *FM7* is used instead of *y cv v f car*, as diagrammed in Figure 17. In the first generation, *FM7* is transmitted from a male to eliminate any maternal cytoplasmic elements. In the next two

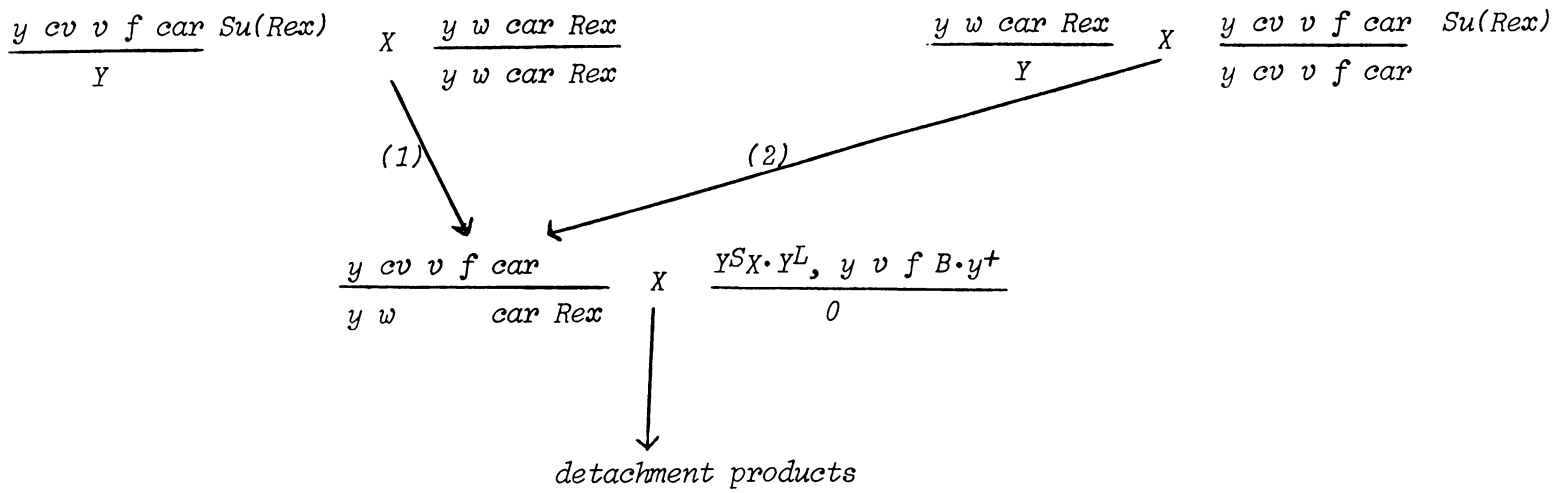


Figure 16. Mating scheme to test for maternal suppression effect.

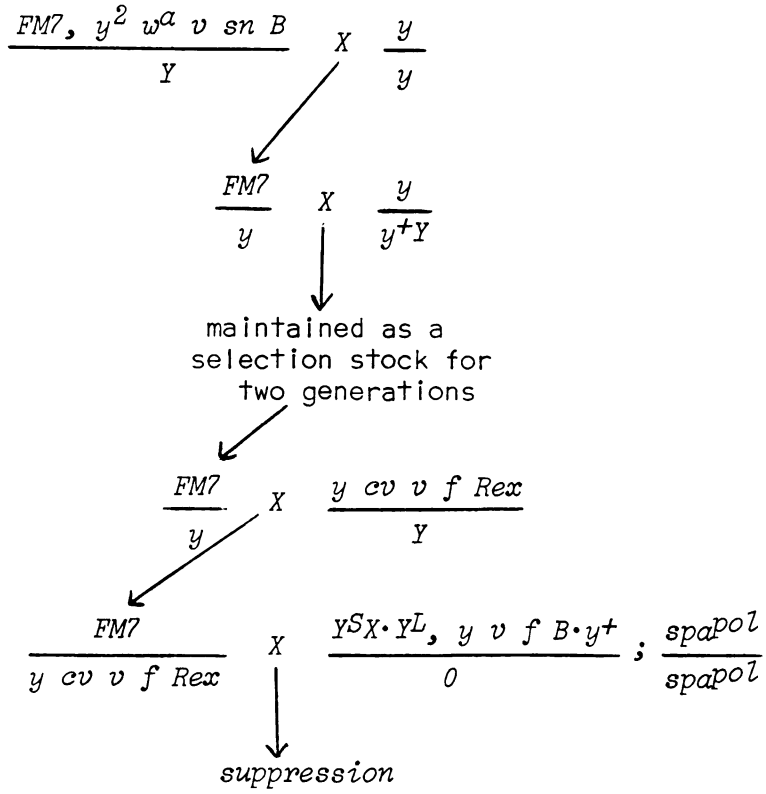


Figure 17. Suppression by *FM7*.

generations, it is again outcrossed to a non-suppressing stock and transmitted through females to permit free re-assortment of the autosomes. The data for the last mating in Figure 17 are shown on the third line of Table 11. *FM7* does not lose the ability to suppress after passing through the *FM7/y* heterozygote. In fact, the data in lines 2 and 3 of Table 11 are homogeneous. In the *FM7* stocks, then, a single suppressor is present on the *X* chromosome. Because *FM7* is a very effective crossover suppressor, however, it would be quite difficult to map the position of *Su(Rex)* within this chromosome.

It may therefore be concluded that more than one genetic locus can function as a suppressor of *Rex* action. A suppressor definitely resides on the *X* chromosome in the *FM7* stock, and the *y cv v f car* stock definitely lacks a suppressor on the *X*. The attempts to map *Su(Rex)* in *y cv v f car* may have failed either because there are actually a number of sites on different chromosomes in that stock, each functioning as suppressors, or because the autosome-balancer stock actually does have a suppressor, but it is located on the *X*, a notion that would also account for the intermediate detachment frequencies found for all the autosome-balancer stock's derivatives.

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