A STUDY OF A DOMINANT SUPPRESSOR OF THE PURPLE EYE-COLOR MUTANT IN DROSOPHILA MELANOGASTER

by

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ABSTRACT

The subject of this study is a new dominant suppressor mutation Su(pr) which acts on the purple eye-colour mutant (pr) of Drosophila melanogaster. The induction of Su(pr) was originally associated with the synthesis of a compound-2R chromosome in SD72/cn bw females. The suppression of pr was first observed in combination with a homologous pr-bearing compound-2L chromosome. Suppressed-pr flies appeared to have a fully wild eye phenotype. The intention of this study was to determine the chromosomal constitution necessary for Su(pr) induction, and to map the suppressor site. To do this, many compound-2R chromosomes were synthesized from several combinations of standard seconds. It was found that SD72 must be present to produce a suppressing compound-2R. The SD72 second carries a pericentric inversion that results in a duplication of 2L heterochromatin, and an associated deficiency of 2R heterochromatin in the compound-2R Su(pr) chromosome. Suppression, therefore, is associated with the pericentric inversion found only on SD72. The role of this segmental aneuploidy was studied by detaching several C(2L)pr; C(2R)SD72/cn bw suppressed strains such that both arms of the Su(pr) compound autosome were recovered independently and established in standard strains. Suppressing and non-suppressing detachment products were recovered with a frequency that varied according to the compound-2R Su(pr) strain from which they were derived. The chromosome mechanics involved in the process of C(2R)SD72/cn bw formation and subsequent detachment implicates alterations to a segment of proximial 2R heterochromatin from SD72 in Su(pr) induction. Loss of Su(pr) in the

detachment process correlates predominantly with deletions generated in 2R heterochromatin. Recombination mapping relative to the two visible heterochromatic markers, light and rolled, revealed that Su(pr) lies to the left of rolled. Spectrophotometric measurements of eye pigments revealed that suppressed-pr and suppressed-pr^{bw} flies had pigment levels that exceeded the wild type. The lethal allele pr^{c4}, was not found to be suppressible.

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CHAPTER 1

GENERAL INTRODUCTION

Suppression occurs when the effects of one mutation are compensated for by a second mutation, such that the wild phenotype is partially or fully restored.[1] The study of suppression has provided valuable information on the mechanisms of mutagenesis and the function of the structures affected. Studying the relationship between two counterbalancing mutations can also identify functional relationships between distinct components of a genetic system. This has revealed several aspects regarding the normal control of gene expression. Examples of suppression in many different organisms have been reviewed by Gorini and Beckworth[2] and Hartman and Roth.[3]

Mutations may be suppressed intragenically by additional changes to the base pair sequence. Missense and nonsense mutations can be suppressed by a second change within the mutated codon that creates an alternative triplet, coding for the correct amino acid.[4, 5] Frameshift mutations can be suppressed by a second frameshift that restores the proper reading frame. Mutations which occur outside the structural sequence of a gene, in the upstream control sequence, may prevent transcription by disrupting the promoter. In such cases, suppression may act at a transcriptional level by giving rise to a new promoter.[6]

Mutations may also impede normal translation if they occur in the polypeptide initiator codon . Suppression at the translational level can occur when a second mutation creates a novel initiator codon.[7] Some mutations which cause the loss of a protein's functional conformation, and therefore its activity, can be suppressed at the level of the polypeptide. Such a mutation occurs at the site of polypeptide chain interaction, preventing the protein from assuming its proper tertiary or

quaternary structure. The mutation at the residue that precludes normal interaction may be counteracted by a change at a second residue that reinstates the normal folding.[8, 9] The same mechanism has been shown to reinstate the functional conformation of doubly mutant tRNAs.[10]

Altered tRNAs are also involved in informational suppression. This is a type of intergenic suppression in which the mutant gene still provides an altered mRNA, but the altered region is misread by a mutant tRNA. The result is a functional protein.[11] Mutant tRNAs can suppress nonsense[12] and missense[13] mutations through base substitutions in the anticodon loop. If a frameshift mutation is caused by the insertion of an additional base pair, then it can be corrected by a complementary tRNA containing a 4 base pair anticodon loop.[14] Few examples of informational suppression have been found in eukaryotes. Suppressor tRNAs have been found for all three nonsense mutations in two species of Saccharomyces, S. cerevisiae and S. pombe. A possible nonsense suppressor that acts on specific alleles of many genes has been described in Caenorhabditis elegans (reviewed by Kubli[15]).

Suppressor tRNAs have not yet been confirmed in Drosophila melanogaster. Several suppressor stocks have been tested for deviation from wild type tRNA patterns by two-dimensional gel electrophoresis. The only aberration from wild type was seen in the dominant suppressor of deltex (Su(dx)), which showed one additional spot in the region of a larger tRNA species.[16] This suggests that Su(dx) may contain an additional isoacceptor, but it has not been characterized, nor has its role in suppression been directly demonstrated. The translation of viral message

by the tRNAs of several suppressor stocks has been tested *in vitro*. The deviant tRNA $^{TYR}_{1}$ isoacceptor found in one allele of the *suppressor of sable* (*Su(s)2*) differed from that found in all other stocks tested by being able to suppress a TMV-RNA stop codon.[17]

As well as the direct interaction of different gene products, intergenic suppression can occur through more general changes in the intracellular millieu (reviewed by Hartman and Roth[3]). In the case of conditional mutants, some suppressor mutations alter certain intracellular conditions, such as pH, ionic concentrations, or the concentrations of effector molecules. Changes in each of these conditions are capable of restoring the functional conformation of some mutant macromolecules. Intergenic suppression often involves changes in the flow of metabolites down a biosynthetic pathway. The suppressing mutation may increase the flow down an alternate pathway, or alter a protein such that it acts on the substrate of the mutated enzyme, or greatly increases the level of mutated enzyme, such that adequate activity levels are maintained.[3]

An example in *Drosophila melanogaster* illustrates how modulation of a biosynthetic pathway can be suppressive. The *black* body mutant (*b*) is caused by a deficiency in beta-alanine, which is necessary for the normal tanning and melanization of the cuticle.[18, 19, 20] There are two sources of beta-alanine in the fly; a small amount is produced by the pyrimidine pathway, while most of the beta-alanine is derived through the alpha-decarboxylation of aspartic acid. In *b* mutants a lesion in this second pathway greatly reduces the levels of beta-alanine produced from aspartate. The amount of this compound normally provided through the pyrimidine pathway is insufficient to prevent the *b* phenotype.[21, 22] The

b mutation is suppressed by the dominant suppressor of black (Su(b)). The Su(b) does not, however, act on the damaged aspartate pathway. Instead, Su(b) elevates the flow down the pyrimidine pathway, such that this normally minor source of beta-alanine compensates for the deficiency in the mutated pathway.[23]

Of the more than 30 suppressor mutations known in Drosophila melanogaster (listed in Lindsley and Grell[24]), the most extensively studied is the suppressor of sable (su(s)). This suppressor acts on mutations at four loci: sable (s) and speck (sp), both of which affect body colour, and purple (pr) and vermillion (v), which both affect eye colour. Most of these studies have used the spontaneous allele $su(s)^2$ in combination with v. The v gene is the structural locus for tryptophan pyrrolase (TP) which catalyses the first step in the production of the ommochrome eye pigments.[25] The action of $su(s)^2$ was long thought to be recessive, but recent studies have revealed a small but appreciable level of suppression in $su(s)^2$ heteroyygotes. The level of suppression in the heteroyygote varied according to the allele used.[26]

A search for the suppressive mechanism concentrated on differences in tRNA species between wild-type and mutant su(s) strains. The only difference found was an altered distribution of the two major isoacceptors of tyrosyl-tRNA, tRNA $^{TYR}_{I}$ and tRNA $^{TYR}_{II}$. In su(s) mutants, tRNA $^{TYR}_{II}$ which is normally the predominant species, is significantly reduced and the level of tRNA $^{TYR}_{I}$ increases proportionately.[27, 28] This shift in isoacceptor pattern was taken to indicate informational suppression.[27] This hypothesis was eventually refuted by the findings of several studies. The most significant of these showed that the mutant, wild-type and

suppressed-v tryptophan pyrrolase were all of the same molecular weight. The hypothesis of informational suppression predicts a lower molecular weight for the mutant enzyme caused by a failure to complete translation.[29] It was found, however, that the mutant enzyme had an altered Km and pH optimum relative to the wild type and su(s):v^2. It was suggested that su(s)^2 might induce changes in the cellular environment which reinstated the functional conformation of the enzyme.[30, 31]

Jacobson[32] alternatively hypothesized that tRNA $^{TYR}_{II}$ acts as an inhibitor of mutant TP, but not wild type TP. In su(s)^2;v, the elimination of most of the second isoacceptor, or its conversion to an inert form, would abolish inhibition and release enzyme function. In this hypothesis, the su(s)^2 gene product functions to produce the mature form of tRNA $^{TYR}_{II}$, or to interconvert it with the other isoacceptor species.[33] Consistent with this, when the level of tRNA $^{TYR}_{II}$ in su(s)^2;v was raised by dietary conditions, there was no change to the suppressed v phenotype. But, in the su(s)$^+$;v, in which the wild type tRNA $^{TYR}_{II}$ was presumably inhibiting mutant TP, reductions in this isoacceptor by dietary modification resulted in a v phenocopy.[34]

The mechanism of su(s) action has still not been determined, but recent work has focused on the possible role of inserted transposable elements discovered at both the suppressor and target loci. Seven spontaneous alleles of su(s) have been cloned, and all have been found to contain an insertion of the gypsy element. Also, six more su(s) alleles have been induced by P element insertion, and subsequently cloned. DNA sequencing has revealed that in all 13 alleles the elements were inserted within a 2.2 kb region adjacent to the 5' end of the transcribed region

of the gene. As well, insertions of the 412 element have been found at each of the four suppressible target loci.[35] It is beginning to appear that the role of these inserted elements in suppression may be to modulate transcription at the target locus.

Evidence for this comes from two different suppressors in Drosophila melanogaster, both of which affect the white eye-colour gene (w). The mutant allele white-apricot (w^a) is caused by an insertion of the copia element into an intervening sequence at the w locus.[36, 37] The w^a phenotype is partially suppressed by the suppressor of white-apricot ($su(w^a)$), which results in a darker eye colour. Conversely, w^a can be enhanced to produce a paler eye colour by the suppressor of forked (su(f)). Also, there is a partial revertant of w^a, containing a single long terminal repeat insertion in place of the whole copia, that is not affected by either $su(w^a)$ or su(f).[38]

Enhancement of w^a coincides with the very low level of mutant w transcription being still further reduced in the presence of su(f). Suppression of w^a is coincident with a several fold increase in the w transcript in the presence of $su(w^a)$.[38] It has been proposed that transcription of the copia element inserted at the w locus causes the w^a mutation. An increase in copia transcription by su(f), to the further detriment of w transcription, would enhance the mutation. Conversely, a reduction in copia transcription by $su(w^a)$ with a concomitant increase in w transcription would suppress w^a. Finally, removal of copia, save one of its long terminal repeats, would eliminate the ability of either $su(w^a)$ or su(f) to modulate transcription of the partial revertant.[40]

It is not yet known what significance there is to the interaction between transposable elements and the suppressor alleles. Nor is it known what function the wild-type allele of the suppressor serves. It has been suggested that suppressor genes may normally produce developmental signals that control the timing and level of transcription at target genes.[38] The normal target genes may be different from those involved in suppression. Also, it may be that the elements discussed here are not indigenous components of a developmental control system. It may only be that they are susceptible to the same types of transcriptional control that governs gene expression during development. Either way, the discovery of several cases of suppressor modulated transcription of target loci suggests that this may be a common suppressive mechanism.[38]

The subject of this study is the suppression of the purple eye mutant (pr) by a new dominant suppressor of purple (Su(pr). The suppressed-pr phenotype was originally associated with the synthesis of a specific type of radiation induced chromosomal rearrangement, a compound-2R chromosome (C(2R)). In a C(2R), the two right arms of the normally metacentric second chromosome are attached to the same centromere. Such a rearrangement can be maintained in a stock bearing the complementary compound-2L chromosome (C(2L)).[39]

The suppressing C(2R) (C(2R)Su(pr)) was synthesized in females that carried the second chromosome combination of In(2LR)SD72/cn bw. The Segregation Distorter components (SD) are carried on SD72. A pericentric inversion on SD72 has its break-points near the heterochromatic junction on both sides of the centromere.[40] This determines that C(2R)SD72/cn bw will be deleted for one copy of 2R-heterochromation (2Rh) and duplicated

for one copy of 2L heterochromation (2Lh). When the C(2R)SD72/cn bw was tested in combination with a homzygous b pr-bearing C(2L), the black body phenotype was present but the eyes looked fully wild type. This suppression was not seen in combination with any other C(2R).[41]

The purpose of this study is to characterize and map Su(pr). In Chapter 2, the constitution of C(2R) necessary for Su(pr) induction is analysed. To do this, many C(2R) were synthesized from SD72 in combination with different homologues. Another SD-bearing second, SD5, was also used in C(2R) synthesis to test the implication of SD in Su(pr) induction. Also, the effects of Su(pr) on eye pigmentation were quantified spectrophotometrically. In Chapter 3, C(2L)pr;C(2R)Su(pr) were detached to reconstitute standard seconds. This tested whether Su(pr) could persist through further chromosomal rearrangement. Deletions generated during the detachment procedure were also analysed in order to localize a region necessary for Su(pr) activity. In Chapter 4, Su(pr) was mapped by recombination to the visible heterochromatic markers, light and rolled.

CHAPTER 2

ANALYSIS OF COMPOUND SECOND AUTOSOMES

Introduction

A compound second autosome is a specific type of chromosomal rearrangement in which two identical autosomal arms are attached to the same centromere. The formation and meiotic behaviour of compound autosomes has been reviewed by Holm.[39] Compound autosome formation involves a translocation-like event which occurs at the four-strand stage of meiosis. For a viable product to be formed the two homologous chromatids involved must each be broken, and the two break-points must lie on opposite sides of the centromere. Rejoining at these break-points between the centric fragment of one chromatid and the acentric fragment of the other chromatid results in the formation of a new compound autosome. Flies carrying one type of compound autosome (eg C(2L)) may maintain diploidy by also carrying the complementary compound autosome (C(2R)).[39]

Both compound autosome formation, and the detachment of compound autosomes to reconstitute standard chromosomes occurs spontaneously, but only at low frequency. Both types of chromosomal rearrangement can, however, be induced by gamma irradiation. In Drosophila melanogaster females most chromosomal rearrangements have their break-points in heterochromatin.[42] On chromosome-2, heterochromatin constitutes approximately 20% of the prometaphase length of the left arm and approximately 25% of the prometaphase length of the right arm.[43] Heterochromatic break-points often occur at a distance from the centromere. In come cases, the break-points fall distal to the vital genes that have been identified within this region. A compound autosome synthesized from fragments thus broken will not be isogenic, insofar as

it is not diploid for all functional genetic loci. In an analagous manner, duplications and deletions of proximal genes may also be generated in the detachment process.[44] The detailed mechanics of these processes, and the utilization of deletions generated this way to map gene function is the topic of Chapter 3.

The break-points that generate the two chromatid fragments probably occur at random between any two chromatids in the tetrad. It has been shown that subsequent compound autosome formation can result from both sister and non-sister attachment.[39] However, in the case of C(2R)SD72 formation, the possibilities for strand attachment are limited by the presence of a pericentric inversion on SD72. The break-points of this inversion have been determined on the polytene chromosome. The break-point on the left arm is very near the euchromatic-heterochromatic border at 39D3-4. The break-point on the right arm is a short distance into the euchromatin at 42A.[42]

The juxtaposition of 2Lh to the right arm of chromosome-2 determines that a C(2R) formed by sister strand attachment will be deleted for essentially all of 2Rh and a small segment of euchromatin. The euchromatic deletion would encompass stw. Such a deletion is lethal. Viable C(2R)SD72 chromosomes must, therefore, be formed from non-sister chromatid attachments to ensure the presence of at least one copy of every vital, proximal gene. In addition to being deleted for one copy of 2Rh, a C(2R)SD72 will be duplicated for one copy of 2Lh. Compound-2R formation by sister strand attachment of the unrearranged homologue is possible.

In this study the formation of several types of C(2R) were tested for their ability to modulate expression at the purple locus. Changes in

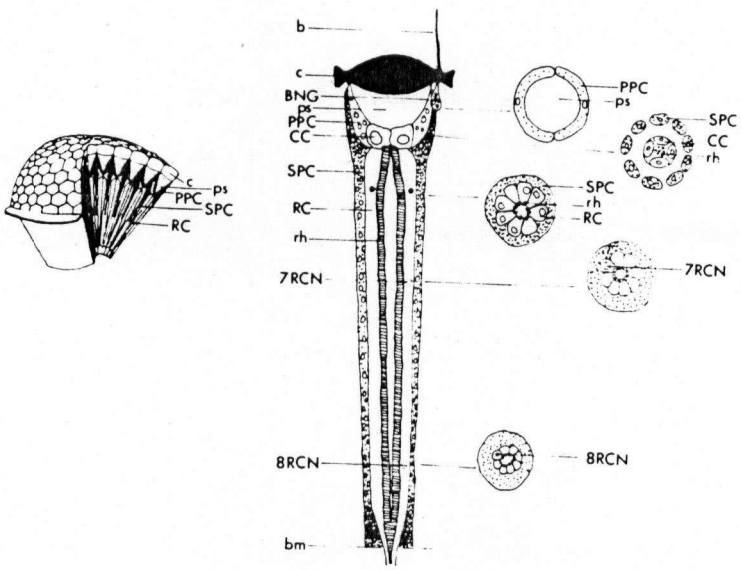
purple gene expression were measured through differences in eye pigmentation that exist between mutant and wild-type flies. Here, pr will denote any purple mutant whereas the specific mutant alleles used are designated as pr^1, pr^2, pr^{c4}, and pr^{bw}.

The normal eye phenotype of Drosophila melanogaster is due to the accumulation of two classes of pigment, the brown ommochromes and the red pteridines. The biochemistry and genetics of eye pigmentation have been reviewed by Phillips and Forrest.[45] The ommochrome present is xanthomattin. The pteridines present are a subset of this group called pterins, or more commonly, drosopterins. The term drosopterin also designates a specific member of this group. Both types of eye pigment are synthesized in the eye. Protein granules with a diameter of 0.4 - 0.8 m are synthesized simultaneously in the eye and association of the pigments with the protein results in a mature pigment granule. The ommochrome and pteridine pigments are separately associated with the protein granules.[46]

The two types of pigment granules have a distinctive deposition within the eye. The fly's eye is composed of about 700 cylindrical units known as ommotidia, that radiate from the optic lobe of the brain to the surface. The ommatidia are organized into a hexagonal array of lenses, or facets, as shown in figure 1. In the wild-type eye, each ommatidia derives its pigmented appearance from the deposition of the two types of pigments in two groups of cells. These are referred to as primary and secondary pigment cells (Figure 1). Brown xanthomattin granules are deposited in the primary pigment cells and the proximal end

Figure 1

Diagram of the adult compound eye and of a single ommatidium. (From Phillips and Forrest, 1980.) b, bristle; bm, basement membrane; BNG, bristler nerve group; C, cornea, CC, cone cell; ps, pseudocone; PPC, primary pigment cell; RC, retinular cell; 7 RCN, 8 RCN, nuclei of seventh and eight retinular cells; rh, rhabdomere; SPC, secondary pigment cell.


c
ps
PPC
SPC
RC
b
c
BNG
ps
PPC
CC
SPC
RC
rh
7RCN
8RCN
bm
PPC
ps
SPC
CC
rh
SPC
rh
RC
7RCN
8RCN

of the secondary pigment cells. Granules carrying the red drosopterins are mostly found in the secondary pigment cells where they are concentrated toward the distal end. A small amount of drosopterin granules are found directly under the basal membrane of the ommatidium.[46]

The pigment granules function to absorb and diffract incident light in a controlled manner. Drosopterins absorb in the blue and near UV region of the spectrum. Xanthomattin absorbs visible light. The presence of eye pigment is essential for visual acuity. White eye mutants have virtually no eye pigment and consequently have no visual acuity. The visual acuity of other eye mutants varies roughly in accordance to the pigment levels of each.[46]

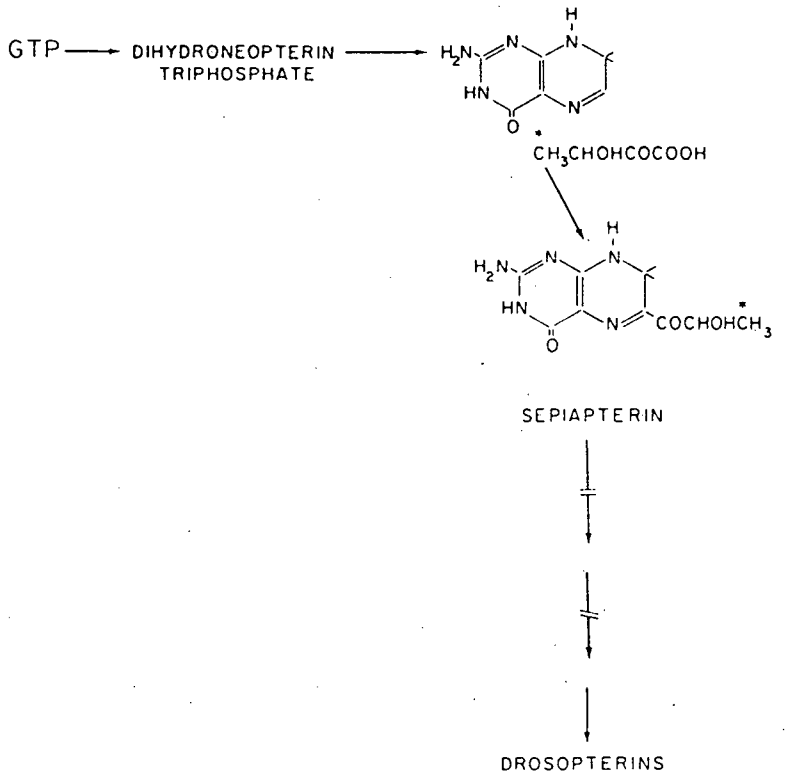
The purple gene participates in the production of the red drosopterins. The purple locus is the site of the structural gene for sepiapterin synthase.[47] As shown in Figure 2, this enzyme catalyses the conversion of dihydroneopterin triphosphate to sepiapterin in the second step of the pteridine pathway. At least five major compounds, all of which are derivatives of alpha-amino-4-hydroxypteridine, result from this pathway (figure 2). Drosopterin and isodrosopterin are enantiomers. The exact pathway from sepiapterin to the end products has not been confirmed. There are other related compounds for which a chemical structure has not been worked out (reviewed in Phillips and Forrest[45]). Purple mutants are characterized by lower levels of drosopterin, isodrosopterin and an unidentified compound (fraction e). Even greater decreases of aurodrosopterin and sepiapterin are found in the pr fly.[48, 49]

Figure 2

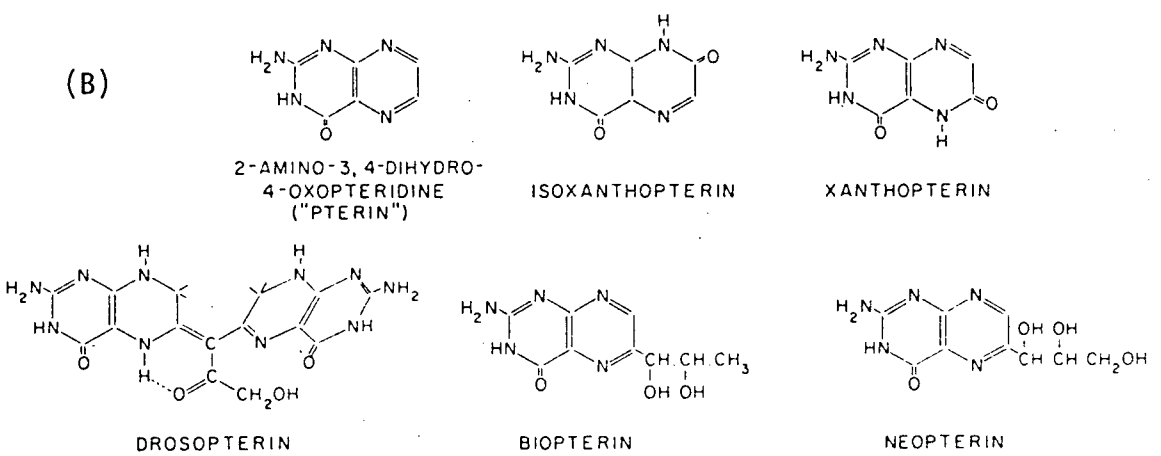
(A) Synthetic pathway for the synthesis of the drosopterins and sepiapterin (after Phillips and Forrest, 1980). The pathway begins with GTP which is converted by GTP cyclohydrolase to dihydroneopterin triphosphate. Sepiapterin synthase catalyses the next step to produce sepiapterin. The broken arrow indicates that the remainder of the pathway is uncharacterized.

(B) Chemical structures of the common drosopterins of Drosophila.

(A)


GTP
DIHYDRONEOPTERIN TRIPHOSPHATE
CH3CHOHCOCOOH
COCHOHCH3
SEPIAPTERIN
DROSOPTERINS

(B)


2-AMINO-3, 4-DIHYDRO-4-OXOPTERIDINE ("PTERIN")
ISOXANTHOPTERIN
XANTHOPTERIN
DROSOPTERIN
BIOPTERIN
NEOPTERIN

Materials and Methods

Mutations and chromosomal rearrangements: A brief description of the genetic markers used in this study is given in Table 1. Further detail on these mutations can be found in Lindsley and Grell.[24] A brief description of the chromosomal rearrangements used in this study is given in Table 2.

Synthesis of C(2L) chromosomes: Three types of pr-bearing C(2L) chromosomes were synthesized for use in this study. The new C(2L) chromosomes were homoyzgous for either pr^1, $b\ pr^1$, or pr^{bw}. Repeated attempts to synthesize $C(2L)pr^2$ were unsuccessful. Each type of C(2L) was synthesized separately by treating about 1,000 homoyygous females with approximately 2,500 rads of gamma radiation from a ^{60}Co source. Groups of 25 treated females were then mated to C(2L)P.b;C(2R)P.px males in half pint bottles at 25 degrees celsius. The flies were transferred to new bottles every five days for a total of three broods. New $C(2L)pr^1$ and $C(2L)pr^{bw}$ were recovered as pr px. Three new $C(2L)pr^1$ were recovered at a frequency of approximately .3 per 100 females treated. Two new $C(2L)b\ pr^1$ were recovered as b pr px progeny at a frequency of about .3 per 100 females treated. Each new C(2L) was established as a separate line with C(2R)P.px. Each new C(2L) was assigned an alphanumeric code according to the system described by Holm.[39]

Synthesis of C(2R) chromosomes: Four standard seconds were used for C(2R) synthesis; SD72 carries SD and also carries a pericentric inversion as described in the Introduction to this chapter and a smaller paracentric inversion on distal 2R. The SD5 chromosome also bears SD and 2

Table 1

Description of second chromosome mutations used.

The chromosome-2 centromere is at 55.1

Symbol	Name	Map Position	Description
v	vermillion	1 - 33.0	bright red eye
b	black	2 - 48.5	black body
pr^1	purple	2 - 54.5	purple eyes
pr^2	purple	2 - 54.5	darker purple eyes
pr^{bw}	purple	2 - 54.5	brownish purple eyes
SD	Segregation Distorter	2 - 55	SD/+ males exhibit meiotic drive
lt	light	2 - 55.1	yellowish-pink eyes
rl	rolled	2 - 55.1	rolled wing edges
cn	cinnabar	2 - 57.5	bright red eyes, colourless ocelli
px	plexus	2 - 100.5	extra wing veins
bw	brown	2 - 104.5	brown eyes

Table 2

Description of compound second chromosomes used.

Symbol	Description
C(2L)SH3,+	Left arm, no genetic markers
C(2L)VY1,b pr^1	Left arm, homozygous for b pr^1
C(2L)VH2,lt	Left arm, homozygous for lt
C(2L)P,b	Left arm, homozygous for b
C(2R)VK43,SD72/cn bw	Right arm, SD72/cn bw
C(2R)SH3,+	Right arm, no genetic markers
C(2R)P,px	Right arm, homozygous for px

non-overlapping inversions on the right arm.[50] An unrearranged second bearing cn bw was used as was a wild-type second from an OR-R stock. New C(2R) chromosomes were synthesized in females from three different SD heterozygotes: SD72/cn bw, SD72/+ and SD5/cn bw. Females in each experiment were treated as discussed above. Groups of 25 females were mated to compound-2 males and cultured as above. New C(2R)SD72/cn bw were recovered with C(2L)P,b as b progeny, while sister strand attachments from SD72/cn bw treated females were recovered as b cn bw individuals. New C(2R)SD72/+ were recovered with C(2L)VH2,lt as suppressed-lt progeny, while the sister strand attachments were recovered as lt individuals. New C(2R)SD5/cn bw were recovered in combination with C(2L)P,b as b progeny and sister strand attachments were recovered as above. Each new C(2R) was established in a separate line with C(2L)P,b and assigned an alpha-numeric code. The number and frequency of each type of new C(2R) is presented in the Results and Discussion.

Cytological Analysis: Polytene chromosomes from salivary glands of late third instar larvae were examined using the method described by Hilliker.[51] The salivary glands were dissected in 45% acetic acid on a depression slide. The isolated glands were transferred to a drop of 2% aceto-lacto-orcein on a siliconized slide and covered with a coverslip. The coverslip was gently tapped, then more firmly pressed to spread the preparation. The preparation was observed and photographed using a Zeiss photomicroscope equipped with phase contrast optics.[51]

Visual Examination for Su(pr): The newly synthesized C(2R), and C(2R)VK43,SD72/cn bw were tested for the ability to suppress pr in combination with several C(2L). All the C(2R) were tested in combination

with C(2L)VY1,b pr^1. In addition, each C(2R) formed by non-sister strand attachment was tested in combination with at least one other C(2L)b pr^1, two C(2L)pr^1 and both C(2L)pr^{bw}. New C(2R) formed by sister strand attachment were tested in combination with one C(2L)pr^{bw}. Each C(2L) was used in combination with several C(2R). When a C(2R) demonstrated Su(pr) activity, the C(2L) bearing the suppressed pr was outcrossed to C(2L)P,b;C(2R)P,px and the px progeny were visually examined for the re-emergence of the pr phenotype. Several C(2R)Su(pr) were also tested for their ability to suppress v by visual examination of v/v;C(2L);C(2R)-Su(pr) females and v/Y;C(2L);C(2R)Su(pr) males.

Spectrophotometric measurement of eye pigments: Several C(2R) were selected for measurement. Each was tested in combination with C(2L)VY1,b pr^1, C(2L)VF1,b pr^1, C(2L)VF1 pr^1 and C(2L)VF1, pr^{bw}. Measurements were also taken for pr^1 and OR-R controls. Stocks of flies cultured as described above were collected at 0 to 24 hours post eclosion. Measurements were taken for one genotype at a time plus a simultaneous measurement of the wild type control. Five determinations were done for each genotype tested. Typically 20 flies, 10 males and 10 females were decapitated for each determination. The heads were placed in 1.0 ml of 1% NH_4OH/0.25 M beta-mercaptoethanol in a microcentrifuge tube on ice and sonicated for 20 seconds. The homogenate was centrifuged at high speed for one minute. The absorbance of 0.5 ml of the clear supernatant was immediately recorded at 495 nm on a Unicam SP1750 ultraviolet spectrophotometer.

Results and Discussion

Thirty-one putative C(2R)SD72 were recovered from approximately 1,600 treated females. The frequency of recovery was about 2 per 100 females treated. Examination of polytene preparations confirms the constitution of these C(2R). As shown in Figure 3, the paracentric inversion on the right arm of SD72 forms an inversion loop in pairing with its unrearranged homologue. A difference in the length of the heterochromatic region caused by the pericentric inversion on SD72 is also evident. As shown in Figure 4, the difference in arm length of the homologues caused by the pericentric inversion prevents the proximal pairing. Five cn bw sister strand attachments were also recovered at a frequency of approximately .3 per 100 females treated. The number of C(2R) of both types was undoubtedly higher than the number recovered, but only one quarter of the eggs bearing newly formed C(2R) are expected to be fertilized by a complementary sperm to produce a diploid viable zygote.[39]

Twenty-eight of the thirty-one new C(2R)SD72/cn bw were found to suppress pr^1. All these C(2R)Su(pr) were seen to fully suppress all C(2L)pr^1 and C(2L)b pr^1 against which they were tested. This shows that Su(pr) action is not peculiar to a specific C(2L). In addition, all C(2R)Su(pr) also looked fully wild-type in combination with both C(2L)pr^{bw}. This shows that Su(pr) is not allele specific.

Each of the suppressed C(2L) was outcrossed to C(2R)P,px. Visual inspection of these flies revealed that full pr^1 and pr^{bw} had fully re-emerged. This demonstrates that the pr locus had not undergone a

Figure 3

Photomicrograph of the proximal region of a polytene chromosome preparation of C(2R)VF5,SD72/cn bw. The arrow points to the inversion loop formed by pairing of the right arm of SD72 with its unrearranged homologue.

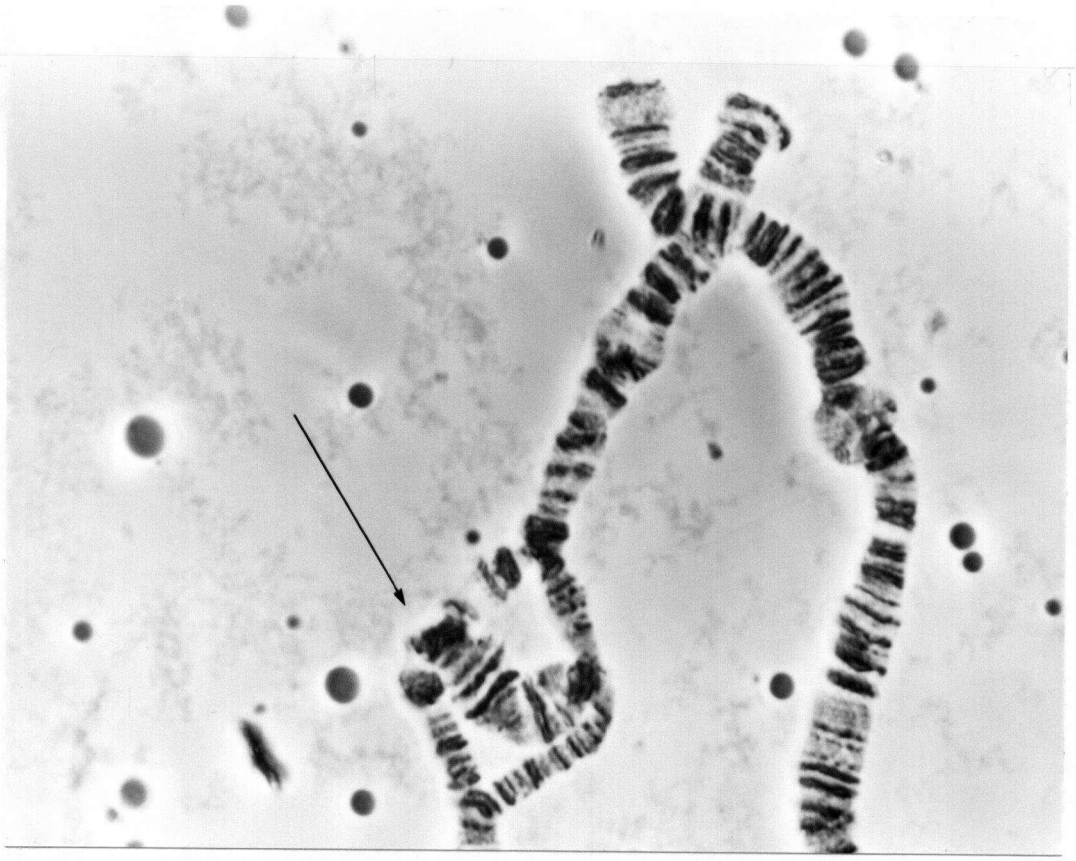
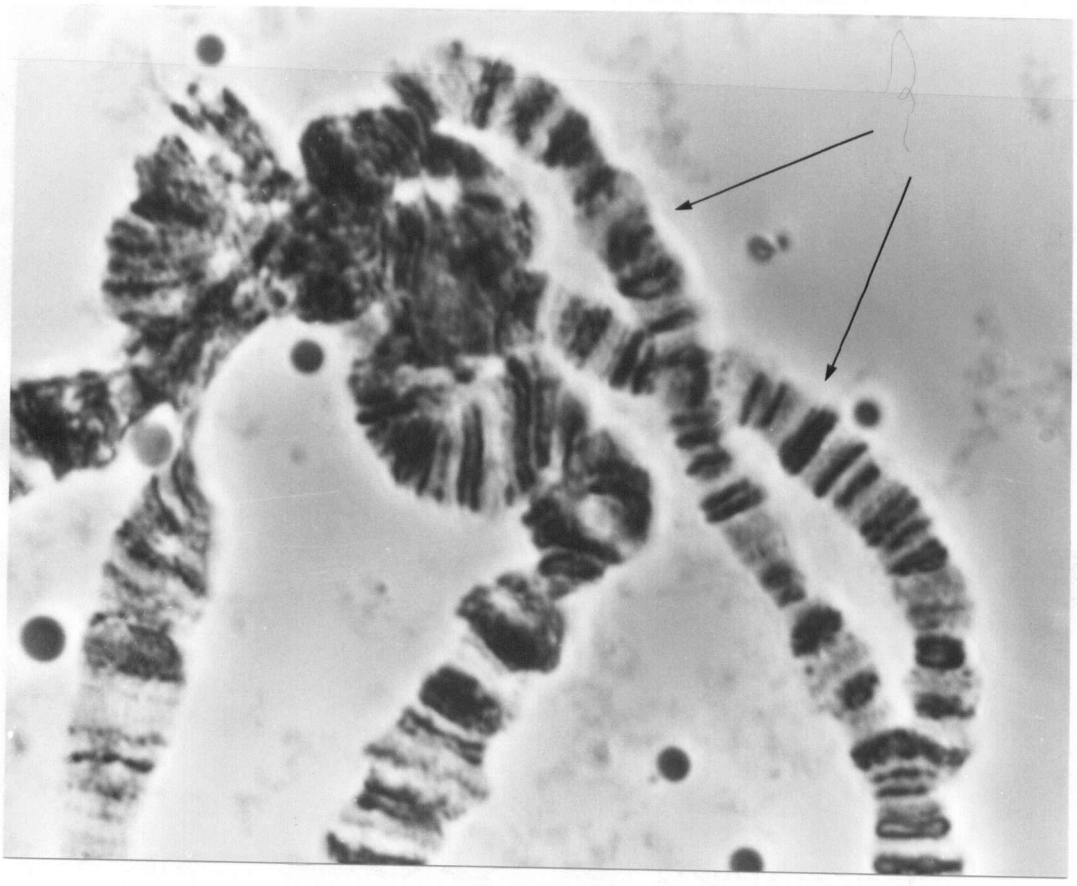


Figure 4

Photomicrograph of the proximal region of a polytene chromosome preparation of C(2R)VF5, SD72/cn bw. The arrows indicate an unpaired region adjacent to the chromocenter.



permanent alteration, and that the presence of Su(pr) is necessary for continued suppression. The three non-suppressing C(2R)SD72/cn bw had a fully mutant phenotype. In no case was an intermediate eye colour observed. It was also found that in no case did a C(2R)Su(pr) suppress v. This is interesting because pr and v are both target mutations of $su(s)^2$. This difference in target specificity plus the fact that Su(pr) action is dominant while $su(s)^2$ is a recessive mutation, suggests that the mode of action of these two suppressor genes is different.

The specificity of C(2R) constitution necessary for Su(pr) induction was tested two ways. First, C(2R) were synthesized in SD72/+ females. Both C(2R)SD72/+ formed by non-sister strand attachment, and C(2R)+ formed by sister strand attachment were expected. Testing the C(2R)SD72/+ for suppression in combination with C(2L)pr chromosomes, was intended to determine whether a wild-type second could substitute for the cn bw-bearing homologue originally used. Testing the C(2R)+ for the ability to suppress was intended to determine whether the presence of a SD72-donated fragment was essential for the formation of a C(2R)Su(pr).

The two types of C(2R) expected were classified according to their ability to suppress lt on C(2L)VH2,lt. A C(2R)SD72/+ can carry a lt^+ duplication on the inverted chromatid fragment donated by SD72. Only if SD72 is broken to the right of the centromere, distal to lt, will the resulting C(2R)SD72/+ not carry a lt^+ duplication. A lt^+ duplication may also be donated to a new C(2R)SD72/+ by the other homologue. This can occur when the chromatid fragment donated by the wild-type second carries a break-point to the left of the centromere, distal to lt. Similarly, a C(2R)+ can carry a lt^+ duplication if one of the wild-type progenitor

standards was broken distal to lt. Gibson[52] found that approximately 90% of all C(2R) chromosomes formed from unrearranged standards will carry their break-points proximal to lt. Consequently, the suppression of lt will accurately classify most of the compound seconds synthesized here as C(2R)SD72/+. However, absolute confidence in this classification could only be gained through the cytological analysis of each of these compounds.

Five C(2R)SD72/+ were recovered out of about 1,500 females treated, a frequency of approximately .33 per 100 females treated. It is interesting to note that the frequency of recovery of C(2R)SD72/+ was lower by an order of magnitude than that of C(2R)SD72/cn bw. Three of the five C(2R)SD72/+ showed Su(pr) activity with all the C(2L) that they tested with: two C(2L)b pr^1, two C(2L)pr^1 and both C(2L)pr^{bw}. This demonstrates that the presence of the cn bw-bearing homologue is not necessary for Su(pr) induction. Nine C(2R)+ chromosomes were also recovered in this experiment, a recovery frequency of approximately .6 per 100 females treated. None of them was able to suppress pr^1 or pr^{bw} on any C(2L). This suggests that the presence of a chromatid fragments from SD72 is essential for C(2R)Su(pr) formation.

The recovery of sister strand attachments was greater than the recovery of non-sister strand attachments in this experiment. This is the opposite of what is expected. One sixth of recovered attachments induced are expected to be of the sister strand type and four sixths of the non-sister strand type.[39] Another one sixth are lethal as discussed above. The deviation from the expected ratio and the low recovery frequency relative to C(2R)SD72/cn bw, suggests that C(2R)SD72/+ may have poor viability.

The constitution necessary for Su(pr) induction was tested in a second way. Compound-2R chromosomes were synthesized from the cn bw bearing homologue and another SD bearing second, SD5. Unlike SD72, SD5 does not carry a pericentric inversion.[50] Twenty-three C(2R)SD5/cn bw were recovered from approximately 1,000 females treated at a frequency of about 2.3 per 100 females treated. This agrees with the recovery rate for C(2R)SD72/cn bw. This agreement substantiates the suggestion that the much lower recovery rate of C(2R)SD72/+ is indicative of poor viability. None of the 23 C(2R)SD5/cn bw could suppress either pr^1 or pr^{bw}. The cn bw sister strand attachments recovered in this experiment cannot be used to study suppression since cn bw is epistatic to pr.

These findings suggest that the presence of SD is not responsible for Su(pr) induction. It substantiates the suggestion that SD72 makes an essential contribution to Su(pr) induction. It also raises a question regarding the presence of the pericentric inversion, present only on SD72. This inversion confers upon the C(2R)SD72 a proximal constitution unlikely in C(2R)SD5, or any other C(2R) not synthesized from an inverted standard second. The C(2R)SD72 carry a 2Lh duplication and a 2Rh deletion that is probably unique to this type of C(2R). Also, the C(2R)SD72 can carry 2Lh and 2Rh in a juxtaposition not possible in any other type of C(2R). A detailed study of the possible constitution necessary for Su(pr) induction is covered in Chapter 3.

Suppression of pr was quantified using the spectrophotometric measurement of eye pigment. This technique is based on the fact that aqueous ammonia extracts of wild type Drosophila heads absorb strongly in the blue region of the spectrum. Analysis of such extracts has revealed

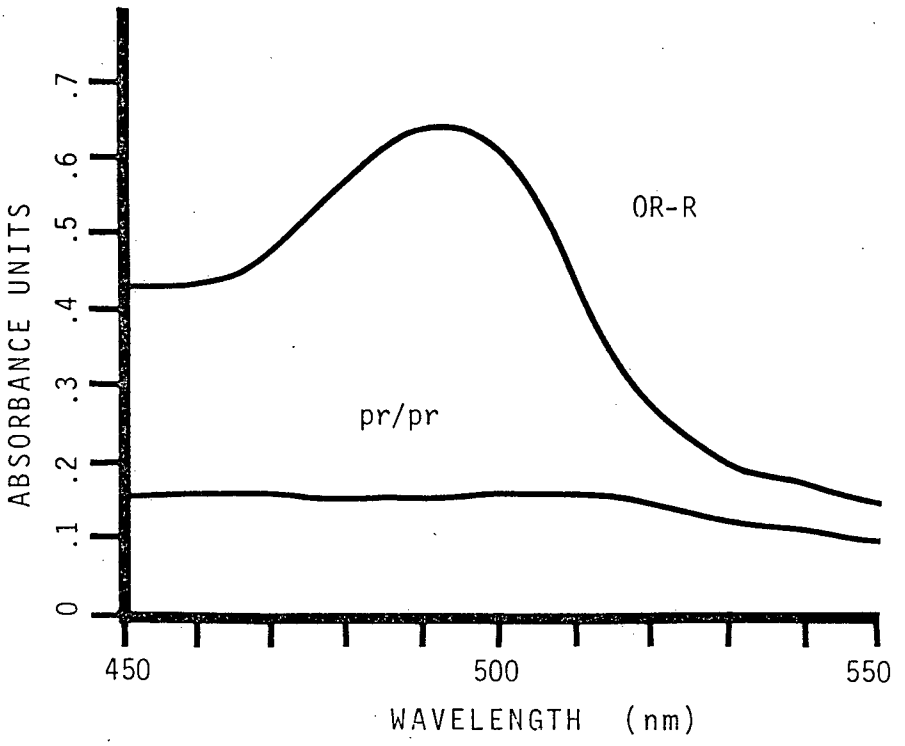
that most of the absorbance is due to the presence of drosopterins.[46] As shown in Figure 5, extracts made from the wild type have an absorption maximum at 495 nm. In the $\underline{pr^1}$ extract, however, there is a very large reduction in absorbance throughout this region, and the peak at 495 nm is undetectable. This large difference in absorbance also exists between suppressed and unsuppressed $\underline{pr^1}$ flies. A comparison between the $\underline{pr^1}$ absorbance curve for a standard second and a C(2R) reveals that they are identical. Likewise, the absorbance curve of suppressed-$\underline{pr^1}$ and the wild type are indistinguishable (Figure 5). Hence, the mean absorbance of head extracts measured at 495 nm (A_{495}) is an effective technique for quantifying drosopterin levels.

Drosopterin measurements were consistent for all C(2R)Su(pr) recovered. Consequently, the results of tests on C(2R)VK43.SD72/cn bw, and three newly synthesized C(2R)SD72 are presented as representative of all C(2R)Su(pr) tested. The results include a representative of each type of C(2L) used. Drosopterin levels are expressed as a percentage of the wild-type levels. Table 3 shows the control values for the wild type and unsuppressed pr. The C(2L)SH3,+;C(2R)SH3,+ drosopterin levels agrees with the wild-type level. This shows that C(2R) formation does not alter eye pigmentation per se. This fact is supported by the synthesis of many difference C(2R) in other studies without any alteration of eye pigmentation.[41]

Although the black gene does not participate in drosopterin synthesis,

Figure 5

Absorbance of drosopterin extracts from 450-550 nm.


ABSORBANCE UNITS
0
.1
.2
.3
.4
.5
.6
.7
OR-R
pr/pr
450
500
550
WAVELENGTH (nm)

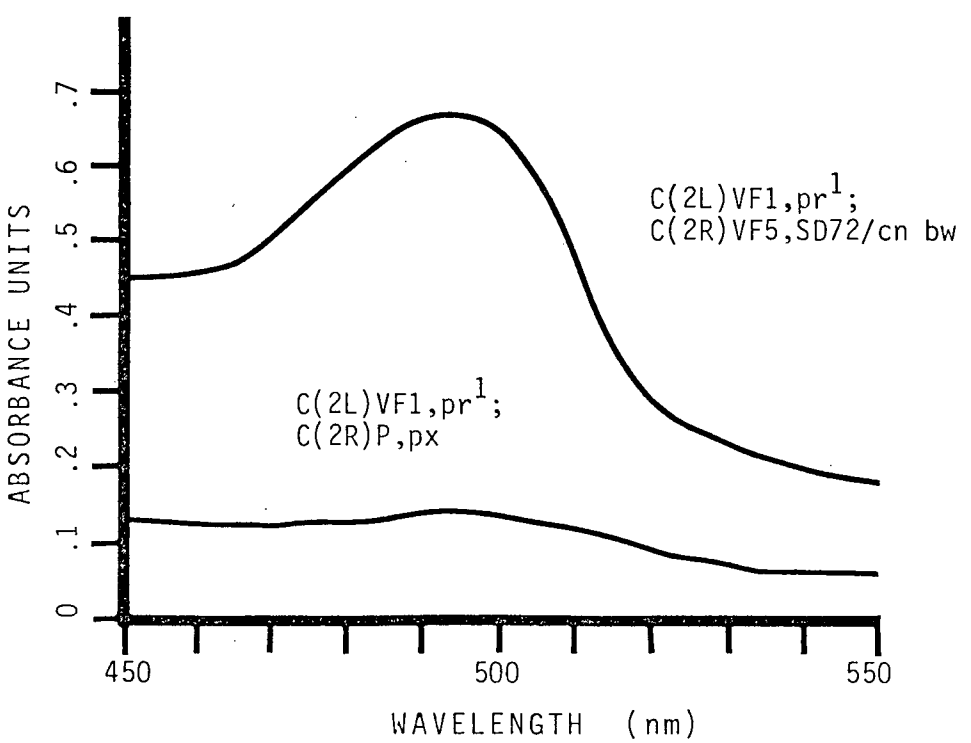

ABSORBANCE UNITS
0
.1
.2
.3
.4
.5
.6
.7
C(2L)VF1,pr1;
C(2R)VF5,SD72/cn bw
C(2L)VF1,pr1;
C(2R)P,px
450
500
550
WAVELENGTH (nm)

Table 3

Drosopterine levels is unsuppressed C(2L) strains

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.780	.073	100
C(2L)SH3,+; C(2R)SH3,+	5	.760	.073	97
C(2L)P,b; C(2R)P,px	5	.885	.078	113
C(2L)VY1,b pr^1; C(2R)P,px	5	.395	.069	51
C(2L)VF1,b pr^1; C(2R)P,px	5	.391	.059	50
C(2L)VF1,pr^1; C(2R)P,px	5	.252	.068	32
C(2L)VF1,pr^{bw}; C(2R)P,px	5	.142	.059	25

the presence of b on some C(2L) was found to slightly increase the A_{495}. This increase was consistently about 0.1 absorbance units above b^+. This results in the A_{495} of C(2L)P,b being approximately 13% greater than C(2L)SH3,+ and the C(2L)b pr^1 about 15% above C(2L)pr^1 (Table 3). The elevation of A_{495} in the presence of b must be considered in the discussion of suppressed-pr drosopterin levels.

The C(2L)VF1,pr^1:C(2R)P,px strain was found to have 32% of the wild type drosopterin levels. This is consistent with the levels found for homozygous pr^1 on standard seconds.[26] The C(2L)VF1,pr^{bw}:C(2R)P,px was found to have drosopterin levels that were 25% of those in the wild type (pr+) strains. Again, this agrees measurement for this allele on standard seconds.[47]

The drosopterin measurements for the four C(2R)Su(pr) presented are contained in Tables 4 - 7. All C(2R)Su(pr) showed drosopterin levels in excess of the wild type. In all tests, the C(2L)pr^{bw} which had the lowest unsuppressed drosopterin levels, also had the lowest suppressed levels. The Su(pr):pr^{bw} drosopterin levels consistently exceeded the wild-type levels by only 1 - 3%. This increase, of 75 - 80% over the mutant levels, was found for both C(2L)pr^{bw} in combination with every C(2R)Su(pr) tested.

The drosopterin levels in Su(pr):vpr^1/pr^1 also exceeded those in the wild-type, but by a greater amount. The suppressed pr^1 levels were in the range of 106 - 110% of wild type for every C(2R)Su(pr) tested. The C(2L)bpr^1 was found to have the highest suppressed pigment levels. All

Table 4

Drosopterin levels in C(2R)VK43,SD72/cn bw strains

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.792	.045	100
C(2L)VY1,b pr^1; C(2R)VK43,SD72/cn bw	5	1.012	.052	128
C(2L)VF1,b pr^1; C(2R)VK43,SD72/cn bw	5	1.009	.080	127
C(2L)VF1,pr^1; C(2R)VK43,SD72/cn bw	5	.872	.076	110
C(2L)VF1,pr^{bw}; C(2R)VK43,SD72/cn bw	5	.802	.071	101

Table 5

Drosopterin levels in C(2R)VF5,SD72/cn bw strains

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.788	.055	100
C(2L)VY1,b pr^1; C(2R)VF5,SD72/cn bw	5	.985	.070	125
C(2L)VF1,b pr^1; C(2R)VF5,SD72/cn bw	5	.961	.043	122
C(2L)VF1,pr^1; C(2R)VF5,SD72/cn bw	5	.839	.061	106
C(2L)VF1,pr^{bw}; C(2R)VF5,SD72/cn bw	5	.831	.083	105

Table 6

Drosopterin levels in C(2R)VF10,SD72/cn bw strains

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.785	.059	100
C(2L)VY1,b pr^1; C(2R)VF10,SD72/cn bw	5	.966	.046	123
C(2L)VF1,b pr^1; C(2R)VF10,SD72/cn bw	5	.950	.048	121
C(2L)VF1,pr^1; C(2R)VF10,SD72/cn bw	5	.865	.059	110
C(2L)VF1,pr^{bw}; C(2R)VF10,SD72/cn bw	5	.809	.062	103

Table 7

Drosopterin levels in C(2R)VF30,SD72/cn bw strains

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.795	.060	100
C(2L)VY1,b pr^1; C(2R)VF30,SD72/cn bw	5	.969	.071	122
C(2L)VF1,b pr^1; C(2R)VF30,SD72/cn bw	5	.962	.045	121
C(2L)VF1,pr^1; C(2R)VF30,SD72/cn bw	5	.859	.062	108
C(2L)VF1,pr^{bw}; C(2R)VF30,SD72/cn bw	5	.818	.078	103

Su(pr);b pr^1/b pr^1 drosopterin measurements exceeded 120% of the wild type and the range induced by the four C(2R)Su(pr) presented here is 121 - 128%. When the contribution to absorbance by b is subtracted, there is good agreement between suppressed drosopterin levels in C(2L)pr^1 and C(2L)b pr^1. Suppressed drosopterin measurements were in agreement in C(2L)VY1,b pr^1 and C(2L)VF1,b pr^1 although the former had slightly higher levels throughout. This variation is partly due to peculiarities of the C(2L), since it was also seen in the absence of C(2R)Su(pr) (Table 3). Overall, the suppression of pr^1 was associated with drosopterin levels approximately 10% greater than wild type, once the contribution to absorbance by b (if present) was accounted for. These results were consistent for each combination of pr^1-bearing C(2L) and C(2R)Su(pr) tested.

The drosopterin measurements in each of the three nonsuppressing C(2R)SD72/cn bw strains confirmed the visual inspection by exhibiting fully mutant pigment levels. Table 8 shows the drosopterin levels produced by one of them, C(2R)VF12,SD72/cn bw. A comparison between these values and those of unsuppressed C(2L)pr (Table 3) reveals no significant difference. Table 9 shows the drosopterin measurements in C(2R)VF3,+. These measurements agree with those in the C(2R)SD72/cn bw. Drosopterin measurements were found to be consistent in all suppressing C(2R)SD72, irrespective of whether they carried a wild-type chromatid fragment, or one from the cn bw second.

These findings agree with earlier studies in which suppressed-pr^1 and suppressed pr^{bw} drosopterin levels induced by various su(s) alleles

Table 8

Drosopterin levels in C(2R)VF12,SD72/cn bw strains

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.778	.017	100
C(2L)VY1,b pr^1; C(2R)VF12,SD72/cn bw	5	.403	.080	52
C(2L)VF1,b pr^1; C(2R)VF12,SD72/cn bw	5	.365	.037	47
C(2L)VF1,pr^1; C(2R)VF12,SD72/cn bw	5	.259	.049	33
C(2L)VF1,pr^{bw}; C(2R)VF12,SD72/cn bw	5	.205	.052	26

Table 9

Drosopterin levels in C(2R)VF3,SD72/+

Genotype	Extracts Tested	Mean A_{495}	Standard Deviation	Drosopterin levels (% of OR-R)
wild type (OR-R)	5	.795	.045	100
C(2L)VY1,b pr^1; C(2R)VF3,+	5	1.088	.066	137
C(2L)VF1,b pr^1; C(2R)VF3,+	5	1.050	.0458	132
C(2L)VF1,pr^1; C(2R)VF3,+	5	.836	.059	105
C(2L)VF1,pr^{bw}; C(2R)VF3,+	5	.802	.057	101

were measured. As in this study, Yim et al[47] found that suppressed pr levels exceeded the wild type, and suppressed-pr^1 levels exceed those of suppressed-pr^{bw}. Yim et al[47] and Jacobson et al[26] also measured the sepiapterin synthase activity levels and compared them to drosopterin levels in suppressed and unsuppressed mutants. Jacobson et al[26] found that a su(s) mutant allele need only raise enzyme activity from the 15% activity level found in $su(s)^+;pr^1$, to 20% of wild-type activity in order to produce wild-type levels of pigment. This shows that above 20% of wild-type activity, the sepiapterin synthase activated step is not rate limiting on drosopterin production.

Jacobsen et al[26] also found that suppressed-pr^1 enzyme activity differed when in combination with different su(s) alleles. Enzyme activity and drosopterin levels were found, in most cases, to be roughly proportional. The weakest allele used, $su(s)^{e5.6}$ raised pr^1 enzyme activity to approximately 34% wild type, and accumulated 105% wild-type pigment levels. The most effective allele, $su(s)^2$ raised sepiapterin synthase activity to 75% and pigment levels to 125% of the wild type.

The discrepancy between enzyme and drosopterin levels in $su(s);pr^1$ suggests that the drosopterin measurements for $Su(pr);pr^1$ may, likewise, not accurately reflect suppressed enzyme activity. If Su(pr) is similar to su(s) in this respect, then different C(2R)Su(pr) chromosomes may differ widely in their ability to elevate enzyme activity, while exhibiting pigment levels that agree quite closely. Also, Su(pr) may not have to raise enzyme levels by much to produce the suppressed-pr phenotype. The $su(s)^{x4};pr^1$ produced drosopterin levels in the range of 110% of wild

type, (like Su(pr);pr^1), by raising enzyme activity to only 50% of the wild type.

It is also interesting to note that the difference between enzyme and pigment levels induced by $su(s)^2$ varies between suppressed pr^1 and suppressed pr^{bw}. Yim et al[47] found that, unlike the large difference found in su(s);pr^1, in su(s);pr^{bw} there was a close correlation between drosopterin pools and sepiapterin synthase activity. Suppressed-pr^{bw} flies had enzyme and pigment levels that were both very close to the wild type. Hence, in contrast to the suppression of pr^1, the suppression of pr^{bw} can not be produced with less than wild type levels of enzyme activity.

The findings of the studies on su(s) pose two questions that are pertinent to the study of Su(pr). First, why does $su(s)^2$ raise the activity of one target allele (pr^1) to 75% of the wild type, yet raise the activity of another target allele (pr^{bw}) to approximately 100% of the wild type? This is especially puzzling because pr^{bw} has a lower unsuppressed activity level than pr^1. Second, how can the lower enzyme activity in $su(s)^2$;pr^1 result in greater drosopterin accumulation (125%) than is found in $su(s)^2$;pr^{bw} (approximately 100% of the wild type).

Perhaps, answers to both these questions involve su(s) induced changes in the development profile of target alleles. Tobler et al[53] have found that there are two main peaks of sepiapterin synthase activity. The first peak occurs very early in larval development, and the second peak begins late in the pupal stage then recedes at about three days post eclosion. It is this second peak that is responsible for eye pigmentation.[53] Drosopterin levels in excess of the wild type could

accumulate in flies with less than wild-type enzyme activity, if the second activity peak began earlier than normal. Alteration in the developmental profile could also explain the different suppressed-$\underline{pr^1}$ pigment levels found in combination with different $\underline{su(s)}$ alleles. Such variation could be accomplished if peak expression of $\underline{pr^1}$ was induced at different times by different $\underline{su(s)}$ alleles. This explanation posits that the greater the difference is between enzyme activity levels and drosopterin levels, the earlier the increase in suppressed enzyme activity must begin.

The difference in the effect of $\underline{su(s)^2}$ on $\underline{pr^1}$ and $\underline{pr^{bw}}$ can also be explained by differences in the developmental profile between the two suppressed target alleles. The close agreement of $\underline{su(s)^2;pr^{bw}}$ enzyme activity levels and drosopterin pools to those of the wild type, suggests that the developmental profile of both is virtually identical. In $\underline{su(s)^2;pr^1}$, however, it may be that enzyme activity does in fact reach wild type levels, but much earlier than normal. If so, it might already be receding at the point when it is measured. The suppressed-$\underline{pr^1}$ activity levels might be lower than either the wild type or suppressed $\underline{pr^{bw}}$ at this time. At the same time, the earlier onset of suppressed-$\underline{pr^1}$ peak activity would result in higher drosopterin accumulation than found in normal or suppressed development.

Considering the enzyme assay studies in $\underline{su(s)^2;pr}$, it is possible to envision how $\underline{Su(pr)}$ might modulate $\underline{pr}$. The possibilities include changes in the onset of peak sepiapterin synthase activity, the profile of the activity peak, the maximum enzyme activity, or the duration of suppressed activity levels. Time course enzyme activity studies in $\underline{Su(pr);pr}$ would make it possible to discern between these possibilities and, as such, would provide a better understanding of $\underline{Su(pr)}$.

CHAPTER 3

ANALYSIS OF C(2R)Su(pr) DETACHMENT PRODUCTS

Introduction

In the detachment process, the steps giving rise to compound autosome formation are reversed, and reconstituted standard chromosomes are recovered.[44] There are two main reasons for undertaking detachment studies on C(2R)Su(pr). First, since Su(pr) was induced during C(2R) formation, it is pertinent to ask whether its function is dependent on that specific chromosomal rearrangement. If Su(pr) activity is dependent on C(2R)Su(pr) constitution, then detachment of the component arms should eliminate its expression. If, however, Su(pr) activity is not dependent on a specific chromosomal rearrangement, but arose through the process of rearrangement, then Su(pr) expression might persist in other types of rearrangement. If so, suppressing detachment products might be recovered.

The second reason for detaching C(2R)Su(pr) is that it allows each component arm to be studied separately over other homologues. These detachment products can be tested with the known vital markers in chromosome-2 heterochromatin. Lethality over these vital genes can reveal deletions of heterochromatin. From this, the constitution of the detachment products may be partially deciphered. Correlations between specific types of deletions, and the loss of suppression, may help to localize Su(pr).

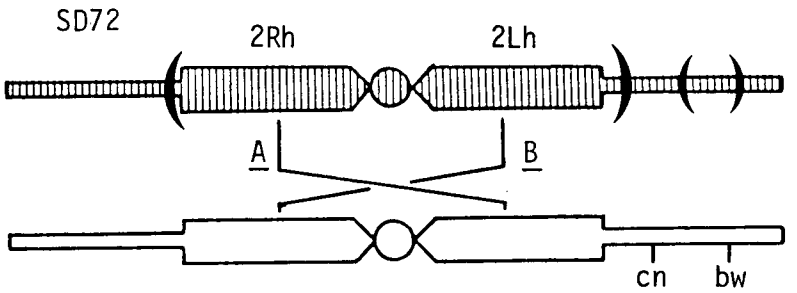
To understand how differences in heterochromatic constitution may help to characterize Su(pr), it is necessary to examine what types of rearrangement may be recovered in the detachment products. The particular heterochromatic constitution of any detachment is initially determined, in

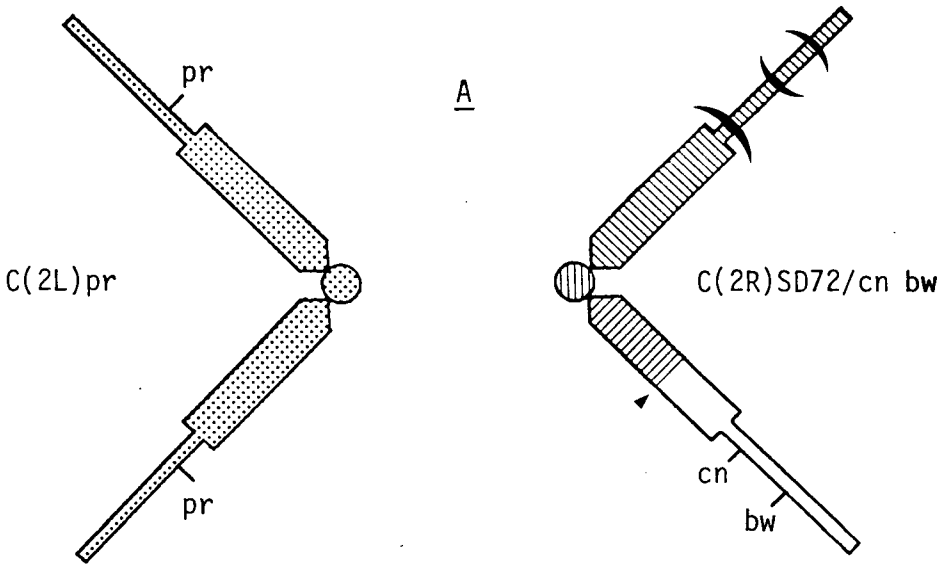
part, by the way in which the progenitor C(2R) is made. The way in which duplications and deletions can be generated during compound autosome formation has been thoroughly analyzed by Hilliker and Holm.[44] The two central points revealed by that analysis pertinent to this study are: (1) all genes lying proximal to a break-point on the centric fragment will be carried as duplications on the newly formed compound autosome, and (2) conversely, the free arms generated by such a break will be deleted for those proximal genes, as will a compound autosome that receives that arm. Since the centric fragment is donated by one homologue and the free arm by the other, differences in the positions of the break-points on each determines that the new compound autosome may be isogenic, or carry a duplication, or a deletion, or both a duplication and a deletion. The same mechanism occurs during the detachment procedure, with the result that a reconstituted standard chromosome may undergo further alterations.[44]

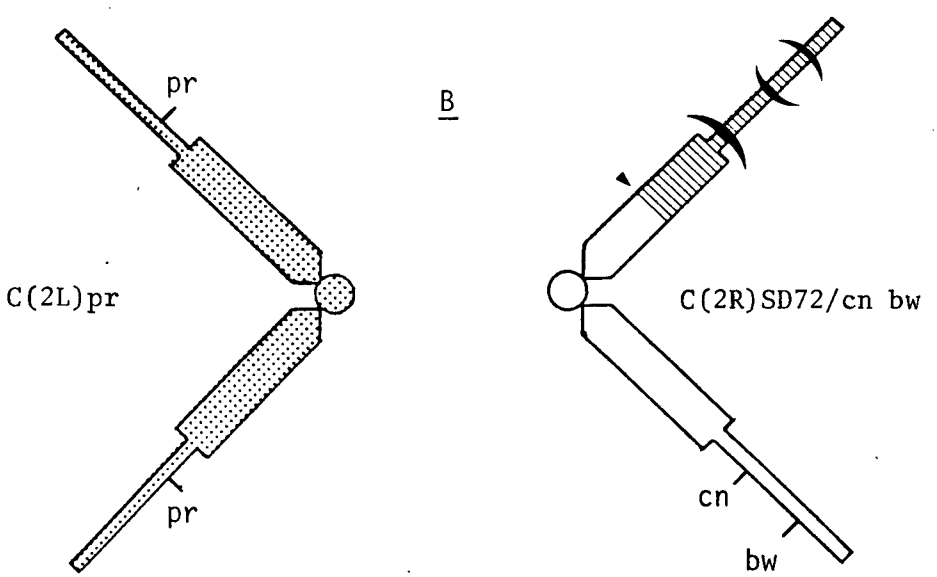
The mechanics involved in C(2R)SD72 synthesis are further complicated by the presence of the pericentric inversion. This inversion is fundamental in determining the content and configuration of heterochromatin in C(2R)SD72. As shown in Figure 1, the pericentric inversion determines that there are two possible ways that the C(2R)SD72 can be composed, depending on whether SD72 or its homologue donate the centric fragment. If SD72 donates the centric fragment, as is part A of Figure 1, then the break-points on both homologues will have occurred in 2Rh. The resulting C(2R) will carry a proximal segment of 2Rh from SD72. This segment may include vital genes, if the break-points on SD72 fell distal to their loci. Vital 2Rh genes may also be duplicated on the captured arm, if the

Figure 1

Alternate configurations of chromatid breakage and reunion in C(2R)SD72/cn bw synthesis. (A) Chromatid fragments are generated by break-points in 2Rh of both homologues. (B) Both chromatid fragments are generated by breaks in 2L,h.


SD72
2Rh
2Lh
A
B
cn
bw


pr
C(2L)pr
pr
A
C(2R)SD72/cn bw
cn
bw


pr
C(2L)pr
pr
B
C(2R)SD72/cn bw
cn
bw

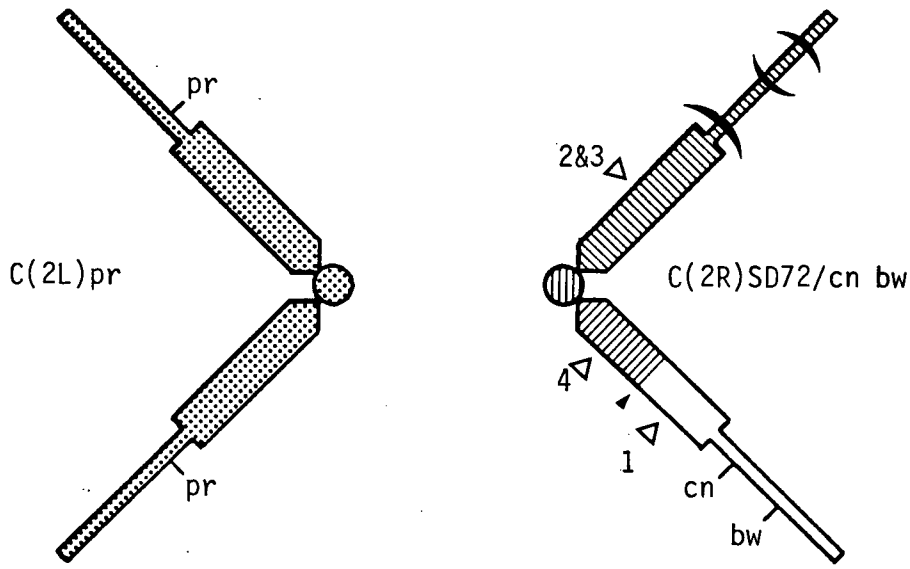
break-point on the cn bw-bearing homologue fell proximal to them. This C(2R) will be duplicated for all of 2Lh. The second possibility is shown in Figure 1B. In this case, SD72 donates the captured arm, and the centric fragment is derived from its homologue. The break-points on both standards will occur in 2Lh. The resulting C(2R) will not carry any 2Rh from SD72 It will be duplicated for part, but not necessarily all of 2Lh. Either way, the C(2R) will be deleted for one copy of 2R to a point just distal to stw. It is also known that no viable C(2R)SD72/cn bw will be deleted for any vital gene in the remaining 2Rh segment.

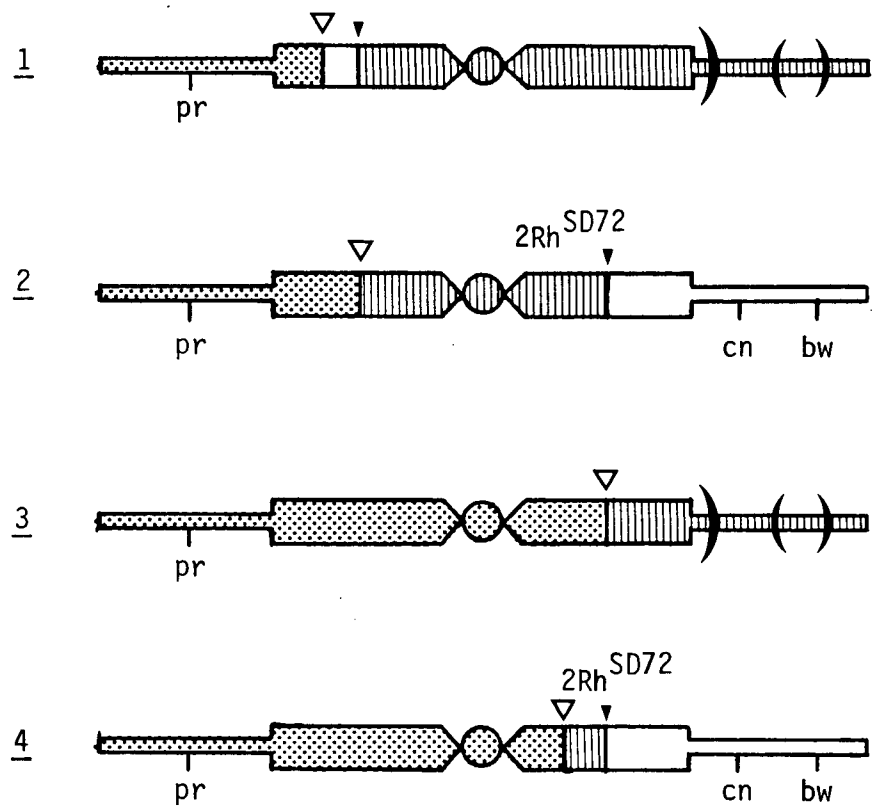
The two possible ways of constructing a C(2R)SD72 determines that two different sets of detachment products are possible. Figure 2 shows the four detachment classes that could possibly be recovered from the C(2R) in Figure 1A. Among the possible variations considered here are (1) the donation of the centric fragment from the C(2L) and the acentric fragment from the C(2R), (2) the reciprocal association, (3) the donation of an acentric fragment derived from either arm of the C(2R) and, (4) variations in the position of the break-point on the acentric fragment. Since it is the constitution of the C(2R) that is under investigation, variation in the position of break-points on the C(2L) are not considered here. Such variations do occur, however, and in practice they will complicate detachment analysis.

Detachments 1 and 2 (Figure 2) both had their centric fragment donated by the C(2R)Su(pr). As well, both classes may be composed of chromosomal segments from three sources: the C(2L), SD72, and its cn bw-bearing homologue. As shown, class 1 detachment resulted from break-points occurring in the 2Rh, on the cn bw side of the C(2R)Su(pr). If

Figure 2

Detachment product classes possible from the C(2R)SD72/cn bw configuration shown in Figure 1(A). The junction between the acentric and centric chromatid fragments that formed the C(2R) is shown by dark arrowheads. Outlined arrowheads show the position of the break-point necessary to generate the four detachment classes. These are numbered on the C(2R) to designate the four classes.


pr
C(2L)pr
pr
2&3
C(2R)SD72/cn bw
4
1
cn
bw


1
pr
2
2Rh SD72
pr
cn
bw
3
pr
4
2Rh SD72
pr
cn
bw

the detachment-generating break-point falls proximal to the C(2R)-generating breakpoint, then this class will not carry the segment derived from the cn bw side that is shown. In any case, the location of this breakpoint will determine which 2Rh genes are donated from this segment. A break-point proximal to a gene in this region makes it possible for the detachment product to carry a non-polar deletion, assuming that it was not duplicated elsewhere during C(2R)Su(pr) formation. This class will carry the SD side of the C(2R)Su(pr) which means that it will be deleted from some, or all, of 2Rh to a point just distal to stw. Presence of this arm can be detected by stw pseudo-dominance. Class 2 detachments will carry the right arm from the cn bw side of the C(2R)Su(pr). This class will maintain any duplications generated in 2Rh during C(2R)Su(pr) synthesis. This class may also carry non-polar deletions for 2Lh.

Class 3 and class 4 detachments are both composed of a captured free arm derived from the C(2R)Su(pr). Class 3 carries the free arm from the SD72 side. All 2Rh will be deleted in this class and some, or all, of 2Lh genes may be duplicated. If a deletion is generated on the 2Lh segment donated by SD72, it will be covered by the corresponding C(2L)-donated segment.

Class 4 detachments will carry the free arm from the cn bw side of the C(2R)Su(pr). It may be composed of chromosomal segments from three sources if the detachment-generating break-point is proximal to the C(2R)-generating break-point. Polar deletions in the 2Rh segment donated by SD72 may be generated in the detachment procedure. However, such deletions may be masked by compensating duplications on the cn bw frag-

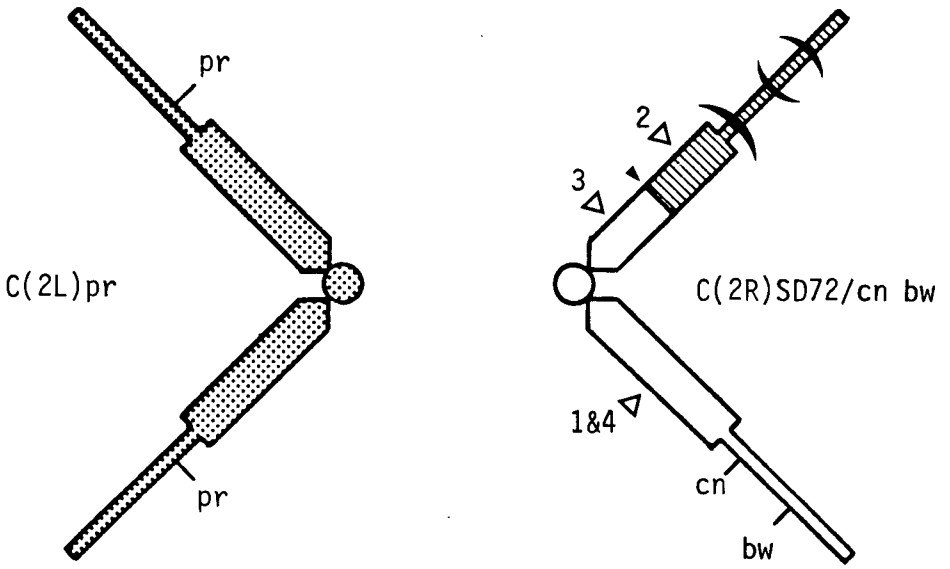
ment. Alternatively, if the break-point that generated the detachment is distal to the one that generated the C(2R), the SD72 2Rh will be completely omitted. In that case, polar deletions may be generated in the distal 2Rh of the cn bw fragment.

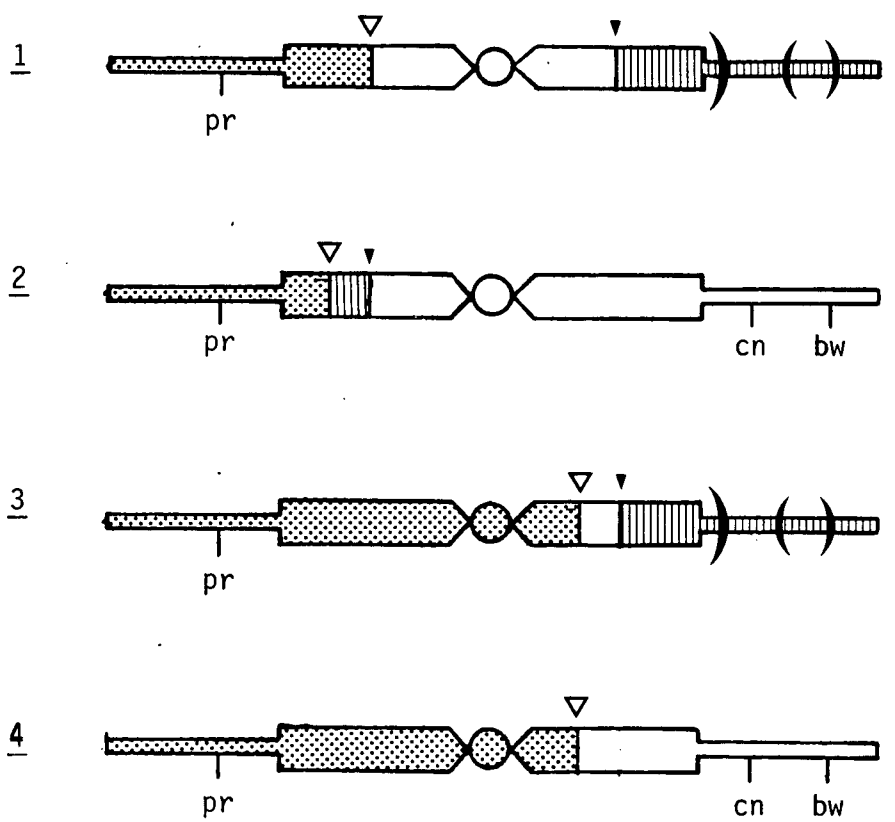
Figure 3 shows the four possible detachment classes expected from a C(2R)Su(pr) synthesized as shown in Figure 1B. As before, classes 1 and 2 contain a centric fragment derived from the C(2R)Su(pr). Detachments of these classes may contain segments from three donor chromosomes. Class 1 detachments contain the centric fragment bearing the SD72 side of the C(2R)Su(pr). The detachment-generating break-point may give rise to non-polar deletions in the 2Rh from the cn bw side. This class may also carry non-polar deletions in the SD72 2Lh that were generated during C(2R) formation.

Class 2 detachments will bear the cn bw arm of the C(2R)Su(pr). Polar and non-polar deletions of 2Lh are possible. Classes 3 and 4 detachments both contain a free arm donated by C(2R)Su(pr). Class 3 detachments are composed of chromosome segments from three sources. They may also carry 2Lh duplications from the cn bw side. They may also carry segments of SD72 2Lh. They should, however, carry no 2Rh. Class 4 detachments contain the free arm from the cn bw side of the C(2R)Su(pr). On these detachments, polar deletions for cn bw-donated 2Rh may be generated.

Figure 3

Detachment products expected from the C(2R)SD72/cn bw configuration shown in Figure 1(B). The junction between the acentric and centric chromatid fragments that formed the C(2R) is shown by dark arrowheads. Outlined arrowheads show the position of the break-point necessary to generate the four detachment classes. These are numbered on the C(2R) to designate the four classes.


pr
C(2L)pr
pr
2
3
C(2R)SD72/cn bw
1&4
cn
bw


1
pr
2
pr
cn
bw
3
pr
4
pr
cn
bw

Materials and Methods

Mutations and chromosomal rearrangements: A brief description of some of the genetic markers and chromosomal rearrangements used is given in Table 1. The rest are described in Chapter 2, Materials and Methods. Further details can be found in Lindsley and Grell.[24] The lethal deletions and EMS-induced lethal mutations used are shown in Figure 4. These are discussed in detail in Hilliker.[54]

Recovery of detachment products: C(2R)VK43,SD72/cn bw was detached in separate experiments with C(2L)VY1,b pr^1 and C(2L)VD3,nub^2 b^{66h} pr^1. The three newly synthesized C(2R)Su(pr), described in Chapter 2, were detached in separate experiments with C(2L)VD3,nub^2 b^{66h} pr^1. Virgin compound-2 females were treated with 2,500 rads of gamma radiation and mated to Df(2L)161,pr/In(2LR)0,Cy $dp^{1v1}pr^1cn^2$ (In(2LR)Cy0) males. The number of treated females varied between experiments. The procedures for mating and culturing are described in Chapter 2, Materials and Methods. Each detachment product was established in a separate lines with In(2LR)Cy0.

Scoring of detachment products for Su(pr): All detachments were scored for suppression over In(2LR)Cy0 both by visual inspection as described in Chapter 2, Materials and Methods.

Lethality tests: Each detachment product was tested for recessive lethality by crossing males and females that carried a detachment over In(2LR)Cy0,Cy pr cn^2, and scoring for Cy^+ progeny. Each lethality test was done in duplicate. If the detachment carried a recessive lethal, no Cy^+ progeny were recovered. If the detachment product was homozygous

Table 1A

Description of second chromosome mutations used.

The chromosome 2 centromere is at 55.1

Symbol	Name	Map Position	Description
Cy	Curly	2 - 6.1	wings curled upward homozygous lethal
nub^2	nubbin	2 - 47.0	allele of nub; small spoon-shaped wing; less extreme than nub
b^{66h}	black	2 - 48.5	black body

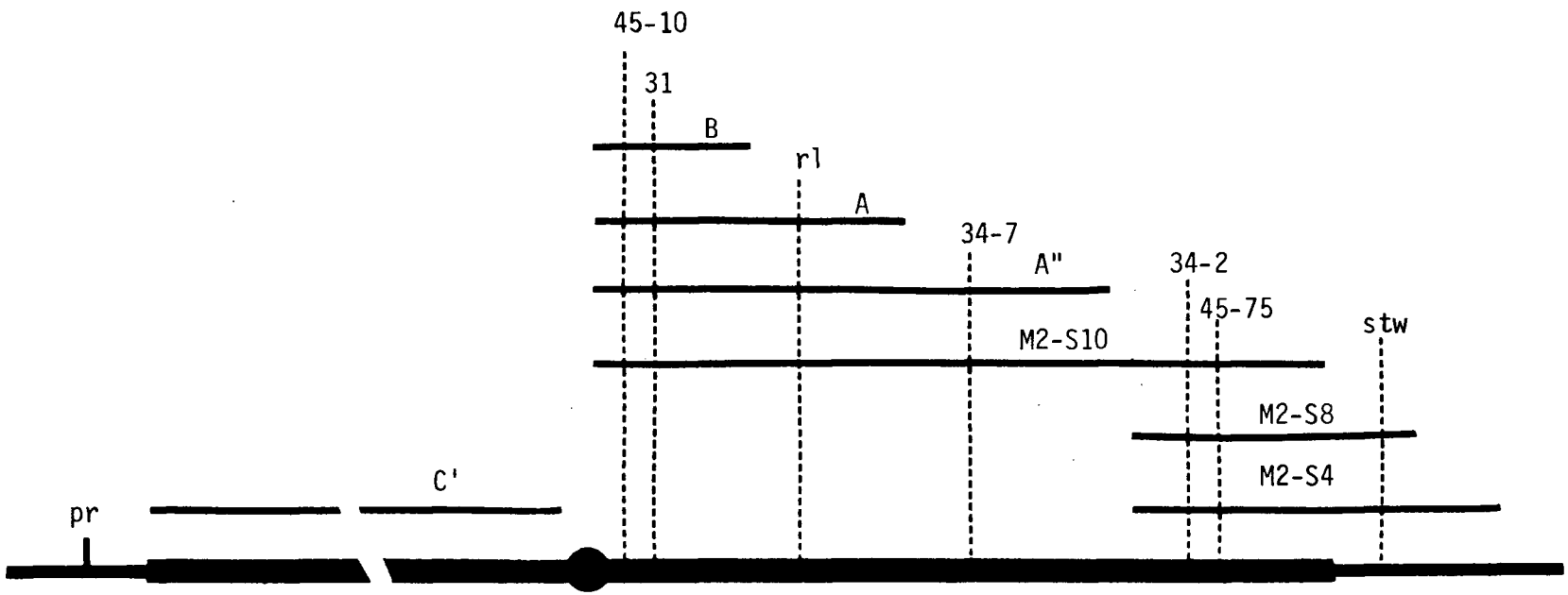
Table 1B

Description of chromosomal rearrangements used.

Symbol	Description
In(2LR)0,Cy $dp^{1v1}pr^1cn^2$	Chromosome-2 balancer with multiple rearrangements
Df(2L)161, pr	pr deletion

Figure 4

Genetic map of the centric region of chromosome-2. Shown are the relative positions and lengths of proximal deficiencies, and EMS-induced lethal mutations used for complementation tests of detachment products.


45-10
31
B
rl
A
34-7
A"
34-2
45-75
stw
M2-S10
M2-S8
M2-S4
C'
pr

viable, the flies heteroyygous of the balance (Cy) and flies homozygous for the detachment product (Cy^+) were recovered in approximately a 2:1 ratio.

Complementation tests: All homozygous lethal detachment products were tested with second chromosome heterochromatic deletions and EMS-induced lethal point mutations shown in Figure 4. Each lethal detachment was first tested with Df(2L)C' and Df(2L)M2-S10. Every detachment that was lethal with either of the big deletions, was then tested for all lethal point mutations uncovered by that deletion. Duplicate crosses were done in every test.

Results and Discussion

The recovery of detachment products is summarized in Table 2. Each detachment class is designated by the letter D, followed by the same code assigned to its progenitor C(2R). The first four columns show the results of detaching the four C(2R)Su(pr)s in combination with C(2L)VD3,nub^{2} b^{66h} pr^{1}. These four classes, termed collectively as the VD3 class, are summarized in column 5. Column 6 contains the class comprised of detachments synthesized from the C(2L)VY1, b pr;C(2R)VK43,SD72/cn bw strain. Detachments of this class are designated as DVK43A to distinguish them from the DVK43 detachments of the VD3 class. The combined totals of all the detachment classes are shown in column 7. Altogether, a total of 6,805 virgin females were irradiated, including 5,625 of the C(2L)VD3,nub^{2} b^{66h} pr^{1} bearing strains, and 1,180 of the C(2L)VY1,b pr^{1} bearing strains. The five compound strains used varied in their viability, and this is reflected in the number of females collected from each class for irradiation. Hence, the smallest number of virgins were collected from C(2L)VD3,nub^{2} b^{66h} pr;C(2R)VF30,SD72/cn bw and the largest from C(2L)VD3,nub^{2} b^{66h} pr^{1};C(2R)VF10,SD72/cn bw. Also, C(2R)VK43,SD72/cn bw proved to be more viable with C(2L)VD3,nub^{2} b^{66h} pr^{1}, than with C(2L)VY1, b pr^{1}.

Variation in the frequency of detachment recovery was seen, as shown in lines 2 and 3. The viability of the progenitor C(2R) did not correlate with the frequency with which detachments were recovered from it. The C(2L)VD3,nub^{2} b^{66h} pr^{1};C(2R)VF5,SD72/cn bw strain, which was not the weakest used, nevertheless produced the lowest frequency of detachments

at 3.30 per 100 treated females. In contrast, the DVF30 class members were recovered at more than twice that frequency (8.80 per 100 treated females), even though the progenitor compound stock was by far the weakest used. The other detachment classes were recovered at frequencies that fell between these two extremes. It is interesting that DVK43 detachments were recovered at a considerably higher frequency (6.76 per 100 treated females), and those of the DVK43A class (4.92 per 100 treated females). This indicates that differences between C(2L)VD3,nub^{2} b^{66h} pr and C(2L)VY1,b pr^{1} determine, in part, the altered viability of detachment products. A total of 320 detachments of the VD3 class were recovered at an average frequency of 5.69 per 100 females treated. An additional 58 DVK43A chromosomes were recovered at a rate of 4.92 per 100 females treated. Overall, a total of 378 detachments were generated at a frequency of 5.55 per 100 females treated.

The most significant result of the detachment experiment is the recovery of both suppressing and non-suppressing chromosomes. This is shown in Table 3, lines 1 and 3. Of the 378 detachment products recovered, 178 (47.09%) carried Su(pr). Of these suppressing detachments, 123 were of the VD3 class and 55 were of the DVK43A class. The majority of detachments recovered were non-suppressing. A total of 200 (52.91%) had lost Su(pr). The retention of Su(pr) in the VD3 class (38.4%) was much less than in the DVK43A class (94.38%).

There is striking variation between detachment classes regarding the retention a loss of Su(pr) (Table 3, lines 2 and 4). At one extreme of this variation is DVF30, wherein 90.48% of the chromosomes have lost the ability to suppress. At the other extreme is DVK43A, wherein 94.83% of

Table 2

Recovery of detachment products from C(2R)Su(pr)

	VD3 *				VD3		COMBINED
	DVF5	DVF10	DVF30	DVK43	TOTALS	DVK43A†	TOTALS
Number of females treated	1,000	2,150	775	1,700	5,625	1,180	6,805
Number of detachments recovered	33	109	63	115	320	58	378
Detachment recovery per 100 females treated	3.30	5.07	8.13	6.76	5.69	4.92	5.55

* VD3 denotes 4 classes of detachment products that were recovered from females bearing a C(2R)Su(pr) in combination with $C(2L)VD3.nub^{2}\ b^{66h}\ pr^{1}$.

† The DVK43A class of detachment products was recovered from females bearing a C(2R)Su(pr) in combination with $C(2L)VY1.b\ pr^{1}$.

Table 3

Recovery of suppressing and non-suppressing detachments

	VD3 *				VD3		COMBINED
	DVF5	DVF10	DVF30	DVK43	TOTALS	DVK43A†	TOTALS
Number of Su(pr) detachments recovered	15	37	6	66	123	55	178
Frequency (%) of Su(pr) detachment recovery	45.45	33.94	9.52	57.39	38.40	94.83	47.09
Number of non-suppressing detachments recovered	18	72	57	49	197	3	200
Frequency (%) of non-suppressing detachment recovery	54.58	66.06	90.48	42.61	61.56	5.17	52.91

* VD3 denotes 4 classes of detachment products that were recovered from females bearing a C(2R)Su(pr) in combination with C(2R)VD3, nub^2 b^{66h} pr^1.

† The DVK43A class of detachment products was recovered from females bearing a C(2R)Su(pr) in combinationwith C(2L)VY1, b pr^1.

the detachments retained Su(pr). There is a large difference in Su(pr) retention between DVK43 (57.39%) and DVK43A (94.83%), indicating that the C(2L) used may be significant in the production of suppressing detachments. Even greater variation, however, is seen within the VD3 class. This indicates that different C(2R)Su(pr) have different propensities to retain Su(pr) during detachment.

Many of the putative detachment-bearing individuals recovered were not viable, and 150 of them either died before mating, or failed to produce offspring. The number of surviving detachments, and the number of suppressing chromosomes and the number of non-suppressing chromosomes successfully established in stocks are shown in Table 4 on lines 1, 3 and 5, respectively. A total of 228 detachment-bearing stocks were established for further analysis. Overall, slightly more non-suppressing (63.50%) than suppressing detachments (56.74%) were viable. Great variation is seen in the rate of putative detachments which survived within each class, as shown on lines 2, 4 and 6. In the DVF5 class, for example, 100% of the suppressing detachments survived, but only 42.11% of the non-suppressing detachment-bearing individuals survived to establish stocks. The opposite is seen in DVK43A. Here the great majority (90.91%) Of all non-suppressed flies survived, while only 54.55% of the suppressed individuals survived. Therefore, the viability of these detachment products does not appear to correlate with the loss or persistence of Su(pr) activity.

These findings show that suppression is not dependent on the overall constitution or integrity of the C(2R)Su(pr) chromosome. Rather, it

Table 4

The establishment of detachment-bearing stocks

	VD3 *				VD3		COMBINED
	DVF5	DVF10	DVF30	DVK43	TOTALS	DVK43A†	TOTALS
Number of detachment stocks established	23	71	36	65	195	33	228
Percent viable detachments viable	69.69	65.14	57.14	60.94	56.52	56.90	60.32
Number of Su(pr) stocks established	15	16	6	34	71	30	101
Percent of viable Su(pr) detachments viable	100.0	43.24	100.0	52.31	57.72	54.55	56.74
Number of non-suppressing detachment stocks established	8	55	30	31	124	3	127
Percent viable non-suppressing detachments	42.11	76.39	52.63	63.26	62.94	90.91	63.50

* VD3 denotes 4 classes of detachment products that were recovered from females bearing a C(2R)Su(pr) in combination with C(2L)VD3, nub^2 b^{66h} pr^1.

† The DVK43A class of detachment products was recovered from females bearing a C(2R)Su(pr) in combination with C(2L)VY1, b pr^1.

appears that a stable alteration on one of the component arms induced Su(pr) activity, and this change may persist through subsequent chromosomal rearrangements. Since non-suppressing detachments were also recovered, it may be reasonably asked whether there are any detectable differences between suppressing and non-suppressing detachments. It may also be reasonable to expect that due to the variation in the position of break-points occurring during C(2R) attachment and detachment, the detachment stocks will vary significantly in heterochromatic content and arrangement. This variation provides an opportunity to search for patterns of heterochromatic constitution that correlate with the loss or retention of Su(pr). Some types of alterations, such as heterochromatic inversions and duplications cannot be detected in this study. However, lethality with the vital heterochromatic markers can be used to detect deletions on the detachment products.

The position of radiation-induced break-points in heterochromatin is unpredictable, if not random.[39] Because of this, generation of detachments from either arm of the C(2R)Su(pr) are expected in similar frequencies. However, when the detachment stocks were tested for stw pseudo-dominance, only two, DVF10-62 and DVK43A-147 were revealed to be carrying the SD72 side of the progenitor C(2R)Su(pr) Both were non-suppressors. Such a disproportionately low recovery of detachments bearing the SD72 arm indicates that these products had a very low viability. The attempt to localize the suppressor site, therefore, employed detachments all bearing the cn bw side of the C(2R)Su(pr).

As noted above, lethal deletions will be generated in the detachment procedure if the break-points on the captured arm occur distal to a vital

gene.[44] Deletions of a vital site may also include a considerable amount of flanking heterochromatin. Hence, if any such deletion encompassed a site or region responsible for Su(pr), it might be localized by correlating the loss of suppression with specific lethal deletion classes.

The detachment products were first tested for homozygous lethality. Lethality may indicate the presence of heterochromatic deletions and these chromosomes were designated for further testing. The results are shown in Table 5. Line 3 shows that the percentage of homozygous lethality varied between detachment classes, from a low of 45.45% for DVK43A, to a high of 100% for DVF5. As shown on lines 4 and 5, there is no apparent correlation between homozygous lethality and the loss or retention of Su(pr).

Heterochromatic deletions carried on detachment products were identified by using two large chromosome-2 deletions. Deletions for 2Lh were tested by using Df(2L)C', which is deficient for at least seven vital loci. Deletions for 2Rh were tested by using Df(2R)M2-S10 which lacks all 2Rh and uncovers at least six vital gens. When the homozygous lethals were tested over Df(2L)C' and Df(2R)M2-S10, it was found that the majority did not carry heterochromatic deletions. Whereas, 165 (72.37%) of the detachments were homozygous lethal (Table 5), only 79 (34.65%) detachments were deleted for chromosome-2 heterochromatin.

This latter percentage agrees with recovery of detachment-generated deletions on the third chromosome by Hilliker and Holm.[44] In that study, however, most homozygous lethal detachment products were found to carry heterochromatic deletions.[44] The findings of this study differ, in that 86 lethals are not deleted for heterochromatin. Of these, 48 are

Table 5

Lethality tests of detachment products

	DV5	DVF10	DVF30	DVK43	DVK43A	TOTALS
Number of detachments tested	23	71	36	65	33	228
Number of lethal detachments	23	55	18	54	15	165
Percent lethal detachments	100.0	76.47	50.0	83.08	45.45	72.37
Number of lethal Su(pr) detachments	15	8	0	22	12	57
Number of lethal non-suppressing detachments	8	47	18	32	3	108

suppressors and 38 are not. Hence, there is not a strong correlation between this lethality and retention or loss of Su(pr). It is known that compound chromosomes accumulate recessive lethals in the proximal region with time.[55] The two C(2L) used in the detachment study had been in existence for some time and it is possible that they donated recessive lethal mutations to the detachment products.

Table 6 shows the results of tests for heterochromatic deletions. The detachments are classified as carrying either no vital heterochromatic deletion, a 2Lh deletion, a 2Rh deletion, or a centromere spanning deletion (2LRh). Of the 228 chromosomes established in viable lines, 149 have no detectable heterochromatic deletions. Another 79 have lethal heterochromatic deletions. As shown on the bottom row, there was variation in the percentage of lethal deletions found in each class. Three of the detachment classes (VF5, VF30, VK43) show close agreement with each other, and to the findings of Hilliker and Holm.[44] In each of these cases, a little more than 30% of the detachments carried heterochromatic deletions. The greatest frequency of deletions (45.10%) was seen in DVF10. The DVK43A showed a much lower frequency of lethal deletion bearing detachments (15.15%). The C(2L)VY1,bpr^{1} used to generate detachments of this class is known to carry a large 2Rh duplication (including the genetic marker rl^{+}). This duplication could rescue many 2Rh deletions generated during detachment.

Table 6

Heterochromatic deletions carried on detachment products

	DVF5	DVF10	DVF30	DVK43	DVK43A	TOTALS
Number tested	23	71	36	65	33	228
2Lh deletion	0	7	4	5	0	16
2Rh deletion	8	24	9	16	5	62
2 LRh deletion	0	1	0	0	0	1
no deletion	15	39	23	44	28	149
Percentage deletions in class	34.78	45.10	36.11	32.31	15.15	65.35

The detachments were next analyzed for the relationship between deletions and the presence or absence of suppression. Table 7 shows the detachment strains that carry no detectable heterochromatic deletions. Among the VD3 detachments, there is considerable variation with regards to the retention of Su(pr). All viable VF5 detachments were suppressors. In contrast, the majority of non-deleted DVF10 members had lost Su(pr). In the other two VD3 classes, DVF30 and DVK43, the majority of undeleted attachments were able to suppress. The combined VD3 results, shown in column 5, reveal that only a slight majority (52.89%) of detachments retained Su(pr), as opposed to those losing it (47.11%). Like DVF5, the non-deleted DVK43A detachments all maintained the ability to suppress. The combined totals, shown in the last column of Table 7, indicate a tendency to maintain Su(pr) on non-deleted detachments.

Although suppressing detachments are in the majority in non-deleted classes, it is not a convincing correlation. Furthermore, while it might be true that non-deleted detachments are more likely to retain the ability to suppress, there remains the problem of demonstrating what has occurred on the non-suppressing chromosomes. It may be that the site or region responsible for Su(pr) can be deleted, but does not contain vital genes. If so, Su(pr) could be lost without generating lethal deletions. A second possibility is that the deleted area is masked by a compensating duplication. If this were the case, loss of the dominant suppressor would be evident, but the lethal deletion would be reduced. A third possibility is that some unknown mechanism which induces Su(pr) during C(2R) formation, is reversed during the detachment presence of

Table 7

The retention of Su(pr) on detachments not carrying heterochromatic deletions

	VD3 *				VD3		COMBINED
	DVF5	DVF10	DVF30	DVK43	TOTALS	DVK43A†	TOTALS
Su(pr)	15	14	25	30	64 (52.89%)	28 (100%)	92 (61.74%)
pr	0	25	18	14	57 (47.11%)	0 (0%)	57 (38.26)
TOTALS					121 (100.0%)	28 (100.0%)	149 (100.0%)

* VD3 denotes 4 classes of detachment products that were recovered from females bearing a C(2R)Su(pr) in combination with C(2L)VD3, nub^2 b^{66h} pr^1.

† The DVK43A class of detachment products was recovered from females bearing a C(2R)Su(pr) in combination with C(2L)VY1, b pr^1.

lethal procedure. The validity of any of these explanations could only be demonstrated through an extremely detailed analysis of the progenitor compounds and each of their detachment products. Since this is not possible in this study, the undeleted detachments remain a class that cannot be satisfactorily explained.

The relationship between the presence of lethality and the loss of Su(pr) is more revealing, as shown in Table 8. Here a clear correlation emerges between lethal heterochromatic deletions and the loss of Su(pr). The great majority (90.54%) of deletion-bearing VD3 detachments had lost Su(pr), compared to the small group that retained it (9.45%). The DVK43A detachments did not show such a strong correlation, but the class size was small. The overall findings indicate that loss of heterochromatin strongly predisposes suppressor chromosomes to the loss of Su(pr). The 70 non-suppressing detachments provide an opportunity to test whether their deletions fall in specific areas and hence define a region necessary for Su(pr) action. The nine suppressing detachments are also of use in this regard, especially if their deletions fall in regions distinct from the non-suppressing detachments.

In order to localize Su(pr) in this way, all detachments were classified according to the presence of lethal deletions in 2Lh and/or 2Rh and the retention of Su(pr). Table 9 shows this comparison for 2Lh deletions uncovered by Df(2L)C'. All 16 detachments that were found to carry 2Lh deletions came from the VD3 class. Of these, slightly more had lost Su(pr) (10) than retained it (6), but the difference is not significant. Since the majority of all VD3 detachments have lost Su(pr), with or without deletions, these few deletion-bearing non-suppressors

Table 8

The retention of Su(pr) in the presence of heterochromatic deletions

	VD3 *				VD3		COMBINED
	DVF5	DVF10	DVF30	DVK43	TOTALS	DVK43A†	TOTALS
Su(pr)	0	2	1	4	7 (9.45%)	2 (40.0%)	9 (11.39%)
pr	8	30	12	17	67 (90.54%)	3 (60.0%)	70 (88.61%)
TOTALS					74 (100.0%)	5 (100.0%)	79 (100.0%)

* VD3 denotes 4 classes of detachment products that were recovered in females bearing a C(2R)Su(pr) in combination with $C(2L)VD3.nub^{2}\ b^{66h}\ pr^{1}$.

† The DVK43A class of detachment products was recovered from females bearing a C(2R)Su(pr) in combinationwith $C(2L)VY1.b\ pr^{1}$.

Table 9

The presence of Su(pr) on detachment products bearing deletions of 2Lh

	VD3 *				VD3		COMBINED
	DVF5	DVF10	DVF30	DVK43	TOTALS	DVK43A†	TOTALS
Su(pr)	0	1	1	4	6 (39.50%)	0	6 (37.50%)
pr	0	6	4	1	10 (62.50%)	0	10 (62.50%)
TOTALS					16 (100.0%)	0	16 (100.0%)

* VD3 denotes 4 classes of detachment products that were recovered from females bearing a C(2R)Su(pr) in combination with C(2L)VD3, nub^2 b^{66h} pr^1.

† The DVK43A class of detachment products was recovered from females bearing a C(2R)Su(pr) in combination with C(2L)VY1,b pr^1.

may simply be part of the overall trend towards the loss of Su(pr). This is supported by the fact that the DVK43A class contained no non-suppressors with 2Lh deletions. However, since no 2Lh deletions were found in this class, the possible significance of 2Lh deletions remains undefined.

A much clearer trend is seen in the case of 2Rh deletions uncovered by Df(2R)M2-S10. This is shown in Table 10. Of the 62 detachments carrying 2Rh deletions, 59 (95.16%) had lost Su(pr), while only 3 (4.84%) retained the ability to suppress. Moreover, the trend seen for detachments not bearing deletions differed from the test involving 2Lh. Whereas, in the previous test suppressors lacking deletions had been in the minority, here they were in the majority. Of the 166 non-deleted detachments, 100 (60.24%) retained Su(pr), while 66 (39.76%) had lost it. Table 10 also shows that the trend towards the loss of Su(pr) being associated with 2Rh deletions is consistent for all VD3 classes. For three of these classes, DVF5, DVF30 and DVK43, all of the 2Rh deletion-bearing detachments have lost Su(pr). In the DVF10 class, only 1 of 24 2Rh deletion-bearing detachments maintained suppression. Only the DVK43A class varied from this trend in that two of the five deleted detachments which maintained suppression.

The presence of Su(pr) in all deletion classes is summarized in Table 11. A G-statistical analysis was done on the distribution of detachments within the classes shown.[56] The analysis tests the hypothesis that the retention or loss of Su(pr) is not related to the absence or presence of a lethal heterochromatic deficiency. The VD3 class alone was

Table 10

The retention of *Su(pr)* on detachment products bearing deletions of *2Rh*

	VD3 *				VD3 TOTALS	DVK43A†	COMBINED TOTALS
	DVF5	DVF10	DVF30	DVK43			
Su(pr)	0	1	0	0	1 (1.75%)	2 (40.0%)	3 (4.84%)
pr	8	23	9	16	56 (98.24%)	3 (60.0%)	59 (95.16%)
TOTALS					57 (100.0%)	5 (100.0%)	62 (100.0%)

* VD3 denotes 4 classes of detachment products that were recovered from females bearing a *C(2R)Su(pr)* in combinationwith *C(2L)VD3, nub*^{2} *b*^{66h} *pr*^{2}.

† The DVK43 class of detachment products was recovered fromfemales bearing a *C(2R)Su(pr)* in combination with *C(2L)VY1, b pr*^{1}.

Table 11

A G-statistical test for the retention of Su(pr) and all DVD3 deletion classes

Deletion Type	Su(pr)	pr	TOTALS
None	64	57	121
2Lh	6	10	16
2Rh	1	56	57
TOTALS	71	124	194

$p < .005$ at 2 df

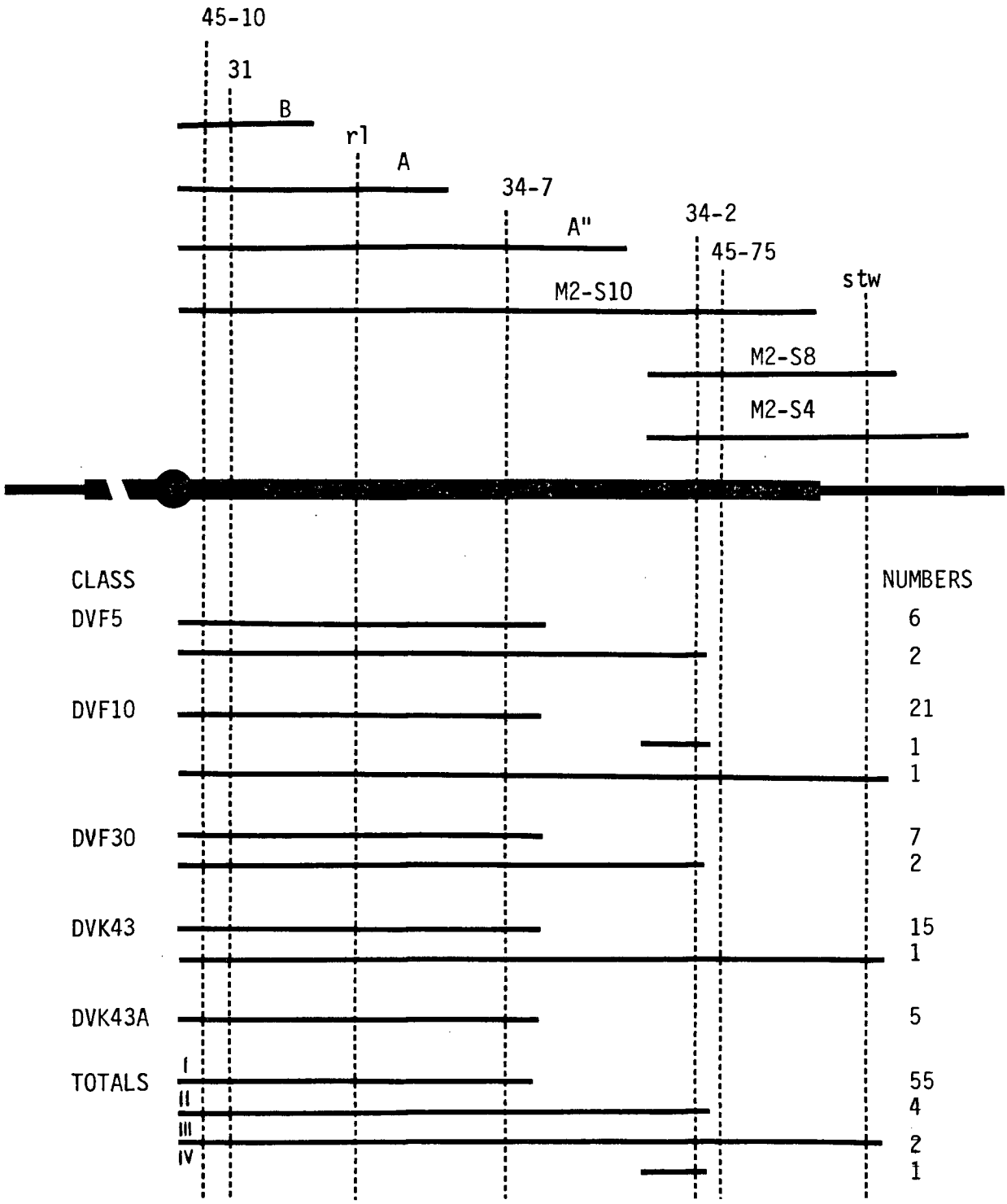
used because there were insufficient deleted DVK43A detachments for analysis. The G-statistical test confirms that these detachments do not represent a homogeneous population. The probability of this distribution being random is less than .005 (Table 11). Overall, these results indicate that detachments not bearing a 2Rh deletion have an increased probability of retaining Su(pr). Conversely, loss of vital 2Rh almost always results in the loss of Su(pr).

The size of the 2Rh deletions on the 62 non-suppressing detachments was approximated by testing them over the previously generated deletions and EMS-induced point mutations shown in Figure 5. The five polar deletions were generated by the detachment of C(2R) chromosomes, and uncover 5 groups of vital genes. Subsequent EMS mutagenesis revealed at least 6 vital loci in 2Rh.[54] The position of these vital loci divides the 2Rh into 6 regions as shown, corresponding to the intervals between them. This allows the size of the deletion on the non-suppressing detachments to be approximated by lethal pseudo-dominance over the EMS lethal mutations. A range of deletion sizes, like those in Figure 5, is expected if all detachment products are recovered.

The intent was to ascertain whether the loss of Su(pr) correlated with a specific type of deletion. It is also of interest to test whether or not the three suppressing chromosomes carry a type of deletion that is distinct from the non-suppressors. The results of these studies are summarized in Figure 5. Four classes of deletion were found corresponding to the interval in which their distal break-point fell. The distal break-point can fall anywhere within this interval. Of the 62 detachments tested, 61 carried polar deletions. As in the study of

Figure 5

Deletion classes recovered in detachment products.


45-10
31
B
rl
A
34-7
A"
34-2
45-75
M2-S10
stw
M2-S8
M2-S4
CLASS
NUMBERS
DVF5
6
2
DVF10
21
1
1
DVF30
7
2
DVK43
15
1
DVK43A
5
TOTALS
I
55
II
4
III
2
IV
1

Hilliker and Holm[44], the deletions vary considerably in size. These results differ, however, in that no deletion with a distal break-point proximal to EMS 34.7 was recovered. In the original study, two deletions of this type (A and B) were recovered out of 18 2Rh deletions. It is interesting that not one deletion of the type was recovered in this study, even though almost four times as many deletion-bearing detachments were generated.

The 62 deletions are grouped into four classes as shown in the summary of Figure 5. Two detachments comprised the type III class. These were both recovered from the SD72 side of the C(2R)Su(pr), as discussed above. One of these detachments came from the DVF10 class and one from the DVF30 class. These results confirmed that both detachments are deleted for all vital loci proximal to stw, that none of these loci had been duplicated from the C(2L). The constitution and source of the heterochromatin proximal to EMS 45.10 cannot be determined. If the detachment was generated from the centric fragment of the C(2R)Su(pr) with a captured 2L free arm, then these two detachments could carry a small undetected piece of 2Rh proximal to EMS 45.10 from the C(2R)Su(pr). Depending on the way in which the progenitor C(2R)Su(pr) was made, this 2Rh segment could have originated from either SD72 or its homologue. Alternately, if in the detachment process the captured arm came from the SD72 side of the C(2R)Su(pr), and if the C(2L) carried a 2Rh duplication, the detachments of class III might carry a segment of 2Rh proximal to EMS 45.10 donated by the C(2L) centric fragment. This serves to illustrate that the role of 2Rh proximal to EMS 45.10 cannot be discerned in this study.

Four deletions of type II were recovered, two from DVF10 and two from DVF30. This class was identified by the fact that its members were lethal over EMS 34.2 and Df(2L)MS-24, but not over EMS 45.75. These four deletions are analagous to the A' deletion generated by Hilliker and Holm.[44] Genetically, these deletions removed the great majority of 2Rh. The position of EMS 34.2 is quite distal in 2Rh and EMS 45.75 is near, if not at, the euchromatic junction.[54] The small number of detachments in this class is due, at least in part, to two factors. First, the assumed random distribution of break-points along the heterochromatin should result in the great majority of break-points occurring to the left of EMS 34.2. Secondly, the proximity of EMS 34.2 to EMS 45.75 presumably presents a relatively small target, hence very few detachments will be generated from a break-point that falls between them. These detachments have lost Su(pr) while retaining this small fragment of 2Rh. Therefore class II rules out only the most distal region of 2Rh for a Su(pr) site.

One of the type IV detachments was picked up from the DVF30 class. This chromosome is unusual in that its deletion is apparently non-polar, being lethal over both 34.2 and Df(2R)M2-S4. From this, we know that this deletion cannot extend as far as EMS 45.75, and its proximal limit must be distal to EMS 34.7 in the large region depicted by the dotted line. Hence the parameters that define this deletion are very wide. It is hard to explain how a deletion was generated. Theoretically, if the C(2L) was duplicated past EMS 34.7, it could have donated the proximal markers, and a break-point on the captured arm between EMS 34.7 and EMS 45.75 would then have generated the observed deletion. But the C(2L)

used is known not to be duplicated as far as rl, therefore some other explanation is required.

A feasible explanation is suggested by the recovery, in a previous study[44], of an apparent deletion of a similar type in distal 2Lh. Though genetically behaving as a non-polar deficiency, examination of the polytene chromosomes revealed that it was in fact a quasi-reciprocal translocation. A three hit even during the detachment process had resulted in the translocation of a large block of 2Lh to the right arm of chromosome 3. This juxtaposition of hetochromatin into a euchromatic region resulted in a position effect variegation for two of the three known loci in the transposed segment. The vital gene closest to the break-point was totally inactivated, and the eye marker lt showed the classical position effect variegation for light eyes. The third vital gene was not affected, presumably because it lay far enough away from the break-point to evade the spreading effect. Examination of the polytenes was not done for the DVF30 detachment and so its constitution is not known, but its apparent similarity suggests that it might likewise be the result of a multiple hit event. Alternatively, this detachment may have undergone a mutation at the EMS 34.2 loci coincident with the detachment procedure. Without a detailed characterization, this detachment does not help locate Su(pr).

The type I deletions comprise the largest class with 55 members, 53 non-suppressing and 2 suppressing. This group is composed of detachments that are lethal over the four proximal vital genes. As shown in Figure 5, the distance between the two loci that define the limits of this class, EMS 34.7 and EMS 34.2 define approximately one third of 2Rh. The

extent of this region means that the size of deletions in this class may vary greatly. Even without knowing the exact size of each deficiency within this group, however, certain inferences can be drawn regarding the placement of Su(pr). Such inferences are based on the assumption that the break-points that determine the distal boundary of deletions in this class occur with equal frequency throughout the region between EMS 34.7 and EMS 34.2. If this is true, and Su(pr) resides in this interval, then it could reasonably be expected that some detachments of this class would retain the ability to suppress. Furthermore, the frequency with which suppressing and non-suppressing detachments of this class were recovered would indicate the approximate position of Su(pr) within this interval.

If Su(pr) was located equidistant from EMS 34.7 and EMS 34.2, then the occurrence of break-points on either side of the putative Su(pr) loci would result in the generation of approximately equal numbers of suppressing and non-suppressing members of this class. If the Su(pr) loci resided in the distal portion of the interval, then a greater proportion of break-points would fall proximal to it. Correspondingly, a greater number of detachments retaining Su(pr) would be recovered. Conversely, if Su(pr) resided proximally in the interval, then more break-points would be expected to fall distal to it. Hence it would be deleted from the majority of detachment products generated from the cn bw free arm. In fact, however, only 2 out of 55 members of this class retained Su(pr). Because the proportion of suppressing detachments in this class is so small, it argues that if Su(pr) resides in this region, it must be located very close to the proximal demarcation of this class, EMS 34.7.

There are, however, other considerations that would make the placement of Su(pr) distal to EMS 34.7 puzzling. The C(2R)Su(pr) studies in Chapter 2 implicated a segment of 2Rh from SD72 in the induction of Su(pr). If Su(pr) is a distinct site lying distal to EMS 34.7, and is donated by SD72, then each C(2R)Su(pr) would have to be duplicated for that segment of SD72 2Rh to a position past EMS 34.7. This would not be expected in more than 50% of all C(2R)SD72 synthesized. Yet over 90% of C(2R)SD72/cn bw chromosomes recovered were suppressors. When this is taken into consideration, along with the fact that only 2 pr^+ detachments of this class were recovered, there is not a convincing case that Su(pr) resides distal to EMS 34.7. As an alternative explanation of the two exceptional detachments, perhaps they have had Su(pr) deleted, but coincidentally are pr^+ revertants.

The bulk of the data from the detachment studies argues that Su(pr) resides in the proximal region of 2Rh. This is consistent with what is known of the chromosome mechanics involved in C(2R)SD72/cn bw formation and detachment. Unfortunately, could not be more accurately placed relative to the three proximal vital loci because no deletions uncovering them were recovered. However, the large number of non-suppressing detachments in deletion class I argues that Su(pr) lies either distal but very close to, or proximal to EMS 34.7.

The classification of the deletions recovered (Figure 6) can be used to partially deduce the constitution of the detachments on which they are carried. There are 8 possible detachment types, as described in the introduction to this chapter. The classification of the recovered detachments is restricted to the 4 classes bearing the cn bw side of the

C(2R)Su(pr) (Figures 2 and 3). Two of the possible detachment types are those of class 2 and class 4, shown in Figure 3. It would be possible to generate the observed polar deletions on the cn bw free arm in both these classes.

These 2 classes are similar in that neither is expected to carry any segments of 2Rh from SD72. It cannot be determined in this study whether the vital gene-containing segments of 2Rh necessary for Su(pr) activity originated from SD72 on the cn bw-bearing homologue. However, in Chapter 2 it was found that the cn bw second could be substituted with a wild-type second in a C(2R)Su(pr). It was also found that the presence of a chromatid fragment from SD72 was strongly implicated in Su(pr) induction during C(2R) formation. Hence, while it is possible that the two detachment classes under consideration might be found in the deletion classes, the findings presented in Chapter 2 suggest that class 2 and class 4 detachments (Figure 3) would be unlikely to carry Su(pr) even when they are deletion free. It is consistent with the findings of this study that these two rearrangement types at least partially comprise the non-deleted, non-suppressing detachment class (Table 7).

The other 2 detachment candidates are found in class 2 and class 4 of Figure 2. Again, the recovery of both of these classes could generate polar deletions on the cn bw free arm derived from a C(2R)Su(pr). In contrast to the 2 classes discussed above, both these classes could carry segments of 2Rh from SD72. It is most consistent with the finds of this study that one or both of these detachment types comprise the non-deleted suppressing class (Table 7). These findings also suggest that either or both of these detachment classes at least partially comprise the

deletion-bearing class that has lost Su(pr) (Table 10).

CHAPTER 4

RECOMBINATION MAPPING OF *Su(pr)*

Introduction

Recombination mapping was undertaken in order to locate Su(pr) more accurately within the region of 2Rh defined by the deletion mapping. This study employed the two visible heterochromatic markers, lt, which resides near the border of 2Lh, and rl, which resides in 2Rh. Rolled is thought to reside at a point which approximately bisects the smallest deletion class recovered. Mapping relative to rl, therefore, would reduce by half the region in which Su(pr) must reside. A chromosome was constructed for this purpose bearing the markers Ift pr^{c4} lt rl.

The recombination studies will also serve 2 other purposes. First, detachment products that have had pr^{1} replaced by pr^{c4} will be recovered. In the pr^{c4} homozygote, these recombinants will test whether Su(pr) can suppress this EMS-induced allele. Second, the recombinant class in which pr^{1} has been crossed off the Su(pr) detachment can be made homozygous, and tested for the re-emergence of the mutant phenotype. This will show that a fully mutant allele was present on the suppressing detachment.

Figure 1 shows the Ift pr^{c4} lt rl in combination with a suppressing detachment, and the five types of recombinants expected. It is the b lt (interval 1) and b rl (interval 1 and 2) recombinants that will determine the position of Su(pr). The recovery of b lt Su(pr) recombinants would place Su(pr) to the right of lt, confirming the results of the deletion mapping in Chapter 3. The greater the proportion of suppressing b lt recombinants, the further it is expected the Su(pr) will lie to the right of lt. If no suppressing b rl recombinants are recovered, then Su(pr) will be located to the right of rl. If, however, Su(pr) lies proximal to

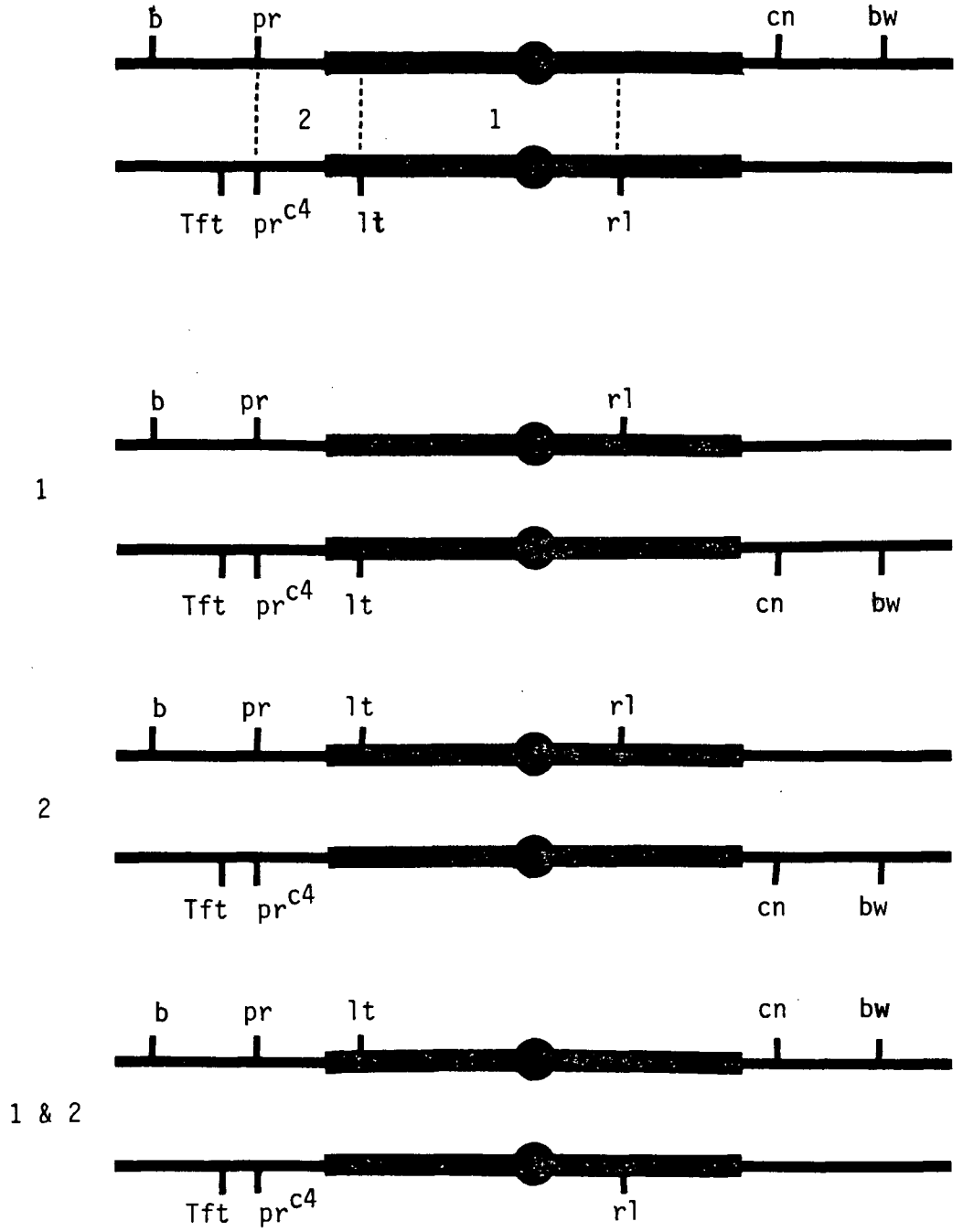
rl, then both suppressing and non-suppressing b rl recombinants are expected. The more proximal the Su(pr) locus, the greater the number of expected suppressing b rl recombinants.

Single exchanges in interval 1 or interval 2 replace pr^1 with pr^{c4} on the suppressing detachment (Figure 1). The Ift locus is tightly linked to pr so that it may safely be assumed that pr^{c4} is present, even if it is suppressed on a Ift pr^{c4} lt Su(pr) (interval 1) or Ift pr^{c4} Su(pr) (interval 2) recombinant. The b lt rl recombinant generated by a single exchange in interval 2 that will be used to test the re-emergence of pr^1 phenotype.

Recombination events occur far less frequently in heterochromatin than euchromatin. Tattersall[57] found that spontaneous exchanges between lt and rl occurs at a frequency of 0.1%. However, radiation induced recombination occurs at 2-6 times the spontaneous frequency in the heterochromatic interval. For this reason, radiation induced recombination was chosen to map Su(pr). Consequently, the recombination frequencies will not accurately reflect map distances. Also, the suppressor detachments used have undergone serial rearrangement, and their heterochromatic region will be perturbed. This may also affect recombination. Hence, recombination products should be interpreted as only indicating relative map positions, not actual map distances.

Figure 1

Recombination products expected from suppressing detachments in combination with Tft*pr*^{c4} *lt rl*. The recombination intervals of interest are the *lt*-*rl* interval (1) and the *pr*-*lt* interval (2). The recombination classes are categorized as products of single exchanges (interval 1 or interval 2), or as double exchanges (interval 1 and interval 2).


b
pr
cn
bw
2
1
Tft
pr^c4
lt
rl
1
2
1 & 2

Materials and Methods

Mutations and chromosomes used: A brief description many of the genetic markers and chromosomes used in this study is given in Table 1. The other mutations used are described in the Materials and Methods section of Chapters 2 and 3. Further details can be found in Lindsley and Grell.[24] The b lt rl chromosome used in this study is described in Tattersall.[57] The pr^{c4} cn and Ift pr^{c4} chromosomes used are described in Yim et al.[47] The Ift pr^{c4} lt rl chromosome was constructed for this study in a Ift pr^{c4}/b lt rl heterozygote female, by a spontaneous single exchange in the pr-lt interval. Four suppressing DVK43A chromosomes designated as DVK43A-4, -16, -19 and -45 were used. None of the 4 possess known deletions and all manifest good viability.

Synthesis of recombinant chromosomes: Recombinants were synthesized from each detachment in separate experiments. Recombinants were synthesized in females bearing a detached second in combination with the Ift pr^{c4} lt rl chromosome. Virgin females were treated 24 hours post eclosion with 2500 rads of gamma irradiation from a ^{60}Co source. Treated females were mated in batches of 25 to b lt rl males and cultured as in Chapter 2, Materials and Methods. Recombinant individuals were recovered in five phenotypic classes; (1) Ift, (2) b lt rl (or lt rl), (3) b rl (or rl), (4) Ift lt, and (5) b lt (or lt). Each recombinant was established in a separate line with as heterozygotes with In(2LR)CyO.

Testing recombinants for Su(pr): Ift-bearing recombinants were tested in combination with the pr^{c4} cn and In(2LR)O, Cy $dp1^{v1}$ pr^{1} cn^{2} chromosomes. Recombinants that did not carry Ift were tested with the Ift pr^{c4} lt rl

Table 1

Description of second chromosome mutations used.

The chromosome-2 centromere is at 55.1

Symbol	Name	Map Position	Description
Tft	Tuft	2 - 53.2	dominant; extra bristles homozygous viability low
pr^{c4}	purple	2 - 54.5	EMS-induced allele pr^{c4}/pr; purple eyes homozygous lethal

and In(2LR)CyO. Flies were scored for suppression by visual inspection as described in Materials and Methods section of Chapter 2.

Results and Discussion

Results from the radiation-induced crossover experiement are presented in Table 2. The number of each type of female treated, the number and frequency of each recombinant type recovered, and the number of each recombinant type tested is shown. Exchanges in regions outside the lt-rl and the pr-lt intervals were observed. Since these other exchanges do not affect the analysis, all recombinants were classified solely with regards to exchanges in the 2 intervals of interest. The b recombinant class is indistinguishable from the parental type suppressing detachment, so b chromosomes were not tested. The analysis of the other 5 recombinant classes is presented below.

The b rl recombinants: This class is the result of an exchange in the lt-rl interval (Figure 1). Twenty-eight chromosomes of this class were recovered as either rl or b rl individuals. Twenty-one chromosomes of this type were tested in a cross to the Tft pr^{c4} lt rl/In(2LR)CyO tester stock. The results are shown in Table 3. Fifteen b rl recombinants showed wild-type eyes in combination with both tester chromosomes. Six b rl recombinants expressed the fully pr-eyed phenotype. The suppressing b rl chromosomes were the result of a cross-over between Su(pr) and rl. This demonstrates that Su(pr) lies proximal to rl in 2Rh.

The recovery of such a large proportion of suppressing recombinants suggests that either there is a hot spot for chromosome breakage between Su(pr) and rl, or that the Su(pr)-rl interval is quite large. If the latter is the case, then either Su(pr) falls very close to the centromere, or the rl locus is more distal than previously thought. The 6 non-suppressing rl recombinants were products of single exchanges

Table 2

Recombinants recovered from detachment products

		DVK43-4	DVK43-16	DVK43-19	DVK43-45	TOTALS
Number of females irradiated		600	600	660	540	2,400
Number of progeny scored		6,050	4,825	6,870	3,670	21,415
Number of recombinants:						
(A) In the pr-lt interval						
(1) Tft	recovered	1(.02%)	5(.10%)	9(.13%)	6(.16%)	21(.10%)
	tested	1	4	9	5	19
(2) b lt rl	recovered	2(.03%)	2(.04%)	5(.07%)	0(0.0%)	9(.04%)
	tested	2	2	5	0	9
(B) In the lt-rl interval						
(1) b rl	recovered	7(.12%)	4(.08%)	13(.19%)	4(.11%)	23(.13%)
	tested	5	3	9	3	21
(2) Tft	recovered	7(.12%)	6(.12%)	8(.12%)	3(.08%)	24(.11%)
	tested	7	6	5	2	20
(C) In the pr-lt & lt-rl interval						
(1) b lt	recovered	3(.05%)	5(.10%)	2(.03%)	1(.03%)	11(.05%)
	tested	3	3	2	1	9

Table 3

Test of b rl recombinants for the presence of Su(pr)

$$\frac{b\ pr^{1}\ rl}{b\ lt\ rl} \times \frac{Tft\ pr^{c4}\ lt\ rl}{Cy\ dp^{1v1}\ pr^{1}cn^{2}}$$

Progeny classes tested:	DVK43-4	DVK43-16	DVK43-19	DVK43-45	TOTALS
(A) b pr^{1}rl/Tft pr^{c4}lt rl Phenotypic classes					
(1) Tft	4	1	6	3	15
(2) Tft pr	1	2	3	0	6
(B) b pr^{1}rl/Cy $dp^{1v1}pr^{1}cn^{2}$ Phenotypic classes					
(1) Cy cn	4	1	6	3	15
(2) Cy pr cn	1	2	3	0	6

between lt and Su(pr). Considering the distance at which lt is thought to reside from the centromere, fewer recombinant chromosomes of this type were recovered than expected. It may be that rearrangements in 2Rh of the four suppressor detachments used might reduce crossing-over in this region. Alternatively, it may be that some exchange products were not recovered.

The Tft lt recombinants: The chromosomes are the reciprocal products of single exchanges in the lt-rl interval. Twenty-four chromosomes of this class were recovered as either b Tft lt or Tft lt F1 individuals. These recombinants were tested in a cross to a pr^{c4} cn/In(2LR)Cy0 tester stock. The results are presented in Table 4. Examination of the Cy Tft F2 progeny tests for the presence of Su(pr) to the right of lt. The ability of Su(pr) to suppress pr^{c4} can be tested in the Tft F2 progeny.

First, considering the Cy Tft F2 class carrying the Tft pr^{c4} lt and Cy0 Cy pr^{1} cn^{2} chromosomes; of the 20 chromosomes tested, 18 had wild type eyes and 2 expressed the pr mutant. Hence, 18 chromosomes in this class picked up Su(pr) as the result of an exchange between lt and Su(pr). The two non-suppressing recombinants of this class resulted from an exchange in the Su(pr)-rl interval. Regarding the ability of Su(pr) to suppress pr^{c4}, no Tft F2 progeny were recovered. This demonstrates that the Tft pr^{c4} lt pr^{c4} cn class was lethal. Hence Su(pr) cannot suppress this EMS-induced allele of pr, nor can it rescue its lethal effects. This interpretation assumes that there are no other EMS-induced lethals tightly linked to pr^{c4}.

The b lt recombinants: Eleven recombinants of this type were recovered as b lt or lt individuals. They are the result of a double exchange,

Table 4

Test of Tft lt recombinants for the presence of Su(pr)

$$\frac{\text{Tft pr}^{c4}\ \text{lt cn bw}}{\text{b lt rl}} \times \frac{\text{pr}^{c4}\text{cn}}{\text{Cy dp}^{1v1}\ \text{pr}^{1}\text{cn}^{2}}$$

Progeny classes tested:	DVK43-4	DVK43-16	DVK43-19	DVK43-45	TOTALS
(A) $\frac{\text{Tft pr}^{c4}\text{lt rl cn bw}}{\text{Cy dy}^{1v1}\text{pr}^{1}\text{cn}^{2}}$					
Phenotypic classes					
(1) Tft cn	7	5	5	1	18
(2) Tft pr cn	0	1	0	1	2
(B) $\frac{\text{Tft pr}^{c4}\text{lt rl cn bw}}{\text{pr}^{c4}\text{cn}}$					
Phenotypic classes					
(1) Tft cn (lethal)	0	0	0	0	0
(2) Tft pr cn (lethal)	0	0	0	0	0

with one in pr-lt interval, and one in the lt-rl interval. Although fewer double exchange products were recovered than single exchange products (Table 2) the number of chromosomes in this class was higher than expected. The analysis of this class with the pr^{c4} cn/In (2LR) CyO tester stock is shown in Table 5. Of the 9 b lt recombinants tested, 8 were found to suppress pr. These suppressing recombinants result from an exchange between lt and Su(pr). The one non-suppressing chromosome in this class resulted from and exchange between Su(pr) and rl.

As shown in Table 6, there is a disparate recovery of reciprocal products of exchanges in the lt-rl interval. The suppressing recombinant chromosomes in the Tft pr^{c4} lt and lt recombinant class were expected in approximately the same frequency as the non-suppressing recombinant chromosomes in the rl recombinant class, since they were both generated by an exchange in the lt-Su(pr) interval. Similarly, approximately equal numbers of suppressing and non-suppressing chromosomes were expected as the products of exchange in the Su(pr)-rl interval. In both cases, however, suppressor recombinant chromosomes were recovered predominantly.

For the two classes produced from an exchange in the lt-Su(pr), 26 suppressed pr and 3 did not. For the class resulting from an exchange in the Su(pr)-rl interval, suppressing chromosomes were also predominant. There were 18 suppressing and only 2 non-suppressing rl recombinants. As noted above, speculations based on the frequency of recombinant class recovery must be made cautiously in this case. However, these results

Table 5

Test of b lt recombinants for the presence of Su(pr)

$$\frac{b\ pr^{1}\ lt\ cn\ bw}{b\ lt\ rl} \times \frac{pr^{c4}cn}{Cy\ dp^{1vl}\ pr^{1}cn^{2}}$$

Progeny classes tested:	DVK43-4	DVK43-16	DVK43-19	DVK43-45	TOTALS
(A) $\frac{b\ pr^{1}\ lt\ cn\ bw}{Cy\ dy^{1vl}pr^{1}cn^{2}}$					
Phenotypic classes					
(1) Cy cn	3	3	1	1	8
(2) Cy pr cn	0	0	1	0	1
(B) $\frac{b\ pr^{1}\ lt\ cn\ bw}{pr^{c4}cn}$					
Phenotypic classes					
(1) cn	3	3	1	1	8
(2) pr cn	0	0	1	0	1

Table 6

Disparate recovery of suppressing and non-suppressing recombinant classes.

Exchange interval	Su(pr)	pr
lt-Su(pr)	26	3
Su(pr)-rl	15	6
TOTALS	41	9

raise the possibility that suppressing recombinant chromosomes were recovered preferentially.

The lft recombinants: Twenty-one recombinant chromosomes of this class were recovered as b lft or lft individuals (Table 2). These recombinants are the result of a single exchange in the pr-lt interval (Figure 1). The results of testing these recombinants with the pr^{c4} cn/ln(2LR)Cy0 is shown in Table 7. Of the 19 recombinants tested, all Cy lft progeny had wild-type eyes confirming the presence of Su(pr) to the right of pr. No lft progeny were recovered from any of the recombinants tested, confirming the inability of Su(pr) to rescue the lethal effects of pr^{c4}. These results agree with the testing of the lft lt class (Table 4).

The b lt rl recombinants: The chromosomes that comprise this class are the reciprocal product generated by a single exchange in the pr-lt interval (Figure 1). Nine recombinants of this type were recovered as b lt rl or lt rl individuals. No recombinants of this type were recovered from the DVK43A-45 suppressing detachment. All 9 recombinants were tested with the lft pr^{c4} lt rl/ln(2LR)Cy0 tester stock as shown in Table 8. All progeny of both the lft and Cy classes had fully pr mutant eyes for each recombinant tested. This shows that the previous suppression of these pr^{1} alleles is not due to reversion. It also confirms the findings in Chapter 2 that the suppression of pr requires the continued presence of Su(pr).

Table 7

Test of *Tft* recombinants for the presence of *Su(pr)*

$$\frac{Tft\ pr^{c4}cn\ bw}{b\ lt\ rl} \times \frac{pr^{c4}cn}{Cy\ dp^{1vl}\ pr^{1}cn^{2}}$$

Progeny classes tested:	DVK43-4	DVK43-16	DVK43-19	DVK43-45	TOTALS
(A) $\frac{Tft\ pr^{c4}cn\ bw}{Cy\ dy^{1vl}pr^{1}cn^{2}}$					
Phenotypic classes					
(1) Cy Tft cn	1	4	9	5	19
(2) Cy Tft pr cn	0	0	0	0	0
(B) $\frac{Tft\ pr^{c4}cn\ bw}{pr^{c4}cn}$					
Phenotypic classes					
(1) Tft cn (lethal)	0	0	0	0	0
(2) Tft pr cn (lethal)	0	0	0	0	0

Table 8

Test of b lt rl recombinants for the presence of Su(pr)

$$\frac{b\ pr\ lt\ rl}{b\ lt\ rl} \times \frac{Tft\ pr^{c4}\ lt\ rl}{Cy\ dp^{1}vl\ pr^{1}cn^{2}}$$

Progeny classes tested:	DVK43-4	DVK43-16	DVK43-19	DVK43-45	TOTALS
(A) $\frac{bpr\ lt\ rl}{Tft\ pr^{c4}\ lt\ rl}$					
Phenotypic classes					
(1) Tft lt rl	0	0	0	0	0
(2) Tft pr lt rl	2	2	5	0	9
(B) $\frac{br\ lt\ rl}{Cy\ dp^{1}vl\ pr^{1}cn^{2}}$					
Phenotypic classes					
(1) Cy	0	0	0	0	0
(2) Cy pr	2	2	5	0	9

CHAPTER 5

SUMMARY

The preceding sections of this thesis describe a new dominant suppressor of purple, Su(pr), which is located in the chromosome-2R heterochromatin, to the left of the genetic marker rl. The heterochromatin in Drosophila, and many other species, differs from euchromatin in cytological appearance, genetic content, and types of DNA sequence. Cytologically, heterochromatin is characterized by staining properties that differ from euchromatin. Its denser stained appearance is due, in part, to its tendency to acquire a far more compacted state than euchromatin. The distribution of heterochromatin in the genome is non-random, and similar in many species. Heterochromatin is most often adjacent to the centromere, at the telomeres, and near the nucleolar organizer.[58]

Heterochromatin has long been thought to be genetically inert, but it is now known that there are genetic loci are located in the heterochromatin of all chromosomes of Drosophila melanogaster[43]. In chromosome-2 heterochromatin, deletion mapping and EMS mutagenesis revealed the presence of 13 vital loci.[44, 54] None of the EMS-induced lethals behave as a deficiency, and several loci exhibit extensive and complex interallelic complementation. This strongly suggests that these loci exist as interspersed unique sequence genes with vital functions.[54] It has also been demonstrated that two of the genetic elements associated with the Segregation Distortion (SD) phenomenon are also located within chromosome-2 heterochromatin.[59, 60] These loci exist at only about 1% the gene density found in euchromatin.[43] Heterochromatin may differ from euchromatin in that these unique sequences may be interspersed with the highly repeated satellite DNA sequences. In Drosophila melanogaster, satellite sequences are only 5 - 12 base pairs long, often A-T or G-C

rich, and can be present in a million or more copies per genome (reviewed by Skinner[61]).

This study has identified an additional genetic function associated with chromosome-2R heterochromatin. There is not, however, sufficient evidence to indicate that Su(pr) is a unique sequence gene. Presently, loss of Su(pr) is associated with a specific class of deletion in 2R heterochromatin. Consequently, it is not possible to discern whether it is the deletion of a specific site, or of a larger region which causes the loss of suppression on these chromosomes. An EMS mutagenesis study of Su(pr) would be useful, in this regard, by establishing whether suppression is the product of a mutable site. If an EMS-induced point mutation abolished Su(pr) activity, there would be a strong suggestion that Su(pr) is a unique sequence gene. Failure to abolish suppression by point mutation would suggest that Su(pr) might be caused by repeated sequences, and possibly a position effect.

An example of position effect in Drosophila melanogaster involving chromosomal rearrangement illustrates how this phenomenon might pertain to Su(pr). The recessive-visible eye mutant, facet-strawberry (fa^{swb}), is defined as a very small deletion at the 5' end of the Notch locus. In preparation of the polytene X chromosome, the deletion is seen to be restricted to interband material. It appears that fa^{swb} arises because this deletion abuts the Notch locus against the nearest upstream band. This juxtaposition apparently induces a position effect, which allows genetic activity in the adjacent region to interfere with the normal functions at Notch. When fa^{swb} is placed is cis with upstream deletions in this region the wild phenotype returns. Apparently, this occurs

because the interfering genetic functions that acted on Notch at close range are eliminated. Consequently, normal Notch function is re-established.[62]

It is possible to envision a similar occurence in the induction and subsequent loss of Su(pr) activity. Deletions may be generated during the synthesis of new C(2R) chromosomes.[44] It may be that during C(2R)SD72 synthesis, normally separated sequences are juxtaposed, and one of them establishes a position effect over the other, thus creating a novel genetic function. The entire region responsible for Su(pr) induction could subsequently be deleted during the detachment process, thereby reinstating mutant target gene expression. As well, unique juxtapositions of heterochromatin are possible when C(2R) is synthesized from a chromosome bearing a pericentric inversion. This is true even if no deletions are generated in C(2R) synthesis. This may explain why suppressing C(2R) has been induced only from the inverted SD72, despite the fact that many C(2R) chromosomes have been synthesized from other types of second chromosome.

Alternatively, it can be considered that by chromosome breakage, Su(pr) is induced through the activation of a previously silent. An example of this occurs in Zea mays in which the dominant suppressor, Dotted (Dt) returns normal activity to a mutant allele of the anthocyanin gene (A). The A gene produces pigment which is deposited in the pigment layer of the kernel. The Dt gene is a transposable element that may exist in an active or inactive state at several locations in the genome. Plants in which Dt is introduced along with non-functional anthocyanin alleles (a) produce kernels with dots covering the aleurone layer of the

endosperm. These dots represent patches of the kernal in which Dt has suppressed a and reinstated normal A pigment production (reviewed by McClintock[63]).

The Dt phenotype was first observed in Zea mays plants that were suspected of having undergone chromosome breakage. McClintock[64] performed an experiment to determine whether chromosome breakage could induce de novo Dt activity. This was tested in a plant that was homozygous for an a mutant on chromosome 3, and heterozygous for an inverted duplication on one end of chromosome 9. During meiosis in this plant, crossovers between the standard chromosome 9 and its inverted homologue cause the formation of a dicentric bridge. When the fused homologues migrate during anaphase, the dicentric bridge breaks. The experiment had been designed so that only pollen grains carrying the inverted chromosome 9 would be viable. When the kernals were examined, suppression was confirmed by the appearance of a number of them in which patches of normal gene expression appeared as dots. Each dot was derived from a single cell in which a silent Dt element had been activated on chromosome 9.[64, 65, 66] The Dt element then suppressed the a mutation on chromosome 3. This gene expression persisted in all cells of that lineage.

The activation of Dt bears some similarities to Su(pr) induction: (1) in both cases, suppression is associated with chromosome breakage, (2) both Su(pr) and Dt are dominant in their activity, and, (3) both suppressors are localized in heterochromatin. This study has not provided any evidence for the involvement of transposable element action in Su(pr) activity. However, the similarities between the suppressor studied here and Dt, as well as the widespread implication of transposable elements in suppression,[38] indicates an interesting avenue for future research on Su(pr).

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APPENDIX

Spectrophotometric measurement of eye pigments reported in Chapter 2.

TABLE 3		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
ORR	1)	0.786	.006	0		
	2)	0.715	-.065	.0042		
	3)	0.835	.055	.0030		S^2 = .0053
	4)	0.866	.086	.0074		S = .0728
	5)	0.699	-.081	.0066		
	X)	0.780		.0212	percent wild-type A^{495} =	100
C(2L)VY1,bpr; C(2R)P,px	1)	0.481	.086	.0074		
	2)	0.368	-.027	.0007		
	3)	0.445	.050	.0025		S^2 = .0047
	4)	0.306	-.089	.0079		S = .0686
	5)	0.377	-.018	.0003		
	X)	0.395		.0188	percent wild-type A^{495} =	51
C(2L)VF1,bpr; C(2R)P,px	1)	0.391	.090	.0081		
	2)	0.399	-.008	0		
	3)	0.466	-.075	.0056		S^2 = .0035
	4)	0.387	-.004	0		S = .0587
	5)	0.402	-.011	.0001		
	X)	0.391		.0138	percent wild-type A^{495} =	50
C(2L)VF1,pr; C(2R)P,px	1)	0.318	-.065	.0042		
	2)	0.243	-.010	.0001		
	3)	0.335	.083	.0069		S^2 = .0047
	4)	0.174	.079	.0062		S = .0684
	5)	0.190	-.036	.0013		
	X)	0.252		.0187	percent wild-type A^{495} =	32
C(2L)VF1,pr^{bw} C(2R)P,px	1)	0.253	.061	.0037		
	2)	0.263	.071	.0050		
	3)	0.172	-.030	.0004		S^2 = .0034
	4)	0.131	-.031	.0009		S = .0585
	5)	0.160	-.061	.0037		
	X)	0.192		.0137	percent wild-type A^{495} =	25

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)P,b;	1)	0.959	.074	.0055		
C(2R)P,px	2)	0.921	.036	.0013		
	3)	0.941	-.056	.0031		S^2 = .0060
	4)	0.803	-.082	.0067		S = .0775
	5)	0.799	-.086	.0074		
	X)	0.885		.0240	percent wild-type A^{495} =	113
C(2L)SH3,+;	1)	0.775	.015	.0002		
C(2R)SH3,+	2)	0.879	.119	.0142		
	3)	0.744	-.016	.0003		S^2 = .0054
	4)	0.701	-.059	.0035		S = .0731
	5)	0.703	-.057	.0032		
	X)	0.760		.0214	percent wild-type A^{495} =	97

TABLE 4

ORR	1)	0.732	-.060	.0036		
	2)	0.820	.028	.0008		
	3)	0.837	.045	.0020		S^2 = .0021
	4)	0.755	-.037	.0014		S = .0455
	5)	0.815	.023	.0005		
	X)	0.792		.0083	percent wild-type A^{495} =	100
C(2L)VY1,bpr;	1)	0.981	-.031	.0010		
C(2R)VK43,SD72	2)	0.988	-.024	.0006		
cnbw	3)	0.947	-.065	.0042		S^2 = .0034
	4)	1.052	.040	.0016		S = .0583
	5)	1.091	.079	.0062		
	X)	1.012		.0136	percent wild-type A^{495} =	128
C(2L)VF1,bpr;	1)	0.919	-.090	.0081		
C(2R)VK43,SD72	2)	0.994	-.015	.0002		
cnbw	3)	1.080	.071	.0050		S^2 = .0063
	4)	1.101	.092	.0085		S = .080
	5)	0.950	-.059	.0035		
	X)	1.009		.0253	percent wild-type A^{495} =	127

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)VF1,pr;	1)	0.865	-.007	0		
C(2R)VK43,SD72	2)	0.889	-.017	.0003		
cnbw	3)	0.992	.120	.0144		S^2 = .0058
	4)	0.817	-.055	.0030		S = .0758
	5)	0.799	-.073	.0053		
	X)	0.872		.0230	percent wild-type A^{495} =	110
C(2L)VF1,pr^{bw};	1)	0.854	.052	.0027		
C(2R)VK43,SD72	2)	0.915	.113	.0127		
cnbw	3)	0.740	-.062	.0038		S^2 = .0051
	4)	0.718	-.084	.0007		S = .0712
	5)	0.781	-.021	.0004		
	X)	0.802		.0203	percent wild-type A^{495} =	

TABLE 5

ORR	1)	0.847	.059	.0035		
	2)	0.755	.033	.0011		
	3)	0.724	-.064	.0041		S^2 = .0030
	4)	0.843	-.055	.0030		S = .0548
	5)	0.769	-.019	.0003		
	X)	0.788		.0120	percent wild-type A^{495} =	100
C(2L)VY1,bpr;	1)	0.962	-.023	.0005		
C(2R)VF5,SD72	2)	0.927	-.058	.0038		
cnbw	3)	1.040	.055	.0030		S^2 = .0061
	4)	1.090	.105	.0110		S = .0779
	5)	0.905	-.080	.0064		
	X)	0.985		.0243	percent wild-type A^{495} =	125
C(2L)VF1,bpr;	1)	0.943	-.018	.0003		
C(2R)VF5,SD72	2)	0.947	-.014	.0002		
cnbw	3)	1.011	.050	.0025		S^2 = .0019
	4)	0.999	.038	.0014		S = .0433
	5)	0.905	-.056	.0031		
	X)	0.961		.0075	percent wild-type A^{495} =	122

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)VF1,pr;	1)	0.789	-.050	.0025		
C(2R)VF5,SD72	2)	0.919	.080	.0064		
cnbw	3)	0.789	-.050	.0025		S^2 = .0037
	4)	0.889	.050	.0025		S = .0608
	5)	0.809	.030	.0009		
	X)	0.839		.0148	percent wild-type A^{495} =	106
C(2L)VF1,pr^{bw};	1)	0.915	.084	.0071		
C(2R)VF5,SD72	2)	0.761	-.070	.0049		
cnbw	3)	0.753	-.078	.0061		S^2 = .0069
	4)	0.925	.094	.0088		S = .0832
	5)	0.803	-.028	.0008		
	X)			.0277	percent wild-type A^{495} =	105

TABLE 6

ORR	1)	0.788	.003	0		
	2)	0.704	-.081	.0066		
	3)	0.834	.049	.0024		S^2 = .0044
	4)	0.863	.078	.0061		S = .0665
	5)	0.734	-.051	.0026		
	X)	0.785		.0177	percent wild-type A^{495} =	100
C(2L)VY1,bpr;	1)	0.944	-.022	.0005		
C(2R)VF10,SD72	2)	0.970	.004	0		
cnbw	3)	1.015	.049	.0024		S^2 = .0022
	4)	1.003	.037	.0014		S = .0469
	5)	0.899	-.067	.0045		
	X)	0.966		.0088	percent wild-type A^{495} =	123
C(2L)VF1,bpr;	1)	0.949	.001	0		
C(2R)VF10,SD72	2)	0.903	-.047	.0022		
cnbw	3)	0.995	-.045	.0020		S^2 = .0023
	4)	1.001	.051	.0026		S = .0482
	5)	0.900	-.050	.0025		
	X)	0.950		.0093	percent wild-type A^{495} =	121

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)VF1,pr;	1)	0.949	.084	.0071		
C(2R)VF10,SD72	2)	0.864	-.001	0		
cnbw	3)	0.791	-.074	.0055		S^2 = .0035
	4)	0.855	.020	.0004		S = .0592
	5)	0.834	-.031	.0010		
	X)	0.865		.0140	percent wild-type A^{495} =	110
C(2L)VF1,pr^{bw};	1)	0.835	.026	.0007		
C(2R)VF10,SD72	2)	0.777	-.032	.0010		
cnbw	3)	0.729	-.080	.0064		S^2 = .0034
	4)	0.823	.014	.0002		S = .0581
	5)	0.881	.072	.0053		
	X)	0.809		.0135	percent wild-type A^{495} =	103

TABLE 7

ORR	1)	0.802	.006	.0004		
	2)	0.855	.060	.0036		
	3)	0.715	-.080	.0064		S^2 = .0038
	4)	0.752	-.043	.0018		S = .0618
	5)	0.851	.056	.0031		
	X)			.0153	percent wild-type A^{495} =	100
C(2L)VY1,bpr;	1)	1.008	.039	.0015		
C(2R)VF30,SD72	2)	0.998	.029	.0008		
cnbw	3)	0.897	-.072	.0052		S^2 = .0500
	4)	0.893	-.076	.0058		S = .0707
	5)	1.051	.082	.0067		
	X)	0.969		.0200	percent wild-type A^{495} =	122
C(2L)VF1,bpr;	1)	0.993	.031	.0010		
C(2R)VF30,SD72	2)	0.943	-.019	.0004		
cnbw	3)	0.962	0	0		S^2 = .0021
	4)	0.899	-.063	.0040		S = .0453
	5)	1.015	.053	.0028		
	X)	0.962		.0082	percent wild-type A^{495} =	121

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)VF1,pr;	1)	0.902	.043	.0018		
C(2R)VF30,SD72	2)	0.781	-.078	.0061		
cnbw	3)	0.799	-.060	.0036		S^2 = .0040
	4)	0.899	.040	.0016		S = .0630
	5)	0.912	.053	.0028		
	X)	0.853		.0159	percent wild-type A^{495} =	108
C(2L)VF1,pr^{bw};	1)	0.881	.063	.0040		
C(2R)VF30,SD72	2)	0.749	-.069	.0048		
cnbw	3)	0.818	0	0		S^2 = .0045
	4)	0.753	-.065	.0042		S = .0669
	5)	0.888	.070	.0049		
	X)	0.818		.0179	percent wild-type A^{495} =	103

TABLE 8

ORR	1)	0.705	-.073	.0053		
	2)	0.819	.041	.0017		
	3)	0.819	.041	.0017		S^2 = .0044
	4)	0.839	.061	.0037		S = .0659
	5)	0.707	-.071	.0050		
	X)	0.788		.0174	percent wild-type A^{495} =	100
C(2L)VY1,bpr;	1)	0.475	.072	.0052		
C(2R)VF12,SD72	2)	0.461	.058	.0034		
cnbw	3)	0.445	.042	.0018		S^2 = .0064
	4)	0.321	-.082	.0067		S = .0796
	5)	0.312	-.091	.0083		
	X)	0.413		.0254	percent wild-type A^{495} =	52
C(2L)VF1,bpr;	1)	0.398	.033	.0010		
C(2R)VF12,SD72	2)	0.361	-.040	.0016		
cnbw	3)	0.322	-.033	.0011		S^2 = .0014
	4)	0.339	-.026	.0007		S = .0371
	5)	0.397	-.032	.0010		
	X)	0.365		.0055	percent wild-type A^{495} =	47

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)VF1,pr;	1)	0.195	-.064	.0041		
C(2R)VF12,SD72	2)	0.231	.028	.0017		
cnbw	3)	0.291	.032	.0010		S^2 = .0024
	4)	0.268	.009	0		S = .0485
	5)	0.310	.051	.0026		
	X)	0.259		.0094	percent wild-type A^{495} =	33
	1)	0.161	-.044	.0019		
	2)	0.152	-.053	.0028		
	3)	0.244	.041	.0017		S^2 = .0027
	4)	0.271	.066	.0043		S = .0517
	5)	0.198	.007	0		
	X)	0.205		.0107	percent wild-type A^{495} =	26

TABLE 9

ORR	1)	0.843	.048	.0023		
	2)	0.733	-.062	.0038		
	3)	0.818	.023	.0005		S^2 = .0020
	4)	0.816	.021	.0004		S = .0447
	5)	0.796	-.031	.0020		
	X)	0.795		.0080	percent wild-type A^{495} =	100
C(2L)VY1,bpr;	1)	1.089	.001	0		
C(2R)VF3,SD72	2)	1.155	.067	.0045		
cnbw	3)	1.061	-.027	.0007		S^2 = .0044
	4)	0.992	-.096	.0042		S = .0663
	5)	1.145	.057	.0032		
	X)	1.088		.0176	percent wild-type A^{495} =	137
C(2L)VF1,bpr;	1)	1.048	-.002	0		
C(2R)VF3,SD72	2)	1.090	.040	.0016		
cnbw	3)	1.001	-.049	.0024		S^2 = .0021
	4)	1.103	.053	.0028		S = .0458
	5)	1.010	-.040	.0016		
	X)	1.050		.0084	percent wild-type A^{495} =	132

		A^{495}	$X-\bar{X}$	$(X-\bar{X})^2$	$S^2 = \frac{(X-\bar{X})^2}{n-1}$	n=5
C(2L)VF1,pr;	1)	0.898	.062	.0038		
C(2R)VF3,SD72	2)	0.772	-.064	.0041		
+	3)	0.786	-.050	.0025		S^2 = .0035
	4)	0.894	.058	.0035		S = .0589
	5)	0.831	-.005	0		
	X)	0.836		.0139	percent wild-type A^{495} =	105
C(2L)VY1,pr^{bw};	1)	0.775	-.027	.0007		
C(2R)VF3,SD72	2)	0.854	.052	.0027		
+	3)	0.729	-.073	.0053		S^2 = .0033
	4)	0.787	-.015	.0002		S = .0570
	5)	0.866	.064	.0041		
	X)	0.802		.0130	percent wild-type A^{495} =	101