A GENETIC LOCUS HAVING *TRANS* AND *CONTIGUOUS CIS* FUNCTIONS THAT CONTROL THE DISPROPORTIONATE REPLICATION OF RIBOSOMAL RNA GENES IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The results of deficiency mapping experiments reveal the presence of a compensatory response ($cr+$) locus that is located distal to the cluster of ribosomal RNA (rRNA) genes and is responsible for disproportionately replicating these genes when $cr+$ locus is present in a single dose, as in X/O males or $X/sc^4\text{-}sc^8$ females. The $cr+$ locus is novel in that it exhibits both *trans* and *contiguous cis* acting properties in somatic cells. It acts in *trans* to detect the presence of its partner locus in the opposite homolog, and if that partner locus is absent, it acts in *cis* to drive the disproportionate replication of those rRNA genes (rDNA) that are contiguous with it. The ability of $cr+$ to function is independent of the number of ribosomal RNA genes present. Furthermore, it can be shown that the $cr+$ locus is not required for the magnification or reduction of germ line rDNA. Finally, the implications of $cr+$ for position-effect variegation and the apparent reversion of the abnormal oocyte (*abo*) phenotype are discussed.

IN the fruit fly, *Drosophila melanogaster*, one cluster of the tandemly repeating copies of ribosomal RNA genes (rDNA) is located at the nucleolus organizer (*NO*) locus in the centromeric heterochromatin of the *X* chromosome, and a second *NO* occurs on the short arm of the *Y* chromosome (STERN 1936; RITOSSA, *et al.* 1966). Several years ago, it was shown that an *X* chromosome *NO* can increase its rDNA multiplicity in genetic constitutions in which there is only one *NO* present, as in X/O males or $X/X,NO^-$ females (TARTOF 1971). This increase, termed "rDNA compensation", is a somatic event since it occurs during the ontogeny of a single generation. Interestingly, the *Y* chromosome *NO* is refractory to rDNA compensation as demonstrated for a number of different *Y* chromosomes (TARTOF 1971; PROCUNIER and WILLIAMSON 1974), but in *D. hydei*, where the *Y* chromosome has two *NO* regions, both *NO* regions do compensate (KUNZ and SCHÄFER 1976).

When the compensation effect was first described, it was suggested that it was the result of a disproportionate replication of rDNA (TARTOF 1971). Subse-

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quently, two other instances of independent rDNA replication in *D. melanogaster* have been reported. SPEAR and GALL (1973) showed that in polytene chromosomes, the rDNA content of *X/O* individuals disproportionately increases to reach the same final abundance as in *X/X* genotypes. A rather different example is illustrated by the case of the autosomal *abo* (*abnormal oocyte*, 2–38; SANDLER 1970) mutation. SANDLER (1970) and PARRY and SANDLER (1974) showed that homozygous *abo* females exhibit a maternal effect that results in the production of defective eggs whose probability of supporting zygotic development increases with increasing doses of either *Y* chromosomes or increasing doses of the penultimate heterochromatin of the *X* chromosome (designated as Xh^{abo}). Later, KRIDER and LEVINE (1975) demonstrated that a loss of the *abo* phenotype among inbred *abo* homozygotes was concomitant with a significant increase in rDNA. The fact that disproportionate rDNA increases have been observed for the cases of compensation, independent polytenization and the apparent reversion of the *abo* mutant suggest that a common mechanism may be involved.

This report is concerned with the compensation phenomenon and presents evidence for the existence of a genetic locus, compensatory response (cr^+), located in the centromeric heterochromatin of the *X* chromosome (*Xh*), that regulates rDNA compensation. Utilizing various deficiencies for *Xh*, we find that cr^+ maps adjacent to the distal end of the rDNA genes, which is the same region of the *X* that interacts with *abo*. The cr^+ locus displays novel *cis* and *trans*-acting features. It acts in *trans* to sense the presence or absence of its partner cr^+ locus in the opposite homologue. If only one cr^+ is present, then it acts in *cis* by driving those rRNA genes contiguous with it on the same chromosome to disproportionately replicate. Both *cis* and *trans*-acting functions of cr^+ are independent of the number of rRNA genes present in the genome. Finally, we demonstrate that rDNA magnification and reduction events that stably alter the germ-line number of rRNA genes (RITOSSA 1968; TARTOF 1974) can occur independently of cr^+ function. The implications of these results will be discussed.

MATERIALS AND METHODS

D. melanogaster stocks: *D. melanogaster* cultures were raised at 25° ± 0.5° in half-pint bottles using a cornmeal, agar and molasses medium (TARTOF 1973). The chromosomes used in this study are briefly described below. A more complete description has been given by LINDSLEY and GRELL (1968).

X—wild-type Oregon-R chromosome carrying one *NO* region.

B^SY—a *Y* chromosome carrying one *NO* region and the *Bar* eye mutation.

y^+Ymal^+—a *Y* chromosome having one dose of the *NO* region and carrying the wild-type alleles for *y* and *mal*.

C(1)DX, yf—a reversed acrocentric compound-*X* chromosome deficient for both *NO* regions.

C(1)RM, y—a reversed metacentric compound-*X* chromosome.

bb^{2rl3}—an *X* chromosome in which the number of rRNA genes has been reduced to a lethal level by reduction (TARTOF 1974). This is an internal deletion for nearly all of the rRNA genes of the *X NO* region, leaving the regions adjacent to rDNA intact (see Figure 1). For convenience we will refer in the text to this mutation as bb^{2rl}.

$Df(1)mal^{12}$, $sc^8B\ mal^{12}$—an *X* chromosome carrying the sc^8 inversion and a deficiency that begins within the *NO* and extends through the distal end of the rDNA boundary to

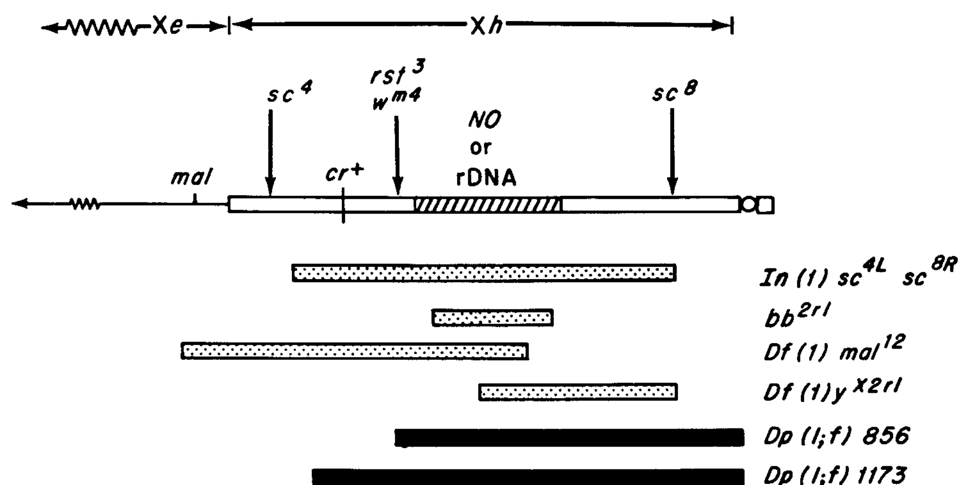

FIGURE 1.—Extents of deficiencies and duplications in the centric heterochromatin of the *X* chromosome. The open regions define the non-rDNA centric heterochromatin of the *X*, the stippled bars indicate deleted segments, and the solid bars depict duplicated portions. *Xe* represents the euchromatin and *Xh*, the heterochromatin of the *X*. At least 27% of the *Xh* is rDNA or nucleolus organizer (*NO*), as indicated by the hatched area. A complete description of the indicated mutations is given in the text.

include the *mal, mel, sw, ot* and *su(f)* loci (SCHALET 1969; see Figure 1). This chromosome is designated in the text simply as mal^{12}.

$Df(1)y^{x2rl}$, y^{x2rl} sc^8 *f*—an *X* chromosome bearing the sc^8 inversion in which there is a deficiency that begins within the *NO* and extends through the proximal boundary of the rDNA cluster (SCHALET 1969; see Figure 1). SCHALET's original y^{x2} mutant contained about 170 rRNA genes. The number of rRNA genes in this mutant was diminished by means of the reduction effect with Ybb^- (TARTOF 1974). This was achieved by synthesizing $y^{x2}/Dp(1;f)164, y^+/Ybb^-$ males and recovering y^{x2} chromosomes that are bobbed lethal (TARTOF 1974). Such a chromosome is designated y^{x2rl} in the text.

$In(1)sc^{4L}sc^{8R}$, *y* sc^4sc^8 *cv v B*—an inverted *X* chromosome with a deletion for all of the DNA in the heterochromatin between the sc^4 and sc^8 breakpoints (see Figure 1). This chromosome is referred to as sc^4-sc^8 in the text.

Dp(1;f)1173, y+—an *X* chromosome (referred to as *Dp 1173* in the text) deleted for most of *X* euchromatin and having the majority of the *X* heterochromatin. The chromosome has the *X* centromere, *bb+* allele and is 3.2–3.6 times the length of the metaphase fourth chromosome (LINDSLEY and GRELL 1968; see Figure 1).

Dp(1;f)856, y+—an *X* chromosome (referred to as *Dp 856* in the text) similar to *Dp 1173* except that it is 3.0 times the length of the metaphase fourth chromosome (see Figure 1).

$In(1)w^{m4}$, *y cv m f*—an inverted *X* chromosome with the heterochromatic break between the *NO* and the sc^4 break and the euchromatic breakpoint distal to *w* (white; see Figure 1). This chromosome is designated simply as w^{m4} in the text.

$In(1)rst^3$, rst^3—an inverted *X* with the left breakpoint between *w* and *rst* and the right breakpoint between the *NO* and sc^4 (see Figure 1). This chromosome is designated as rst^3 in the text.

Nucleic acid isolation and rRNA-DNA hybridization: In order to expedite the matter of obtaining accurate rDNA measurements, we have developed a simple and rapid method for the extraction of DNA that requires only a small quantity of flies. About 1.0 g of frozen adult flies (stored at —70°) of the appropriate genotype were homogenized at 4° in 10 ml of a lysis solu-

tion (0.1 M EDTA, pH 8.0, 2% sodium dodecyl sulfate, 0.5 M sodium perchlorate and 0.15 M NaCl) and 10 ml of water-saturated phenol (pH 8.0) containing 0.1% (w/v) 8-hydroxyquinoline. One to two volumes of 1 × SSC (0.15 M NaCl + 0.015 M Na citrate, pH 7.0) are then added to the homogenate to the point where the viscosity is markedly reduced. The homogenate is shaken for 20 min and then centrifuged. The aqueous phase is re-extracted with an equal volume of chloroform-isoamyl alcohol (24:1) and the DNA finally precipitated with 1 volume of cold 95% ethanol. The precipitate is dissolved in 2 ml of MUP [8 M urea and 0.24 M phosphate buffer (PB), pH 6.8], passed through a hydroxyapatite (0.5 g Bio-Rad HTP per gram of flies, in 0.24 M PB) column that was first washed with MUP, and then with 0.014 M phosphate buffer. With these washes RNA, single stranded DNA, protein and polysaccharide pass through the column. The DNA is eluted from the column with 0.40 M PB and denatured in 0.5 M NaOH (pH 12.2) for 10 min at room temperature. The solution is then neutralized with HCl and immobilized on a nitrocellulose filter as previously described (TARTOF 1973). The amount of DNA bound is determined by the difference in optical density at 260 mμ of the DNA solution before and after passing through the filter. The methods for ^{3}H-rRNA isolation and ^{3}H-rRNA-DNA hybridization reactions have been previously described (TARTOF 1973).

RESULTS

Compensation of the X *chromosome* NO *region (Experiment 1)*: When the *X* chromosome is the only sex chromosome present, as in *X/O* males, or when it is opposite another *X* that is deficient for its *NO* region, as in $X/sc^{4}\text{-}sc^{8}$ females, the rRNA genes of the *X* chromosome *NO* region disproportionately increase in number (TARTOF 1971). In wild-type *D. melanogaster* females having two *X* chromosomes, there are about 228 rRNA genes per *NO* region (Table 1). When this *X* chromosome is maintained with a $sc^{4}\text{-}sc^{8}$ chromosome, as $X/sc^{4L}\text{-}sc^{8R}$ females or *X/O* in males, there is an increase of 148 and 175 rRNA genes, respectively, per *X NO* region (Table 1). This increase of rRNA genes is termed "rDNA compensation." Parenthetically, it should also be noted that the amount of rDNA found in each of the genotypes utilized in Experiment 1 is in excellent quantitative agreement with similar measurements obtained by previous methods of DNA extraction (TARTOF 1971, 1973).

Stability of Y *and* C(1)RM *chromosome rRNA gene redundancy (Experiment 2)*: When determining the rDNA content of a particular chromosome, ambiguous results may occur as a consequence of compensation. In order to describe accurately the rRNA gene multiplicity of a particular *NO* region on the *X*, this region must be placed in genetic conditions not perturbed by the compensation effect. This can be achieved in two ways. One is by making all rDNA measurements in homozygous *X/X* females. In cases of lethal bobbed mutants, this is not possible. Thus, a second and easier means of eliminating compensatory effects is to measure rDNA values in *X/Y* males and subtract the rDNA contributed by the *Y*. This is both practical and effective because we know from previous studies that the *Y* chromosome *NO* region is refractory to compensation (TARTOF 1971; PROCUNIER and WILLIAMSON 1974). As shown in Table 1, Experiment 2, the $B^{S}Y$ and y^{+} *Y* mal^{+} chromosomes, possessing only one *NO* region, have 205 and 160 rRNA genes, respectively. The number of rRNA genes in the $B^{S}Y$ chromosome is in excellent agreement with previous measurements (TARTOF 1971). Because the amount of rDNA in the y^{+} *Y* mal^{+} chromosome was lower

than usually encountered, we made a second and independent measurement of its rDNA content. The compound-*X* chromosome, *C(1)RM*, has 262 rRNA genes (Table 1, Experiment 2). In $C(1)RM/y^{+}Ymal^{+}$ females, the $y^{+}Ymal^{+}$ chromosome rDNA is additive with that of the *C(1)RM* chromosome and found to contain 172 rRNA genes (Table 1, Experiment 2). Thus, the $y^{+}Ymal^{+}$ chromosome does contain fewer rRNA genes than the $B^{S}Y$ (160 compared to 205) but like the $B^{S}Y$ it is refractory to compensation and can be used for determining the rRNA gene numbers of other chromosomes.

Genetic localization of the compensatory response locus (cr^{+}) *by deficiency mapping (Experiments 3 and 4)*: The behavior of the compensatory response suggests that in the heterochromatin of the *X* there exists a locus (or loci) capable of sensing a deficiency of its homologous region and then directing a disproportionate replication. Such a locus (or loci) might be located within one of three regions: distal to the rDNA cluster, proximal to the rDNA cluster, or within the rDNA cluster. Our rationale for determining if there exists a genetic locus responsible for rDNA compensation involves the use of various tester chromosomes or chromosome fragments. The tester chromosome is placed opposite a wild-type *X* chromosome to determine whether or not the wild-type *X* rDNA compensates. Compensation of the rDNA on the wild-type *X* would occur only when there was a deletion for the hypothetical regulatory locus on the tester chromosome, as in $X/sc^{4}\text{-}sc^{8}$, for example. On the other hand, if compensation does not occur in the wild-type *X*, then the locus controlling this function must still be present in the opposite homologue (as in *X/X* individuals). We shall refer to such a hypothetical locus as the compensatory response (cr^{+}) locus. Thus, in the context of this terminology, the rDNA of cr^{+}/cr^{+} genotype would not compensate, while cr^{+}/cr^{-} would exhibit rDNA compensation in the cr^{+} bearing homologue. To determine if the locus regulating compensation is embedded within the cluster of repeating rRNA genes, we utilized the bb^{2rl} chromosome. This chromosome was generated by means of the "rDNA reduction" with Ybb^{-}. As such, this deficiency is probably the result of unequal sister-chromatid exchange between rRNA genes (TARTOF 1974). Such an exchange event leading to a reduced number of rRNA gene copies could delete most of the internal rDNA repeats, but would not involve the DNA regions bounding the rDNA cluster. The bb^{2rl} chromosome contains approximately 46 rRNA genes and is thus deleted for 80% of its wild-type rDNA content (Table 1, Experiment 3). As seen in Table 1 (Experiment 3), the wild-type *X NO* region does not compensate when opposite the bb^{2rl} chromosome, as in X/bb^{2rl} females. Although this experiment is somewhat equivocal because some rRNA genes still remain in bb^{2rl}, the simplest conclusion is that if the hypothetical cr^{+} locus exists, it does not reside within the rDNA cluster.

If cr^{+} does not lie within the rDNA cluster, then it must be located either proximal or distal to it. To test this point, we used the y^{x2rl} and mal^{12} chromosomes of SCHALET (1969). Both are X-ray-induced deficiencies in a sc^{8} chromosome. y^{x2rl} is a deficiency that begins within the rDNA cluster and extends proximally (relative to the wild-type *X*), whereas mal^{12} is a deficiency

TABLE 1

The number of rRNA genes in various deficiencies and duplications of the X *and* Y *chromosomes*

Genotype	Percent DNA hybridized ± S.E.*	rRNA gene number: Total	rRNA gene number: *X* chromosome	rRNA gene number: *Y* chromosome	Compensatory net increase of rRNA genes of wild-type *X NO* region
Experiment 1					
X/X	0.40 ± 0.013	456	228	—	—
X/sc^{4L}-sc^{8R}	0.33 ± 0.011	376	376	—	148
X/O	0.37 ± 0.014	430	430	—	175
Experiment 2					
C(1)DX/B^{S}Y	0.18 ± 0.0064	205	—	205	—
C(1)DX/y+Ymal+	0.14 ± 0.0038	160	—	160	—
C(1)RM/O	0.23 ± 0.0087	262	262	—	—
C(1)RM/y+Ymal+	0.38 ± 0.011	434	262	172	—
Experiment 3					
bb^{2rl}/B^{S}Y	0.22 ± 0.0088	251	46	205	—
X/bb^{2rl}	0.24 ± 0.012	274	228(*X*) 46(*bb^{2rl}*)	—	0
Df(1)y^{x2rl}/y+Ymal+	0.23 ± 0.011	262	102	160	—
X/Df(1)y^{x2rl}	0.26 ± 0.0080	296	194(*X*) 102[*Df(1)y^{x2rl}*]	—	0
Df(1)mal^{12}/y+Ymal+	0.20 ± 0.0082	228	68	160	—
X/Df(1)mal^{12}	0.41 ± 0.0086	468	400(*X*) 68[*Df(1)mal^{12}*]	—	172
Experiment 4					
C(1)RM/Dp(1;f)1173	0.42 ± 0.021	479	262[*C(1)RM*] 217[*Dp(1;f)1173*]	—	—
X/Dp(1;f)1173	0.43 ± 0.0062	491	274(*X*) 217[*Dp(1;f)1173*]	—	46
C(1)RM/Dp(1;f)856	0.43 ± 0.020	491	262[*C(1)RM*] 229[*Dp(1;f)856*]	—	—
X/Dp(1;f)856	0.57 ± 0.012	650	421 229[*Dp(1;f)856*]	—	193
Experiment 5					
In(1)w^{m4}/y+Ymal+	0.36 ± 0.0053	411	251	160	—
In(1)w^{m4}/In(1)sc^{4L}sc^{8R}	0.19 ± —0.0035	217	217	—	0
X/In(1)w^{m4}	0.42 ± 0.0084	479	228(*X*) 251[*In(1)w^{m4}*]	—	0
X/In(1)w^{m4L}sc^{8R}	0.23 ± 0.0032	263	263(*X*)	—	35

TABLE 1—Continued

Genotype	Percent DNA hybridized ± S.E.*	rRNA gene number Total	X chromosome	Y chromosome	Compensatory net increase of rRNA genes of wild-type X NO region
Experiment 6					
$In(1)rst^3/B^SY$	0.35 ± 0.017	399	194	205	—
$In(1)rst^3/In(1)sc^{4L}sc^{8R}$	0.15 ± 0.017	171	171	—	0
$X/In(1)rst^3$	0.36 ± 0.0072	411	217(X) [$In(1)rst^3$]	—	0
$X/In(1)rst^{3L}sc^{8R}$	0.20 ± 0.0015		228(X)	—	0
Experiment 7					
X/Y	0.42 ± 0.018	479	228	251	0
X/Ybb^-	0.43 ± 0.012	491	228	40†	223

* The percent DNA hybridized was determined 4 times from a single DNA preparation obtained from each genotype.
† The Ybb^- chromosome contains approximately 40 rRNA genes (see TARTOF 1973).

that begins within the rDNA cluster but extends distally (Figure 1).

The y^{x2rl} and mal^{12} chromosomes contain 102 and 68 rRNA genes, respectively (Table 1, Experiment 3). Despite the fact that they have similar numbers of rRNA genes, the mal^{12} differs from the y^{x2rl} in permitting the compensatory response in a wild-type *X*. As seen from the data in Table 1, Experiment 3, in X/y^{x2rl} females the *X* rDNA does not compensate, whereas in X/mal^{12} females, it does. From these data, we conclude that cr^+ maps in the heterochromatic DNA segment between the distal end of the *NO* region and the heterochromatic sc^4 breakpoint (Figure 1). Thus, because the *X* rDNA in X/y^{x2rl} flies fails to compensate, the flies are presumed to be cr^+ NO^+/cr^+ bb^l, but because this same *X* does compensate in X/mal^{12} individuals, they are designated cr^+ NO^+/cr^- bb^l. From these results, it would also appear that compensation is independent of the amount of rDNA in the opposite homologue, since there are certainly some (50–100) rRNA genes in the y^{x2r} and mal^{12} chromosomes. Thus, cr^+ would function not by detecting a depletion of rDNA in the opposite homologue, but rather the absence of cr^+.

The location of cr^+ and the fact that the compensatory response is independent of the amount of rDNA present was confirmed using another set of partial deficiencies for distal *X* chromosome heterochromatin that result in the formation of free duplications. *Dp 1173* contains all of the heterochromatic portions of the *X* from the centromere through the *NO* but is deficient for the distal region of X*h* (Figure 1) and contains 217 rRNA genes (Table 1, Experiment 4). When this chromosome is placed opposite an *X* chromosome, as in *X/Dp(1;f)1173* males, there is no significant *X NO* compensation: *Dp1173* is therefore cr^+. However, *Dp(1;f)856*, which has approximately the same number of rRNA genes (229; Table 1, Experiment 4) but is deficient for more of the distal hetero-

chromatin than *Dp 1173*, causes the *X NO* region to compensate: *Dp 856* is therefore cr^-. Males of the genotype *X/Dp(1;f)856* (cr^+ NO^+/cr^- NO^+) contain a total of 650 rRNA genes (0.57%) resulting from 229 rRNA genes in the *Dp 856*, 228 rRNA genes in the *X* plus an increase of 193 rRNA genes resulting from the compensatory increase in *X* rDNA. From these experiments, we draw three principal conclusions: (1) the cr^+ locus is located between the distal breakpoints of the tested duplications outside the *NO*; (2) that cr^+ acts in *trans* to detect a deficiency of its homologous locus in the apposite chromosome; and (3) that cr^+ functions independently of the number of rRNA genes in the opposite homologue.

The contiguous cis *acting nature of the* cr^+ *locus (Experiments 5 and 6)*: As demonstrated above, the cr^+ locus senses a genetic deficiency of the same gene in a homologous *X* chromosome, thereby resulting in compansation. This "sensing" function of cr^+ acts in *trans*. Various *X* chromosome inversions were then tested for the purpose of transposing the cr^+ locus away from the *NO* region to determine if the cr^+ gene must be contiguous with the rDNA cluster that undergoes compensation. The inverted *X* chromosome, $In(1)w^{m4}$, has the *Xh* breakpoint distal to the *NO* region (Figure 1) and contains the wild-type number of rRNA genes (251; Table 1, Experiment 5). Females, $X/In(1)w^{m4}$, show an absence of *X NO* compensation, indicating that the inverted chromosome has the cr^+ locus (cr^+ NO^+/cr^+ NO^+). However, when the $In(1)w^{m4}$ chromosome is placed opposite the sc^{4L}-sc^{8R} chromosome, which is deficient for the cr^+ locus, $In(1)w^{m4}/sc^{4L}$-sc^{8R} females (cr^+ NO^+/cr^- NO^-), there is also no compensation. The same results (Experiment 6) are also demonstrated for another inverted *X* chromosome, $In(1)rst^s$, with an *Xh* breakpoint distal to the *NO* region (Figure 1). These data suggest that the cr^+ locus must be in a certain proximity, that is in a *contiguous cis* position, to the *NO* region in order to drive the compensatory increase in rDNA. It could be assumed, however, the the cr^+ locus has in fact not been relocated away from rDNA sequences by the w^{m4} and rst^s rearrangements. Rather, it could still be adjacent to the rDNA, but lost its control over rDNA compensation due to the presence of euchromatic segments placed next to it. To demonstrate that cr^+ has indeed been relocated to a distal position on the *X* chromosome by the w^{m4} and rst^s inversions, the following chromosomes were synthesized. $In(1)w^{m4}/In(1)sc^{4L}sc^{8R}$ females and $In(1)rst^s/In(1)sc^{4L}sc^{8R}$ females were constructed with appropriate markers such that the recombinant chromosomes $In(1)w^{m4L}sc^{8R}$ and $In(1)rst^{sL}sc^{8R}$, respectively, were recovered. Since these chromosomes contain the left end of either the w^{m4} or rst^s and the right end of sc^8, they will contain no nucleolus organizer, but should contain the cr^+ locus if it has been located distally as a result of the w^{m4} or rst^s inversions. Females of the genotype $X/w^{m4L}sc^{8R}$ and $X/rst^{sL}sc^{8R}$ were then constructed. If compensation takes place, then the cr^+ locus is not at the left end of the *X* chromosome in the w^{m4} and rst^s inversions. If, however, the wild-type *X* chromosome rDNA fails to replicate disproportionately, then this must mean that the cr^+ locus could come only from the distal end of the w^{m4} or rst^s chromosomes. The results of these experiments are shown in Table 1, Experiments 5 and 6, where it is seen that $X/w^{m4L}sc^{8R}$ and $X/rst^{sL}sc^{8R}$ fail to show the typical rDNA

compensatory response. It is concluded, therefore, that w^{m4} and rst^{3} each contain a distally located cr^{+} locus whose existence can be sensed in *trans* but which cannot drive the disproportionate increase in rDNA unless it is in a *contiguous cis* position with respect to such sequences. On the basis of these data, we place cr^{+} (or more correctly, Xh^{cr+}) within the distal penultimate heterochromatic segment bounded by the sc^{4} and w^{m4} (or rst^{3}) breakpoints (see Figure 1).

The difference between X *and* Y cr+ *loci (Experiment 7)*: At this point, it is appropriate to emphasize an interesting difference between the *X* and *Y* cr^{+} loci. As shown in Table 1 (Experiment 7), *X/Y* males are additive in their rDNA contents, indicating that such males are presumably $cr^{+}NO^{+}(X)/cr^{+}NO^{+}(Y)$, and hence there is no compensation in the *X* because of the presence of cr^{+} in the *Y*. That the *Y* contains a cr^{+} locus becomes manifest by examination of the Ybb^{-} chromosome that is deleted for approximately 80% of the wild-type rDNA content such that it contains only about 40 rRNA genes (TARTOF 1973). In X/Ybb^{-} males, the *X* increases its rDNA content by 223 rRNA genes (Table 1, Experiment 7). It seems likely that the Ybb^{-} elicits the compensatory effect in the *X* because it is deficient for its own cr^{+} locus. But, as noted above, the *Y* rDNA is refractory to compensation as in sc^{4}-sc^{8}/Y males (TARTOF 1971; Table 1, Experiment 7). We presume that this results from the fact that the cr^{+} locus is present in the *Y* but located in such a position that, as in w^{m4} or rst^{3} chromosomes, it is unable to drive the disproportionate replication of its own rDNA.

Is compensation required for rDNA magnification or reduction? Compensation is a somatic phenomenon that occurs when an *X* chromosome *NO* nearly doubles its rDNA content in response to being present in a single dose. Characteristic of this somatic event is the fact that the extra rDNA is not stably inherited in subsequent generations (TARTOF 1973). Such a somatic change is distinct from that which takes place in the male germ line, where an *X* chromosome rDNA cluster is maintained opposite a Ybb^{-} chromosome. In this case, increases (*e.g.*, *bb* to bb^{+}) in rDNA redundancy are referred to as magnification, while decreases in rDNA number (*e.g.*, bb^{+} to *bb*) are defined as reduction. In contrast to compensation, these changes of rDNA content in either the reverse or forward direction are stably inherited (TARTOF 1973, 1974). However, since compensation only occurs in the *X* chromosome, and since Ybb^{-} can induce both compensation (Table 1, Experiment 7) and magnification, it is appropriate to ask if compensation is required for magnification to take place. Since we now know that w^{m4} or rst^{3} are refractory to compensation (Experiments 5 and 6, Table 1), one can then ask if reduction and magnification are similarly inhibited. Thus, w^{m4} bb^{+}/Ybb^{-} and rst^{3} bb^{+}/Ybb^{-} males were mated to sc^{4}-$sc^{8}/\Delta 49$ females and the w^{m4} or rst^{3}/sc^{4}-sc^{8} female progeny were screened for the presence of bobbed mutations. In such reduction experiments (Table 2), we found that both the w^{m4} and rst^{3} chromosomes yielded bobbed mutants with a frequency similar to that of two other wild-type chromosomes that have been tested (TARTOF 1974). In order to see if these bobbed mutants could then magnify, w^{m4} bb/Ybb^{-} and rst^{3} bb/Ybb^{-} males were mated to sc^{4}-$sc^{8}/\Delta 49$ females and the progeny scored for bb^{+}/sc^{4}-sc^{8} females. In this case (Table 2), wild-type bb^{m+} revertants do appear,

TABLE 2

Reduction and magnification in w^{m4}/Ybb⁻ *and* rst^3/Ybb⁻ *males as detected by mating to* sc^4-sc^8/Δ49 *females and examining the bobbed phenotype of the* X/sc^4-sc^8 *daughters*

Male parent genotype	Number of flies		Percent reduction
	bb^+/sc^4-sc^8	bb/sc^4-sc^8	
w^{m4} $bb+/Ybb^-$	14,456	2	0.014
rst^3 $bb+/Ybb^-$	10,189	7	0.069
w^{m4} $bb+/Y$	9,747	0	0
rst^3 $bb+/Y$	10,518	0	0
			Percent magnification
w^{m4} bb/Ybb^-	46	2,874	1.6
rst^3 bb/Ybb^-	106	2,240	4.5
w^{m4} bb/Y	0	3,084	0
rst^3 bb/Y	0	2,962	0

but at a somewhat lower frequency than previously studied bobbed mutants (TARTOF 1974). The reason for the lower frequency of magnification is not obvious. In any case, the data clearly demonstrate that magnification and reduction occur independently of the ability of an *X* rDNA cluster to undergo compensation.

DISCUSSION

The experiments detailed in this paper demonstrate that there exists a locus that we call compensatory response (cr^+) that is located in the penultimate region of the distal heterochromatin of the *X* chromosome and regulates the disproportionate increase of the ribosomal RNA genes that are contiguous with it when it is present in a single dose. The cr^+ locus acts in *trans* to sense the presence or absence of its partner cr^+ locus in the opposite homologue. If only one cr^+ *is* present, then its acts from a *contiguous cis* position to drive the disproportionate replication of adjacent rRNA genes. In certain rearranged *X* chromosomes, such as w^{m4} and rst^3, the rDNA is not disproportionately replicated because these chromosomes have been rearranged such that cr^+ is no longer contiguous with the rDNA it regulates. Similarly, the *Y* chromosome contains a cr^+ locus that is also apparently situated so that it is not capable of driving a disproportionate increase of its own rDNA. Finally, we have demonstrated that cr^+ function is not required for the magnification and reduction of germ line rDNA.

Aside from the ribosomal RNA genes, both the physical and genetic structure of *X* heterochromatin remains obscure. The centromeric heterochromatin of the *X* chromosome contains approximately one-third of the DNA content of an entire *X* (RUDKIN 1964). Since the Drosophila genome contains 1.8×10^8 bp (base pairs), and since the *X* chromosome contains 20% of the total DNA content of the genome, there are, then, 1.2×10^7 bp in the *Xh* region. There are approximately 230 ribosomal RNA genes in the *X* chromosome (Table 1, Experiment 1) and the *X* nucleolus organizer is composed of nearly equal numbers of ribosomal RNA genes that have unit repeat lengths of 1.7×10^4 base pairs and

1.1×10^4 base pairs (average = 1.4×10^4 bp; TARTOF and DAWID 1976). This means that there are 3.2×10^6 ribosomal RNA gene base pairs present in the *X* heterochromatin. If anything, this could be an underestimate if there are subclusters of ribosomal RNA genes separated by unusually long spacers. Thus, at least 27% of the DNA in the *Xh* region codes for ribosomal RNA. The nature of the remaining 73% (8.8×10^6 bp) is not known, though certainly a substantial fraction of this is probably satellite DNA (see TARTOF 1975 for a review). From a genetic point of view, there is one or possibly two genes located in *Xh*. The suppressor of forked [*su(f)*] locus is certainly very close, if not within, the distal *Xh* segment. Another gene known to be located in heterochromatin is Xh^{abo} which is a locus in the heterochromatic region of the *X* that interacts with, or is regulated by, the abnormal oocyte (*abo*) mutation located on the second chromosome (SANDLER 1970; PARRY and SANDLER 1974). Homozygous *abo* females exhibit a maternal effect that results in the production of defective eggs, most of which do not hatch. This maternal effect can be reversed by increasing the dose of either *Xh* or a *Y* chromosome in either the mutant mother or her progeny (SANDLER 1970). The region in the heterochromatin responsible for this reversal has been designated as Xh^{abo} and has been mapped to the same position as Xh^{cr+}, between the sc^4 and *Dp 856* breakpoints in the *X* heterochromatin (see Figure 1). It has been also shown that Xh^{abo} has an effect on the multiplicity of ribosomal RNA genes. Flies maintained as *abo/abo* homozygotes over the course of several generations no longer express the *abo* phenotype and also exhibit a several-fold increase in the number of ribosomal RNA genes (KRIDER and LEVINE 1975). As a result of the experiments described in this paper, we have shown that cr^+ maps with Xh^{abo} to the same region of the distal heterochromatin.

On this latter point our results suggest a simple mechanism for the reversion of the *abo* phenotype in *X/X*; *abo/abo* homozygotes. Unequal exchange in the distal penultimate heterochromatic segment containing Xh^{abo} and Xh^{cr+} would lead to increasing tandem duplications of this region and in turn, have the following effects. First, the amount of Xh^{abo} would increase and cause a phenotypic reversion of *abo*. Such revertants would have an obvious selective advantage and dominate the population rather quickly. Second, there would be multiple tandem copies of Xh^{cr+} in a *contiguous cis* position with respect to its neighboring rDNA. An *X* chromosome having several tandem copies of cr^+ ($Xh^{cr+cr+cr+}$) and maintained opposite an *X* having somewhat fewer copies of Xh^{cr+} (a wild-type *X* or one produced from unequal exchanges) could give rise to flies (*e.g.*, $Xh^{cr+cr+cr+}/Xh^{cr+}$ or Xh^{cr+cr+}/Xh^{cr^o}) containing several-fold increases in their rRNA gene number as a result of disproportionate rDNA replication on the homologue containing the greater number of Xh^{cr+} loci. In this model, the genetic basis of the apparent reversion of the *abo* phenotype takes place not at the *abo* locus but at the distal penultimate heterochromatin of the *X*. These ideas bear a certain resemblance to the mechanism proposed for rDNA magnification and reduction (TARTOF 1974), though in the *abo* case the proposed unequal exchanges could be either of the sister-chromatid type or between homologues.

The model makes several straightforward predictions that are eminently testable. It should be noted that since the presence of the extra rDNA in Dp 856 (Table 1) does not suppress the *abo* phenotype (PARRY and SANDLER 1974) it would appear that the increase in rDNA observed during reversion of *abo/abo* homozygotes is not an obligate for suppression, but rather is a derivative consequence of other events taking place in *Xh*.

It is worth considering for the moment the possibility that many different cr^+-like loci may be scattered throughout the genome. It has been previously demonstrated that the 5S RNA genes, which are located at a euchromatic site on the second chromosome (*2*–56EF), are also capable of disporportionately increasing their number when present in a single dose (PROCUNIER and TARTOF 1975). This indicates that the 5S genes are under a compensatory control similar to the ribosomal RNA genes, and it seems reasonable to suggest that the 5S RNA gene cluster also possesses its own specific cr^+ locus. It is not known how many different cr^+ loci may exist, or if such loci are limited only to redundant genes. The fact that the 5S RNA genes are under compensatory control does suggest, however, that cr^+ loci are not limited to the sex chromosomes or exclusively to heterochromatic segments of the genome.

Perhaps one of the most interesting features of the cr^+ locus is that it must be in a *contiguous cis* position with respect to the *rDNA* it regulates. It is particularly curious that the compensatory response is lost in flies that now express position-effect variegation. This could be either a fortuitous or a causal association. In 1936, SCHULTZ noted that variegation results when a wild-type euchromatic gene such as white+ is placed next to heterochromatin. The eye, in such individuals, is now composed of both wild-type (red) and mutant (white) ommatidia (phenotypically white mottled, *e.g.*, w^{m4}). To explain this observation, he suggested that during early development and differentiation of the eye, the white gene may be physically deleted (SCHULTZ 1936) or inactivated (SCHULTZ 1965) in one or a few cells. During subsequent growth, this gives rise to a clone of white eye cells and hence, the production of a mottled red-white eye. We suggest that the rearrangement of cr^+ as it occurs in w^{m4} and other genotypes expressing phenotypic variegation disrupts the usual control of heterochromatin replication, thereby leading to the deletion of neighboring (*e.g.*, w^+) euchromatic sequences, which then results in a variegated phenotype.

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