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Genetics

Nucleolar dominance in polytene cells of *Drosophila*

(ribosomal genes/ribosomal DNA replication/compensation)

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ABSTRACT **Previous studies indicate that genes from only one of the cell's nucleolus organizers undergo multiple rounds of DNA replication in polytene cells of *Drosophila*. This report presents evidence that this effect is mediated by a function that is associated with the ribosomal genes of the dominant or replicating X chromosome. This function can act in *trans* to result in replication of the ribosomal genes on the recessive X chromosome in flies that are bobbed for the dominant X chromosome. In these cases, ribosomal genes from both chromosomes undergo polytenization. Heterochromatic regions that flank the nucleolus organizer have little or no effect on nucleolar dominance. In addition, deletion of the compensatory response (cr^+) locus does not affect the dominance, suggesting that ribosomal gene compensation and nucleolar dominance in polytene cells of *Drosophila* are separate genetic phenomena.**

Nucleolar dominance in *Drosophila* results in the polytenization of ribosomal genes from predominantly one of the cell's nucleolus organizers in wild-type X/Y cells or hybrid X/Y, X/X, or XX/Y cells (1–3). Replication of genes from only one of the cell's nucleolus organizers would account for the observation that ribosomal gene levels of X/0 and X/X polytene cells are the same, although diploid levels show the expected 1:2 ratio (4). The equivalence of rRNA gene (rDNA) levels in X/0 and X/X polytene cells has been proposed to explain ribosomal gene compensation (4), which is the observation that the rDNA levels of X/0 and X/X flies are more nearly equivalent than expected (5).

The present study describes the effect of X chromosomes that carry deletions of the proximal or distal heterochromatin on nucleolar dominance in polytene cells. The results indicate that there is little or no effect of deleting proximal or distal heterochromatin from either the dominant or the recessive X chromosome. Partial deletion of ribosomal genes from the dominant chromosome, however, causes a release of dominance and results in the replication of genes from both nucleolus organizers. Nucleolar dominance thus appears to be mediated by an activity that is associated with the ribosomal genes of the dominant chromosome. This activity is affected by the number of ribosomal genes that are present on the dominant X chromosome and can act in *trans* to result in replication of genes on the recessive chromosome.

The results presented here indicate that nucleolar dominance is not affected by deletion of the compensatory response or cr^+ locus, which controls compensatory replication of the ribosomal genes (6). Nucleolar dominance in polytene cells therefore is probably not the basis of the ribosomal gene compensation described by Tartof (5).

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MATERIALS AND METHODS

***Drosophila* Stocks.** Stocks of *D. melanogaster* Oregon R A, Canton S, and OK-1 were obtained and restarted as described (1, 2). Stocks of $Df(1)mal^{12}$, sc^8 *B* mal^{12} were obtained from A. Hilliker, K. Tartof, and V. Finnerty; stocks of $Df(1)y^{x2}$, y^{x2} sc^8 *f* were obtained from A. Hilliker and K. Tartof. The $In(1)sc^8$, sc^8 *cv* and $In(1)sc^8$, sc^8 w^a *bb* stocks were from the laboratory of F. Ritossa, and stocks of $In(1)sc^{L8}$, sc^{L8} w^a sn^3 *car*, $In(1)sc^{S1}$ + S, y^{c4} sc^{S1} *B*, $In(1)sc^{L8L}$ sc^{S1R} + S, sc^{L8} *B*, and $In(1)sc^{S1L}$ sc^{8R}, *y* sc^{S1} sc^8 *cv v f* were obtained from W. Baker. These stocks are referred to as mal^{12}, y^{x2}, sc^8, sc^8 *bb*, sc^{L8}, sc^{S1}, $sc^{L8L,S1R}$, and $sc^{S1L,8R}$, respectively. Appropriate crosses were carried out to reclone the sc^8 and sc^8 *bb* stocks and to construct flies homozygous for the sc^8, sc^8 *bb*, sc^{L8}, and sc^{S1} chromosome. All eight chromosomes were tested for bobbed (*bb*) deficiency with the $In(1)sc^{4L}$ sc^{8R}, *y* sc^4 *cv* chromosome. The mal^{12} chromosomes from two of the three stocks and the $sc^{L8L,S1R}$ chromosome were found to be bobbed lethal (bb^l). The mal^{12} chromosome from the third stock was weak *bb*, as were the y^{x2} chromosomes from both y^{x2} stocks. The sc^8, sc^{L8}, sc^{S1}, and $sc^{S1L,8R}$ chromosomes were weak *bb* or bb^+, whereas the sc^8 *bb* chromosome carried a strong *bb* allele. The mal^{12} bb^l and mal^{12} weak *bb* chromosomes were tested and found to be *l-20*$^-$, maroonlike with a mal^2 chromosome and lethal with a mal^{10} chromosome. The mal^{12} bb^l chromosomes were y^+ and viable with the y^{x2} chromosome; the mal^{12} weak *bb* chromosome was *y* and nonviable with the y^{x2} chromosome. These data suggest that changes in the mal^{12} weak *bb* chromosome have occurred at *y* and *bb*, but that the original deletion covering *mal* and *l-20* is still present. One of the mal^{12} bb^l chromosomes and the mal^{12} weak *bb* chromosome were tested for the presence of the cr^+ locus as described below.

Compensatory Response. The ability of one of the mal^{12} bb^l chromosomes and the mal^{12} weak *bb* chromosome to induce compensation was tested by constructing hybrids with the Oregon R and Canton S X chromosomes. DNA was extracted from 20–50 adults by a modification of previous methods (2). The flies were crushed in lysing solution and allowed to dissolve 4–5 hr or overnight, the supernatant was removed and frozen, and the crushing and extraction were repeated. DNA from the combined supernatants was purified by equilibrium centrifugation in ethidium bromide/CsCl gradients as described (2). DNA was transferred to nitrocellulose by using the dot blot method (7) and filters were hybridized with the gel-purified 11.5-kilobase (kb) insert from plasmid pDm ra51 no. 1 (3, 8) ^{3}H-labeled *in vitro* by nick-translation and the ^{32}P-labeled insert from a *Drosophila* actin clone (a gift from E. Fyrberg). The actin gene insert corresponded to the 1.8-kb actin-coding region and a 1.6-kb noncoding region from the 3′ end of the gene

Abbreviations: rDNA, 18S and 28S rRNA genes; *cr*, compensatory response locus; *bb*, bobbed locus; kb, kilobase(s).

at 5C on the X chromosome. Control DNAs were from Oregon R and Canton S X/X females, $X^{Or}/y^+ Y\ mal^+$ and $X^{Cs}/y^+\ Y\ mal^+$ males containing the $y^+\ Y\ mal^+$ chromosomes from both the $mal^{12}\ bb^l$ and mal^{12} weak *bb* stocks and the Oregon R or Canton S X chromosome, and $mal^{12}\ bb^l/y^+\ Y\ mal^+$ and mal^{12} weak $bb/y^+\ Y\ mal^+$ males. After hybridization samples were autoradiographed to control for nonspecific ^{32}P binding and ^3H and ^{32}P radioactivities were measured in toluene fluor. The amount of ^3H hybridization was standardized against the ^{32}P hybridization to correct for the amount of DNA present on the filter. ^{32}P hybridization to the X/X female DNAs was corrected by a factor of 12/14 to account for the hybridization of the 3′ noncoding region of the probe. ^{32}P hybridization to X/Y male DNAs was not corrected because the extra hybridization to the X chromosome compensated for the lack of an actin gene on the Y chromosome. Results of these experiments indicated that both the $mal^{12}\ bb^l$ and mal^{12} weak *bb* chromosomes induced a compensatory response in the Oregon R and Canton S X chromosomes. This is consistent with previous reports that both the Oregon R and Canton S X chromosomes can undergo compensatory replication (5, 9) and indicates that the mal^{12} bb^l and mal^{12} weak *bb* chromosomes are cr^-.

DNA Extraction. Extraction of DNA from diploid or polytene tissue dissected from progeny of single pair matings was as described (1, 2). In most cases third-instar larval brains and imaginal discs were the diploid tissues used and salivary glands were the polytene tissue. In cases in which it was necessary to monitor the adult phenotype before tissue dissection, adult brains were used as the source of diploid tissue and the Malpighian tubules were used as the polytene tissue. The Malpighian tubules contain chromosomes of high levels of polyteny (10, 11). Comparison of rDNA patterns of OK-1 female or Oregon R A female or male larval salivary glands and adult Malpighian tubules showed patterns that were almost identical. The rDNA patterns showed differential replication of ribosomal gene repeats and replicative dominance of the Y chromosome nucleolus organizer in the X/Y male polytene cells.

Southern Transfers. Nitrocellulose filters were prepared and hybridized with [^{32}P]rDNA as described (1, 2). The hybridization probe was the gel-purified 11.5-kb rDNA insert from pDm ra51 no. 1 described above. In some experiments hybridization was carried out in the presence of 10% sodium dextran sulfate (12) as described (3).

Backcross Experiment. Two series of experiments were carried out to increase the background of recessive chromosomes in flies heterozygous for a dominant and a recessive X chromosome. In the first set of experiments, $X^{Cs}/sc^8\ cv$ females carrying the Canton S X chromosome were constructed and backcrossed in single pairs for four generations to $sc^8\ cv/B^S\ Y$ males. cv^+ females were selected each generation and mated to produce the succeeding generation. Diploid and polytene tissue was dissected from 10 individuals from each of two vials from the F_5 generation and DNA was prepared and analyzed by restriction enzyme digestion, transfer to filters, and hybridization with [^{32}P]rDNA. A second backcross series was carried out by constructing X^{Cs}/X^{Or} females containing a Canton S and Oregon R X chromosome and backcrossing single pairs for three generations to Oregon R X/Y males. Heterozygous F_2 and F_3 females were selected after 3 days of mating by Southern blot analysis of rDNA patterns in DNA from adult brain. Larval tissue was dissected from progeny of vials in which the rDNA pattern indicated that the parental female had been hybrid for the Canton S and Oregon R X chromosomes. DNA analysis was as before.

Containment. Experiments involving recombinant DNA were carried out under P1 conditions in accordance with current National Institutes of Health guidelines for recombinant DNA research.

RESULTS

Three wild-type X chromosomes were tested with the $In(1)sc^8$ chromosome for nucleolar dominance in polytene cells. These three wild-type X chromosomes are from the Canton S, OK-1, and Oregon R A strains of *D. melanogaster* (1, 2) and are denoted X^{Cs}, X^{OK}, and X^{Or}, respectively. After the pattern of dominance with the sc^8 chromosome had been determined, the wild-type chromosomes were tested with the deficiency chromosomes, $Df(1)mal^{12}$ and $Df(1)y^{x2}$, in order to determine the effect of deleting regions of either the distal or proximal heterochromatin.

The $Df(1)mal^{12}$ and $Df(1)y^{x2}$ chromosomes arose after x-irradiation of the $In(1)sc^8$ chromosome. These two chromosomes were discovered and characterized by Schalet (13). The mal^{12} chromosome carries a deletion that begins within the nucleolus organizer and extends to the euchromatic sequences in the proximal region of the X chromosome including *ot, mal, l-20,* and *su-f.* The deletion in the mal^{12} chromosome therefore covers the distal heterochromatin of the X chromosome, including cr^+. The y^{x2} chromosome carries a deficiency which extends from within the nucleolus organizer to the *y* locus in the euchromatic region at the tip of the X chromosome. The deletion in the y^{x2} chromosome covers almost all of the proximal heterochromatin of the X chromosome. The mal^{12} and y^{x2} deficiency chromosomes are shown in Fig. 1 together with the sc^8 chromosome from which they were derived.

Of the three wild-type chromosomes tested, X^{Cs} was dominant to the sc^8 chromosome in polytene cells, X^{Or} was recessive to the sc^8 chromosome, and X^{OK} was equal in dominance to the sc^8 chromosome (Fig. 2). Examination of DNA from X^{OK}/sc^8, X^{OK}/mal^{12}, and X^{OK}/y^{x2} polytene cells showed rDNA patterns from both nucleolus organizers in each case, indicating that there was no effect of deleting distal or proximal heterochromatin on the ability of the mal^{12} or y^{x2} ribosomal genes to undergo polytenization. This suggests that origins of rDNA replication during polytenization may lie within the ribosomal gene array rather than in the flanking heterochromatin. Because the X^{Cs}/sc^8 polytene tissue showed replicative dominance of the X^{Cs} nucleolus organizer, X^{Cs}/mal^{12} and X^{Cs}/y^{x2} flies were examined to determine the effect on this dominance of deleting the distal or proximal heterochromatin from the recessive chromosome. Analysis of DNA from X^{Cs}/mal^{12} and X^{Cs}/y^{x2} flies showed the X^{Cs} rDNA pattern in polytene tissue in both cases (Fig. 3), indicating that deletion of the distal or prox-

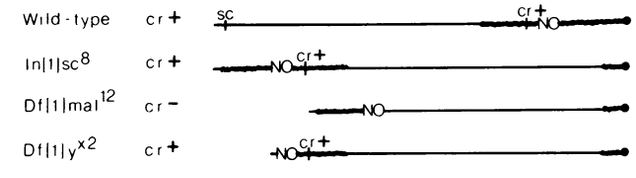

FIG. 1. Deletions carried by the $Df(1)mal^{12}$ and $Df(1)y^{x2}$ chromosomes. A wild-type X chromosome and the $In(1)sc^8$ chromosome are shown together with the $Df(1)mal^{12}$ and $Df(1)y^{x2}$ chromosomes. The $Df(1)mal^{12}$ and $Df(1)y^{x2}$ chromosomes arose from the parental $In(1)sc^8$ chromosome after x-irradiation. The heavier lines indicate the heterochromatic region of the X chromosome, the bulk of which is inverted to the tip of the X chromosome by the sc^8 inversion. The $Df(1)mal^{12}$ chromosome carries a deletion that begins in the nucleolus organizer (NO) and extends to proximal (with respect to wild-type orientation) markers in the euchromatin including *mal.* The $Df(1)y^{x2}$ chromosome is deleted for a region extending from the nucleolus organizer to sequences at the tip of the X chromosome.

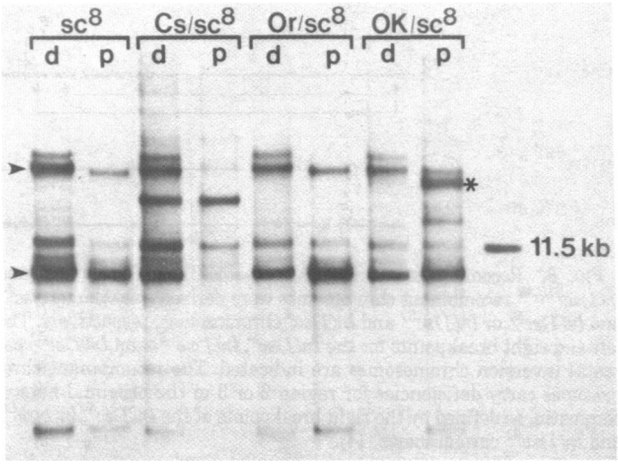

FIG. 2. Dominance of the *In(1)sc*^{8} chromosome with three wild-type X chromosomes. DNA from diploid (d) or polytene (p) tissue was prepared, digested with *Eco*RI, and analyzed by Southern blot analysis. The diploid tissue in each case was larval brains and imaginal discs, and the polytene tissue was larval salivary glands. DNA in lanes labeled *sc*^{8} was from homozygous females. Arrows mark the positions of the major *sc*^{8} ribosomal gene repeats. The Cs/*sc*^{8} polytene lane shows the Cs polytene rDNA pattern, while the Or/*sc*^{8} polytene lane shows the *sc*^{8} polytene pattern. The OK/*sc*^{8} polytene pattern shows both the *sc*^{8} and OK polytene rDNA patterns; the major band just below the 11.5-kb marker is from the *sc*^{8} chromosome, while the band marked with an * is from the OK chromosome.

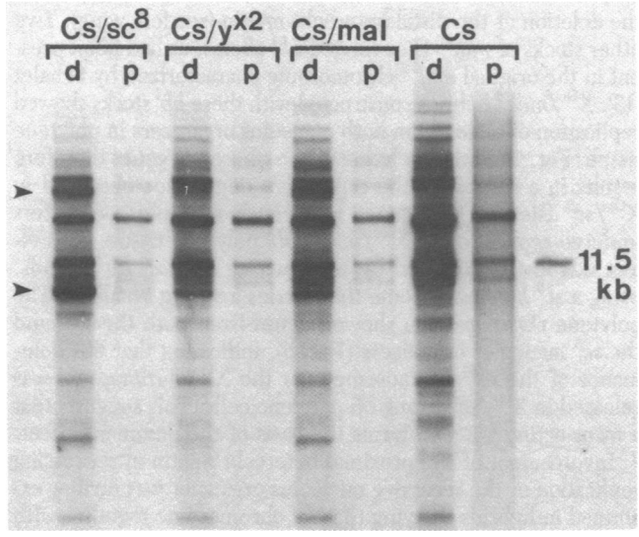

FIG. 3. Effect of the y^{x2} and mal^{12} deletions on dominance of the Cs chromosome in Cs/*sc*^{8} flies. Southern blot analysis of DNA from diploid (d) or polytene (p) larval tissues after digestion with *Eco*RI. DNA in lanes labeled Cs is from homozygous X/X females. The polytene patterns of the Cs/y^{x2} and Cs/mal^{12} DNAs are identical to the Cs polytene rDNA pattern. The arrows indicate the positions of the major *sc*^{8} ribosomal gene repeats.

imal heterochromatin from the recessive chromosome has no effect on the ability of the dominant chromosome to express its dominance.

The effect of deleting the distal or proximal heterochromatin from the dominant chromosome was examined by constructing X^{Or}/mal^{12} and X^{Or}/y^{x2} flies. The X^{Or}/y^{x2} hybrid showed little or no effect due to the deletion of the proximal heterochromatin (Fig. 4). X^{Or}/mal^{12} hybrids showed different effects, depending on the stock that was used as a source of the mal^{12} chromosome. This differential effect was determined to be due to a difference at the *bb* locus. One stock of mal^{12} flies carried a weak *bb* allele. In X^{Or}/mal^{12} hybrids using this stock as a source of the mal^{12} chromosome, the mal^{12} rDNA pattern was the predominant pattern observed in polytene cells (Fig. 4). This indicates that deletion of the distal heterochromatin, including cr^{+}, from the dominant chromosome has only a small effect on the ability of the dominance chromosome to express its dominance. The small amount of replication attributable to Oregon R ribosomal genes may be due to the lower number of ribosomal genes on the mal^{12} weak *bb* chromosome rather than to

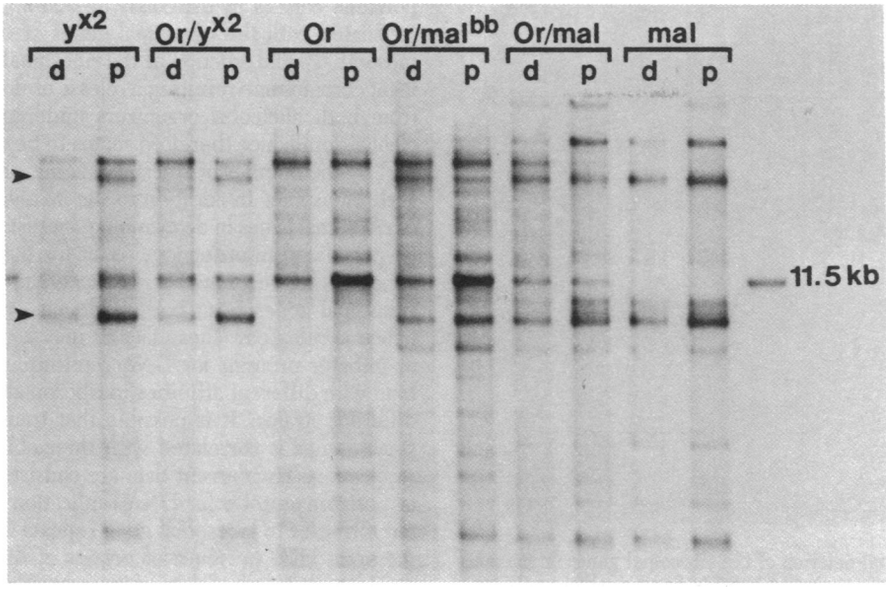

FIG. 4. Effect of the y^{x2} and mal^{12} deletions on dominance of the *sc*^{8} chromosome in Or/*sc*^{8} hybrids. Southern blot analysis of DNA from diploid (d) or polytene (p) larval tissues after digestion with *Eco*RI. The lanes labeled y^{x2} and *mal* contain DNA from $sc^{4,8}/y^{x2}$ or $sc^{4,8}/mal^{12}$ adult brains (d) or Malpighian tubules (p). The lanes labeled *mal* and Or/*mal* contain DNA from flies carrying the mal^{12} weak *bb* chromosome. The lanes labeled Or/mal^{bb} contain DNA from flies carrying the mal^{12} bb^{l} chromosome. DNA in lanes labeled Or is from homozygous X/X females. The positions of the major *sc*^{8} ribosomal gene repeats are indicated by arrows.

the deletion of the distal heterochromatin (see following). Two other stocks of mal^{12} flies carried bb^{l} alleles, as had been present in the original mal^{12} chromosome characterized by Schalet (13). X^{Or}/mal^{12} hybrids constructed with these bb^{l} stocks showed replication of genes from both nucleolus organizers in polytene tissue (Fig. 4). Partial deletion of the ribosomal genes therefore results in a release of the replicative dominance observed in X^{Or}/sc^{8} flies so that genes from both nucleolus organizers undergo replication in X^{Or}/mal^{12} bb^{l} polytene tissue. This effect was further verified by constructing X^{Or}/sc^{8} *bb* hybrids, using a sc^{8} *bb* chromosome that carries a strong *bb* allele. The polytene rDNA pattern showed genes from both the X^{Or} and the sc^{8} nucleolus organizers (Fig. 5), indicating that the dominance of the sc^{8} chromosome over the X^{Or} chromosome was released in X^{Or}/sc^{8} strong *bb* polytene cells. This suggests that a *trans*-acting function forms the basis of the dominant effect.

Involvement of the proximal heterochromatin in repressing replication of the recessive nucleolus organizer was further examined in hybrids carrying the X^{Or} chromosome together with a $sc^{L8L,S1R}$ or $sc^{S1L,8R}$ recombinant X chromosome (14). The $sc^{L8L,S1R}$ chromosome carries a deficiency for the heterochromatic region that lies between the right sc^{L8} and sc^{S1} breakpoints, whereas the $sc^{S1L,8R}$ chromosome is deficient for the region of proximal heterochromatin that lies between the right breakpoints of sc^{S1} and sc^{8} (Fig. 6). The $X^{Or}/sc^{L8L,S1R}$ hybrid showed a release of the dominance observed in X^{Or}/sc^{L8} polytene tissue. When the $sc^{L8L,S1R}$ chromosome was tested for *bb*, it was found to carry a bb^{l} allele, again suggesting that the release in dominance was due to a deletion of ribosomal genes. The effect of deleting the heterochromatic region between the right sc^{L8} and sc^{S1} breakpoints was not tested independently of the ribosomal gene deletion. There appeared to be no effect of the $sc^{S1L,8R}$ deletion on the dominance of the sc^{S1} chromosome observed in X^{Or}/sc^{S1} polytene tissue, indicating that the deleted region of heterochromatin is not involved in maintaining

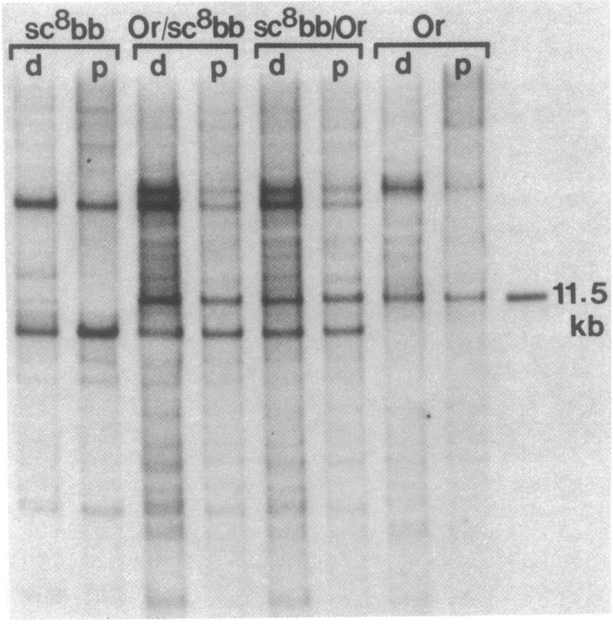

FIG. 5. Effect of partial deletion of the ribosomal genes on dominance of the sc^{8} chromosome on Or/sc^{8} hybrids. Southern blot analysis of DNA from diploid (d) or polytene (p) larval tissues after digestion with *Eco*RI. The lanes labeled sc^{8} *bb* and Or contain DNA from homozygous females. The lanes labeled Or/sc^{8} *bb* and sc^{8} *bb*/Or contain DNA from progeny of reciprocal crosses in which the first chromosome denoted is from the female parent. The Or/sc^{8} *bb* and sc^{8} *bb*/Or polytene lanes show both the Or and sc^{8} *bb* rDNA patterns.

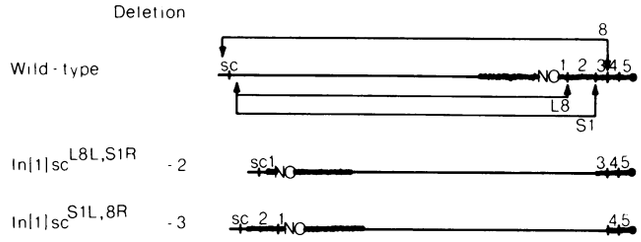

FIG. 6. Recombinant inversion chromosomes. The $In(1)sc^{L8L,S1R}$ and $In(1)sc^{S1L,8R}$ recombinant chromosomes were derived from the $In(1)sc^{L8}$ and $In(1)sc^{S1}$ or $In(1)sc^{S1}$ and $In(1)sc^{8}$ chromosomes, respectively. The left and right breakpoints for the $In(1)sc^{8}$, $In(1)sc^{L8}$, and $In(1)sc^{S1}$ parental inversion chromosomes are indicated. The recombinant chromosomes carry deficiencies for region 2 or 3 of the proximal heterochromatin, as defined by the right breakpoints of the $In(1)sc^{8}$, $In(1)sc^{L8}$, and $In(1)sc^{S1}$ chromosomes (14).

nucleolar dominance. This result is consistent with the observation that there is little or no effect of deleting the proximal heterochromatin in X^{Or}/y^{x2} polytene tissue.

A backcross experiment was carried out to determine whether dominance factors were limited to the X chromosome or were present on the autosomes. After the X^{Cs} chromosome had been backcrossed into a background of chromosomes from the recessive sc^{8} strain for five generations, DNA from diploid and polytene tissue was prepared from 20 female individuals and analyzed. In each case in which both the X^{Cs} and sc^{8} rDNA patterns were observed in DNA from diploid tissue, only the X^{Cs} rDNA pattern was observed in the corresponding polytene tissue (10 out of the 20 samples). Similar results were obtained when the X^{Cs} chromosome was backcrossed through four generations into a background of chromosomes from the X^{Or} strain. These results suggest that factors that mediate replicative dominance in polytene cells are located on the X chromosome and that there is little or no involvement of autosomal factors.

DISCUSSION

These results provide evidence that nucleolar dominance in polytene cells is mediated by an activity that is located in or associated with the ribosomal genes of the dominant X chromosome. Partial deletion of the ribosomal genes from the dominant chromosome results in a release of dominance so that genes from both nucleolus organizers undergo polytenization. Nucleolar dominance therefore seems to be determined at least in part by the number of ribosomal genes present on a given X or Y chromosome. In addition to the involvement of the number of ribosomal genes in determining dominance, other factors must be postulated in order to account for the under-replication of genes in the "recessive" nucleolus organizer and replication of ribosomal genes from both nucleolus organizers when dominance is released. These factors may consist of repressor-like or initiator proteins for DNA replication or transcription that bind with different affinities to ribosomal gene sequences from different strains. It is possible that transcription of the ribosomal genes is correlated with their ability to undergo DNA replication. The current data are consistent with the presence of initiator proteins for DNA replication that bind with different affinities to ribosomal gene repeats from different X chromosomes. The presence of origins of different strengths that reside on different X chromosomes would explain the dominant effect and the release of dominance in flies that are bobbed for the dominant chromosome.

The results presented here demonstrate that the heterochromatic regions covered by the y^{x2} and mal^{12} deletions have little or no effect on either the expression of nucleolar domi-

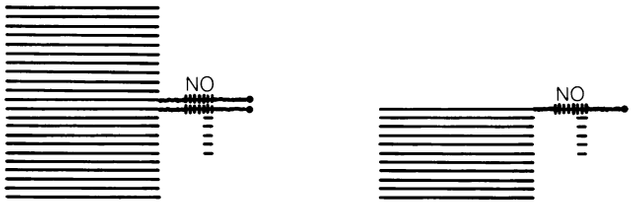

FIG. 7. Replication of the ribosomal genes in X/X (*Left*) and X/0 (*Right*) polytene cells. The lines represent DNA double helices. The thicker lines show the nonreplicating heterochromatic sequences that flank the nucleolus organizer (NO) and lie adjacent to the centromere (●). The figure shows three features of ribosomal gene polytenization: (*i*) nucleolar dominance, (*ii*) under-replication, and (*iii*) differential replication. The number of rounds of DNA replication that the ribosomal genes undergo is not certain because the proportion of genes that polytenize is not known.

nance or the ability of the ribosomal genes in the deficiency chromosomes to undergo replication. This conclusion is supported by analysis of $X^{Or}/sc^{S1L,8R}$ hybrids, which shows that heterochromatic region 3 is not involved in the expression of nucleolar dominance.

The results from X^{Or}/mal^{12} and X^{Cs}/mal^{12} hybrids carrying the mal^{12} weak *bb* chromosome suggest that deletion of the cr^+ locus has little effect on the dominance observed in polytene cells, although these hybrids, which are cr^+/cr^-, appear to undergo ribosomal gene compensation. This observation indicates that compensatory replication and nucleolar dominance are under the control of different regions of the X chromosome and suggests that ribosomal gene compensation is probably not explained by nucleolar dominance in polytene cells.

Features of ribosomal gene replication in polytene cells are shown in Fig. 7. This figure is modified from Fig. 2 of Spear and Gall (4) and summarizes information reported previously (1, 2, 4, 15) and in this paper. Three general features of ribosomal gene replication in polytene cells of *D. melanogaster* are the following. First, in hybrid X/X and X/Y cells as well as in wild-type X/Y cells, ribosomal genes from only one nucleolus organizer undergo polytenization. This may also be true of wild-type X/X cells that are homozygous for a given X chromosome, but this has not yet been formally demonstrated. Replication of genes from only one nucleolus organizer involves a mechanism that is mediated by the number of ribosomal genes and by factors associated with the ribosomal genes of the dominant chromosome. A second feature of ribosomal gene polytenization is that the ribosomal genes are under-replicated with respect to the euchromatic sequences. This is true even when replication of genes from only one nucleolus organizer is taken into account, so that the ribosomal genes undergo the equivalent of 6–7 rounds of DNA replication, in contrast to the 9–10 rounds of the euchromatic sequences. The actual number of rounds of DNA replication that the ribosomal genes undergo is not certain because the number of genes that polytenize is not known. A third feature is that the ribosomal genes present in a given nucleolus organizer are not evenly replicated, but certain gene repeats are differentially replicated. In the cases of the Oregon R and Canton S X chromosomes, the genes which are preferentially replicated are intron$^-$ repeats (ref. 1; unpublished data). This supports the suggestion that replication may be linked to transcription, because intron$^-$ genes are expected to be transcriptionally active, in contrast to intron$^+$ genes, which are transcribed to low levels and are also under-replicated. Differential replication of the ribosomal genes and under-replication of the heterochromatic sequences that surround the nucleolus organizer region suggest that this region of the polytene chromosome must contain an unusual molecular structure. This region of the chromosome may be similar molecularly to the amplified chorion protein genes (16), which to date provide the only molecular data for the structure of differentially replicated chromosomal DNA regions.

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