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ANALYSIS OF Y CHROMOSOME NUCLEOLAR ORGANIZER MUTANTS IN DROSOPHILA MELANOGASTER

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Five Y chromosome nucleolar organizer (Y-NO) mutants were analyzed with respect to their rRNA gene numbers, phenotypes and additivity tests with other NO mutants. Four of these are indicative of a class of mutants in which most of the rRNA genes are transcribing functional rRNA. The other mutant has 80 genes, however, lethality and additivity tests suggests that many if not all of these rRNA genes are non-functional. The basis for the observed suppression of rRNA genes of the Y-NO region is discussed.

INTRODUCTION

In Drosophila melanogaster, the nucleolus organizer region (NO) contains tandemly repeating units which code for 18S and 28S ribosomal RNA (rRNA). There are two NO regions, one localized to the proximal heterochromatin of the X chromosome (Xh) and the other on the heterochromatic short arm of the Y chromosome (1,2). The bobbed (bb) syndrome in the fruit fly has a phenotype of short, thin bristles, abdominal etching and a delayed developmental time. Different bb alleles manifest different phenotypes -- from early developmental lethals to non-etched adult flies with slightly thinner than wild type bristles. The NO region, 18 + 28S rRNA genes and bb alleles are synonymous (3,4). The bb alleles have been shown to be deletions of various sizes within the NO region and thus suggests that less rDNA results in less rRNA, reduced number of ribosomes and therefore a decreased rate of protein synthesis. The larger the deletion, the greater severity of the bobbed phenotype. This report describes five Y-NO mutants with respect to their phenotypes, rRNA gene numbers and additivity tests with other X-NO mutants.

MATERIALS AND METHODS

D. melanogaster stocks:

D. melanogaster cultures were raised at 25°C on a standard cornmeal, agar and yeast medium. A complete description of Drosophila genetic terminology is given in LINDSLEY and GRELL (5). The particular chromosomes used in this study were: X-- a wild type

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Oregon-R chromosome carrying one NO region maintained in an isogenic strain of flies; C(1)DX, yf -- a reversed acrocentric compound X chromosome deficient for both NO regions; In(1)$sc^{4L}sc^{8R}$, y $sc^{4}sc^{8}$cv v B -- an inverted X chromosome with a deletion for all of the DNA in the X heterochromatin between the sc^{4} and sc^{8} breakpoints including the NO region. This chromosome is referred to as sc^{4} sc^{8} in the text; C(1)RM, y -- a reversed metacentric compound X chromosome carrying one NO region; $Y^{S}X{\cdot}Y^{L}$, In (1) EN -- an attached X and Y chromosome having only one NO region, designated as XY in the text; bb^{2rl} -- an X chromosome in which the number of rRNA genes has been reduced to a lethal level. This is an internal deletion for about 80% of the rRNA genes of the X-NO region leaving the heterochromatic regions adjacent to rDNA intact, (6); $y^{2}bb^{8}$ -- an X chromosome with about one half the wild type number of rRNA genes (101), generated by a reduction event (7). Flies having one dose of the bb^{8} allelle are severely bobbed and this chromosome is referred to as bb^{8} in the text; B^{S} Y $bb^{+}y^{+}$ -- a Y chromosome carrying one NO region, B^{S} eye mutation and y^{+} allele.

Isolation and lethal tests of Y-NO mutants:

Five ethyl methanesulfonate induced Y-NO mutants were derived from the wild-type NO region of the B^{S} Y bb^{+} y^{+} chromosome (8). The Y-NO mutant chromosomes are maintained in stock as (XY) $Y^{S}X{\cdot}Y^{L}$, In (1) EN/B^{S} Y bb y^{+} males and (XX) C (1) R M, y /B^{S} Y bb y^{+} females. They were tested for the presence of an intact Y-NO by viability studies of C (1) DX/B^{S} Y bb y^{+} females and sc^{4} sc^{8}/B^{S} Y bb y^{+} males. Since the C(1)DX and $sc^{4}sc^{8}$ chromosomes contain no rDNA, a mutation in the Y-NO of the B^{S} Y bb^{+} y^{+} chromosome will give a phenotype range from lethality to mild bobbness. Males, XY/B^{S} Y bb y^{+} were mated to C (1) DX/B^{S} Y $bb^{+}y^{+}$ females and the phenotype of F1 C (1) DX/B^{S} Y bb y^{+} daughters recorded. Similarly, C (1) RM/B^{S} Y bb y^{+} females were mated to sc^{4} sc^{8}/B^{S} Y bb^{+} y^{+} males and the phenotype of F1 sons recorded.

Additivity tests:

The two X-NO mutants used in this study were the bb^{2rl} and bb^{8} chromosomes. The phenotype of flies having one dose of these mutant X-NO chromosomes was determined by mating bb^{2rl}/B^{S} Y bb^{+} y^{+} and bb^{8}/B^{S} Y bb^{+} y^{+} males to C (1) R M/O females and observing the F1 sons. Additivity tests of the five Y-NO mutants with the two X-NO mutants were carried out. Males of the bb^{2rl}/B^{S} Y bb^{+} y^{+} genotype were mated to C (1) R M/B^{S} Y bb y^{+} females and the bb^{2rl}/B^{S} Y bb y^{+} sons phenotypes recorded. Identical crosses using the bb^{8}/B^{S} Y bb^{+} y^{+} males were also made, recording the bb^{8}/B^{S} Y bb y^{+} male phenotypes.

DNA-rRNA hybridization:

Saturation hybridization experiments for estimating the number of 28 + 18S repeating units can be accomplished quickly while using a small quantity of flies. About 2.0 grams of frozen adult flies (-70°C) are homogenized in 20 ml of 10 mM $MgCl_2$, 50 mM Na acetate, 150 mM KCl, pH 6.0 at 4°C. To this mixture, 2.2 ml of 10X lysis solution (0.1M EDTA, pH 8.0, 2% sodium dodecyl sulfate, 0.5 M sodium perchlorate and 0.15 M NaCl) and 20 ml of water-saturated phenol:chloroform (v/v = 1) containing 0.1% (w/v) 8-hydroxyquinoline are added. The homogenate is shaken for 10 minutes and centrifuged. The DNA in the aqueous layer is precipitated with 1 volume of 100% ethanol. The DNA is dissolved in 2 ml of MUP [8 M urea and 0.24 M phosphate buffer (PB), pH 6.8] and loaded onto a small hydroxyapatite column (0.50 g Bio Rad HTP in 0.24 M PB). The column is washed with 15 ml of MUP and then with 10 ml of 0.014 M PB. After these washes, only the DNA remains bound to the column and is eluted by 0.40 M PB.

The DNA is denatured in 0.5 M NaOH for 10 minutes at room temperature. The solution is neutralized with HCl and the DNA bound to nitrocellulose BA85 25 mm filters by gravity filtration. The filters are dried and baked at 80°C in vacuo for 2 hours. The amount of DNA bound per filter is determined after the hybridization procedure. Filters are incubated in 2X SSC (SSC is 0.15M NaCl, 0.015M Na citrate, pH 7.0) at 60°C for 3 hours with saturation amounts of (^{3}H) 28 + 18S rRNA. The filters are washed in 2X SSC, RNased (20ug/ml, 1 hour at 37°C), washed, and counted in Beckman Ready-Solv ™NA. The number of background counts was estimated by using filters treated in an identical manner but containing no DNA. These control values were less than 20 cpm. The isolation of (^{3}H) 28 + 18S rRNA (45,000 cpm/ug) has previously been described by TARTOF (7). After counting, the filters are washed with chloroform, dried, and placed in 5% perchloric acid (9). The DNA on the filter is hydrolyzed for 30 minutes at 70°C, along with salmon sperm DNA standards treated in the same way. The DNA is quantified by reading the OD_{260}nm. There

TABLE 1

The number of rRNA genes of various chromosomes

Genotype	Percent DNA hybridized ± SE	RNA gene number Total	X or $\overline{XY}$ or C(1)RM chromosome	Y chromosome
A.				
X/X	.40 ± .0060	456	228	-
X/Y	.38 ± .0172	434	228	206
$sc^4sc^8/B^SY\ bb^+\ y^+$	.19 ± .0085	216	0	216
B.				
$\overline{XY}$/O	.22 ± .0022	251	251	-
$\overline{XY}/B^S\ Y\ bb^+y^+$	.41 ± .0022	467	251	216
$\overline{XY}/B^S\ Y\ bb^1y^+$	.32 ± .0044	365	251	114
$\overline{XY}/B^S\ Y\ bb^2y^+$	.30 ± .0038	342	251	91
$\overline{XY}/B^S\ Y\ bb^{1-3}y^+$	.28 ± .0139	320	251	69
$\overline{XY}/B^S\ Y\ bb^{1-4}y^+$	.28 ± .0022	320	251	69
$\overline{XY}/B^S\ Y\ bb^{1-5}y^+$	.31 ± .0077	354	251	103
C.				
C(1)RM/O	.24 ± .0135	274	274	-
$C(1)RM/B^S\ Y\ bb^+y^+$	.43 ± .0022	490	274	216
$C(1)RM/B^S\ Y\ bb^1y^+$	.35 ± .0070	400	274	126
$C(1)RM/B^S\ Y\ bb^2y^+$	.35 ± .0041	400	274	126
$C(1)RM/B^S\ Y\ bb^{1-3}y^+$	.29 ± .0041	331	274	57
$C(1)RM/B^S\ Y\ bb^{1-4}y^+$	.32 ± .0040	365	274	91
$C(1)RM/B^S\ Y\ bb^{1-5}y^+$	.32 ± .0010	365	274	91

is no loss of DNA from the filter during the chloroform treatment. The advantage of this procedure is that we routinely experience DNA loss during the hybridization reaction. This error is circumvented by quantitating the DNA on the filter after the hybridization reaction. Since the Y chromosome comprises about 10% of the total genome, the percent rDNA in genomes with an extra Y chromosome was multiplied by 1.1. The multiplicity of rRNA genes in the NO region can be estimated using the molecular weights of the D. melanogaster genome and 28 + 18S rRNA as 2.4×10^{11} and 2.1×10^6 daltons, respectively (10).

RESULTS

Measuring rDNA content using non-compensating chromosomes:

An X chromosome NO can increase its rDNA content under certain genetic conditions. When there is only a single NO in the genome, as X/O males or X/sc^4sc^8 females, the rDNA multiplicity of the NO region increases almost two fold, referred to as compensa-

TABLE 2

The phenotype and rRNA gene number of Y-NO and X-NO bb mutants

GENOTYPE	PHENOTYPE	NUMBER rRNA GENES PER NO
C(1)DX/$B^{s}Y$ $bb^{1}y^{+}$	extreme bb adult	120
C(1)DX/$B^{s}Y$ $bb^{2}y^{+}$	extreme bb adult	109
C(1)DX/$B^{s}Y$ $bb^{1-3}y^{+}$	early-late pupal lethal	63
C(1)DX/$B^{s}Y$ $bb^{1-4}y^{+}$	egg-early larvae lethal	80
C(1)DX/$B^{s}Y$ $bb^{1-5}y^{+}$	late pupal lethal	97
bb^{2rl}/0	egg-larvae lethal	46*
bb^{8}/0	extreme bb adult	101*

* The bb^{2rl} and bb^{8} gene numbers measured under non-compensating conditions (6,7)

tion (6,11). Thus when determining rDNA content of a particular sex chromosome, one must place this chromosome opposite a non-compensating or stable sex chromosome. For measuring the gene number of particular Y chromosomes, one can use an $\overline{XY}$ or C (1) R M chromosome in which both are refractory to compensation (Table 1). The rRNA gene numbers of the five mutant Y-NO chromosomes was measured by placing these chromosomes opposite both the $\overline{XY}$ and C (1) R M chromosomes. The rDNA content for each of the five Y-NO mutants was virtually the same whether measured in $\overline{XY}$ males or C (1) R M females. The two values were averaged to give 120 genes for the bb^{1} allele, 109 for bb^{2}, 63 for bb^{1-3}, 80 for bb^{1-4} and 97 for bb^{1-5} (Table 2).

Lethality tests of the Y-NO mutants:

The bobbed phenotype can range from an egg lethal (12) to a mild bobbed adult fly depending upon the number of rRNA genes present in the genome. The classifications used in this study were: (1) mild bobbed as adult flies with no abdominal etching, thin bristles (2) extreme bobbed as adult flies with severe abdominal etching, short, thin bristles and (3) bobbed lethal as the absence of adult flies, the lethal phase occurring sometime during the egg, larval, and pupal stages. The bb^{1} and bb^{2} alleles have 120 and 109 genes, respectively and their phenotypes are both extreme bobbed (Table 2). Thus there is enough rRNA genes in these Y-NO mutants for the organism to reach the adult stage. The bb^{1-3} allele (63 genes)

TABLE 3

Additivity tests of X-NO and Y-NO bb mutants

X-NO	Y-NO				
	bb^{1}(120)*	bb^{2}(109)	bb^{1-3}(63)	bb^{1-4}(80)	bb^{1-5}(97)
bb^{2rl}(46)	[+] (166)	[+] (155)	[extreme bb adults] (109)	egg-early larvae lethal (126)	[partially eclosed pupae] (143)
bb^{8}(101)	[+] (221)	[+] (209)	[+] (164)	extreme bb adults (181)	[+] (198)

* The rRNA gene number of bb mutants in parenthesis.
A rectangle encompassing the phenotype indicates additivity of the two alleles with the total rRNA gene numbers in parenthesis.

allows development up to the pupal stage. The bb^{1-4} allele has 80 rRNA genes, however, individuals having only this allele die very early during development. The bb^{1-5} allele has 97 genes and is a late pupal lethal (wings, eyes are formed). The phenotypes of the five bb alleles in $sc^{4}sc^{8}/B^{S}$ Y bb y^{+} males were essentially the same as recorded in C (1) DX females.

Additivity tests:

The two X chromosomes used in the additivity tests were the bb^{2rl} and bb^{8} which have 46 and 101 genes, respectively (6,7). Males of the genotype bb^{2rl}/O have an egg to early larval lethal phase. Males of the genotype bb^{8}/O reach the adult stage, but are extreme bobbed. The additivity results of these two X-NO mutants with the five Y-NO mutants are shown in Table 3. The phenotypes of individuals with either the bb^{2rl} and bb^{8} alleles are reverted to a wild type phenotype with the addition of the bb^{1} or bb^{2} alleles. Flies with about 160 rRNA genes are wild type. Flies with the bb^{2rl} allele are reverted from an egg-early larval lethal to a viable, but extreme bobbed adult upon the addition of the bb^{1-3} allele to the genome. As shown previously, (Table 2), flies with about 109-120 genes show an extreme bobbed phenotype. The bb^{8}/bb^{1-3} flies have 164 genes and are wild type. Similarly, the bb^{1-5} allele demonstrates additivity with either the bb^{2rl} or bb^{8}

alleles. These results suggest that most of the rRNA genes of the $\underline{bb^{1}}$, $\underline{bb^{2}}$, $\underline{bb^{1-3}}$ and $\underline{bb^{1-5}}$ alleles are functional. The $\underline{bb^{1-4}}$ allele has 80 genes, enough for development to the late pupal stage, however, it is an early lethal. Upon addition of this allele with the $\underline{bb^{2rl}}$ or $\underline{bb^{8}}$ alleles, there is no additive effects. The $\underline{bb^{1-4}}/\underline{bb^{2rl}}$ individuals have enough genes for development to adults, however, they die early. The $\underline{bb^{1-4}}/\underline{bb^{8}}$ flies should be wild type adults but have an extreme bobbed phenotype identical to that of $\underline{bb^{8}}/O$ males. The simplest explanation is that most of the rRNA genes of the $\underline{bb^{1-4}}$ allele are non-functional.

DISCUSSION

Wild type male and female flies have two NO regions with a total of about 456 rRNA genes. The X and Y-NO regions have about the same number of genes. One complete NO region, either the X or Y-NO, has enough rDNA for a wild type phenotype. Our results indicate that less than one Y-NO's worth of rDNA gives a bobbed phenotype. The $\underline{bb^{1}}$ (120 genes) and $\underline{bb^{2}}$ (109 genes) alleles allows development to the adult stage however these flies show a severe bb phenotype. The $\underline{bb^{1-3}}$ (63 genes) and $\underline{bb^{1-5}}$ (97 genes) alleles allows development to the pupal stage. The $\underline{bb^{1-4}}$ allele behaves phenotypically as having no rDNA despite the fact that rDNA saturation hybridization tests indicate the allele having a substantial number of rRNA genes. The 80 genes of this allele have enough rDNA to allow development to the pupal stage, however, early lethality is observed. In addition, the $\underline{bb^{1-4}}$ allele causes no additivity of either the $\underline{bb^{2rl}}$ or $\underline{bb^{8}}$ alleles. It is proposed that the 80 rRNA genes of this allele contribute insignificantly to the formation of functional rRNA. Since the basic Y-NO repeating unit is 1.2×10^{4} bp (base pairs) in length this amount of non-functional rDNA is quite large, 9.6×10^{5} bp (18). Reports of non-functional rDNA have been previously suggested however contrary evidence has been reported (13-17). This report describes a Y-NO bb lethal mutant in which the gene number measurements are done under conditions not distorted by rDNA compensation and the lethality of this mutant can only be due to the Y-NO rDNA since the only genes on the Y chromosome necessary for viability of the organism are rRNA genes.

Various explanations for a large number of non-functional tandemly redundant genes can be hypothesized. About 16% of the 28S genes of the Y NO (32 genes) are interrupted by an intervening sequence, these being the Type II inserts (18). Genes containing the Type II insert do not significantly contribute to functional 28S rRNA (19). There is a direct cor-

relation between the severity of the bobbed phenotype, the amount of rRNA synthesis (20), and the number of rRNA genes without an intervening sequence (21). Whether a significant proportion of the 80 inactive genes of the Y-NO bb^{1-4} allele is due to Type II inserts is unknown since the relative abundance of these inserts has not been determined. The B^S Y bb^+y^+ chromosome the origin of the bb^{1-4} allele, has few rRNA genes with intervening sequences (unpublished data). Position-effect variegation may cause the inactivation of a large number of genes (22). A change in the normal rDNA-Y heterochromatic border caused by the bb^{1-4} deletion would place different Y heterochromatin near the rRNA genes thus, leading to the bb^{1-4} phenotype. Further studies are being done to differentiate between these two hypothesis of rDNA suppression.

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