Genet. Res., Camb. (1984), **44**, *pp.* 351–357
With 2 text-figures
Printed in Great Britain

SHORT PAPER

Suppression of ribosomal RNA genes in *Drosophila melanogaster*

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(*Received 6 April 1984 and in revised form 6 August 1984*)

SUMMARY

The rDNA of five *Y* chromosome mutants was examined with respect to their insert free (In^-) repeat type multiplicity. The In^- repeat number of each mutant was correlated with its hemizygous *bobbed* phenotype and additivity with an *X* NO *bobbed* (*bb*) mutant. Four of these mutants showed a direct relationship between their In^- frequency, hemizygous *bb* phenotype and additivity tests. A fifth mutant, bb^{1-4}, had a sufficient number of In^- repeats to ensure viability to the late pupal stage and show additivity; however, the In^- repeats genetically behaved as a complete rDNA deletion. Possible mechanisms resulting in the suppression of the bb^{1-4} In^- repeats are discussed.

1. INTRODUCTION

The *Y* chromosome nucleolar organizer (NO) region located on the *Y* short arm (Y^S) contains about 200–220 repeating units which code for rRNA. About 85% of these units (In^-) have no insertion within the 28S coding region and the remainder are interrupted by a non-28S DNA type II insertion (Wellauer, Dawid & Tartof, 1978). Contrarily, a substantial portion of *X* NO repeats are interrupted within the 28S gene by type I or II insertions. Type I insertions are present in 50% of the *X* NO units and are subdivided into major (5 kilobases, kb) and minor (0·50, 1·0 kb) classes. Type II insertions are non-homologous to type I, are present in about 15% of *X* NO repeats and have a size range from 0·50–3·5 kb. There is a substantial body of evidence that all insert bearing repeats (In^+) do not significantly code for functional rRNA (Long & Dawid, 1979; Long *et al.* 1981; Kidd & Glover, 1981).

Wild type *X* and *Y* NO regions (bb^+) have the full complement of repeats. When either NO region is genetically in the hemizygous condition, adult flies are phenotypically normal. A *bobbed* (*bb*) phenotype results from hemizygous deletions of various sizes within the NO region (Ritossa, Atwood & Spiegelman, 1966). In general, for *Drosophila melanogaster*, the larger the deletion the greater the severity of the *bobbed* phenotype – from early developmental lethals to non-etched adult flies with slightly thinner than wild type bristles. The *bb* phenotype has a direct correlation with the rate of rRNA accumulation (Shermoen & Kiefer, 1975) and the number of In^- repeats (Franz & Kunz, 1981). The rRNA repeats have an additive effect, the severity of the *bobbed* phenotype dependent upon the total number of In^- rDNA repeats carried on both *X* and *Y* chromosomes.

Reports of non-functional rDNA have been previously suggested. Baker (1971) and Nix (1973) proposed the suppression of rDNA repeats when the *X* NO was moved to the tip of the *X* chromosome. Contrary to this, Johnson, Harger & Holm (1979) suggested that the lethality of the $In(1)sc^{S1}$ and $In(1)sc^{L8}$ chromosomes is not due to rDNA

suppression but to the suppression of other vital euchromatic genes near the transposed block of heterochromatin. Ritossa (1968) reported the Y-NO *bobbed* lethal mutant (Ybb^-) contained nearly the wild type number of rRNA repeats which were apparently inoperative. Subsequent measurements of the rDNA multiplicity of this particular mutant demonstrated a low number of rRNA repeats (Tartof, 1973). Marrakechi & Prud'homme (1971) suggested non-functional *X*-NO rDNA for their *bb* mutants; however, the In^- repeat frequency was not measured. We provide evidence of suppression for a substantial number of In^- rDNA repeats. In^- repeats are considered to code for functional rRNA. The In^- repeats of a *Y* chromosome mutant, bb^{1-4}, are inoperative.

2. MATERIALS AND METHODS

(i) *Stocks*

D. melanogaster cultures were raised at 25° on a standard cornmeal, agar and yeast medium. A complete description of *Drosophila* genetic terminology is given in Lindsley & Grell (1968). The particular chromosomes used in this study were – (a) *C(1)DX, yf*, a reversed acrocentric compound *X* chromosome deficient for both NO regions (b) $In(1)sc^{4L}sc^{8R}$, *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, referred to as sc^4 sc^8 in the text (c) *C(1)RM, y*, a reversed metacentric compound *X* chromosome carrying rDNA (262 repeats) which is refractory to rDNA compensation (Procunier & Tartof, 1978) (d) y^2 bb^8, an *X* chromosome with about one half the wild type number of rRNA repeats (101), generated by a reduction event and referred to as bb^8 (Tartof, 1973). Flies having one dose of the bb^8 allele are severely *bobbed* (e) B^S Ybb^+y^+, a *Y* chromosome carrying one NO region, B^S eye mutation and y^+ allele and (f) B^S $Ybby^+$, a derivative of the B^S Ybb^+y^+ which carries a *bobbed* allele (*bb*).

(ii) *Characterization of Y mutants*

The phenotype, total number of rDNA repeats and additivity tests for the five Y mutants have been previously described (Gandhi, Sharp & Procunier, 1982) and the results represented in Table 1. The intensity of the *bobbed* phenotype was classified as (1) mild *bobbed*, adult flies with thin bristles, no abdominal etching, (2) extreme *bobbed*, adult flies with short, thin bristles and severe abdominal etching and (3) *bobbed* lethal, the absence of adult flies, the lethal phase occurring sometime during development.

(iii) *Southern transfers*

The procedures for DNA extraction, restriction endonuclease digestion, Southern transfer, hybridization, autoradiography and scanning have been previously described (Sharp, Gandhi & Procunier, 1983; Kalumuck & Procunier, 1984). The filters were hybridized with ^{32}P nick-translated plasmids pBW416 and 423 (10^8 cpm/μg). Together, they contain essentially an entire In^- repeat unit plus about 250 base pairs of the type I insertion (Fig. 1).

3. RESULTS AND DISCUSSION

EcoRI and *BamHI* digests of *X/X* and *X/Y* rDNA in our laboratory showed that the percentages of the various repeat types are in close agreement with the published values (Sharp, Gandhi & Procunier, 1983; Kalumuck & Procunier, 1984). In addition, there was no significant difference in repeat type multiplicity between whole flies and the diploid

Table 1. *The phenotype, In⁻ repeat number and additivity tests of Y-rDNA bb mutants*

Genotype	Total no. repeats*	In⁻ (12 kb) percentage†	Total no. In⁻ repeats	*Y*-rDNA In⁻ repeats	Phenotype‡	Additivity with *X*-NO bb^8 allele
C(1)RM/0	262	23 ± 0·35	60	0	Wild type adult	—
$C(1)RM/B^S\ Ybb^1y^+$	382	39 ± 1·8	149	89	Extreme *bb* adult	Wild type adult
$C(1)RM/B^S\ Ybb^2y^+$	371	38 ± 4·0	141	81	Extreme *bb* adult	Wild type adult
$C(1)RM/B^S\ Ybb^{1-3}y^+$	325	37 ± 3·6	120	60	Late pupal lethal	Wild type adult
$C(1)RM/B^S\ Ybb^{1-4}y^+$	342	36 ± 2·1	123	63	Egg-early larvae lethal	Extreme *bb* adult
$C(1)RM/B^S\ Ybb^{1-5}y^+$	359	35 ± 1·8	126	66	Late pupal lethal	Wild type adult
$C(1)RM/B^S\ Ybb^+y^+$	476	54 ± 1·4	257	197	Wild type adult	Wild type adult

* The total number of repeats determined from Gandhi, Sharp & Procunier (1982).

† The In⁻ frequency determined by densitometric scanning of autoradiographs represented by Fig. 2. Measurements were obtained from at least four separate autoradiographs. The means and standard errors are given.

‡ The phenotype of the *bb* alleles (hemizygous condition) and additivity tests have been previously described (Gandhi, Sharp & Procunier, 1982).

tissue of 3rd instar larvae brains (unpublished data). This showed that the restriction pattern from whole adult tissue is not distorted by the small number of adult polytene cells (Ashburner, 1970) where underreplication of In^+ repeats occurs (Endow & Glover, 1979). Thus, easier handling and identification of specific genotypes was accomplished by using whole adult flies.

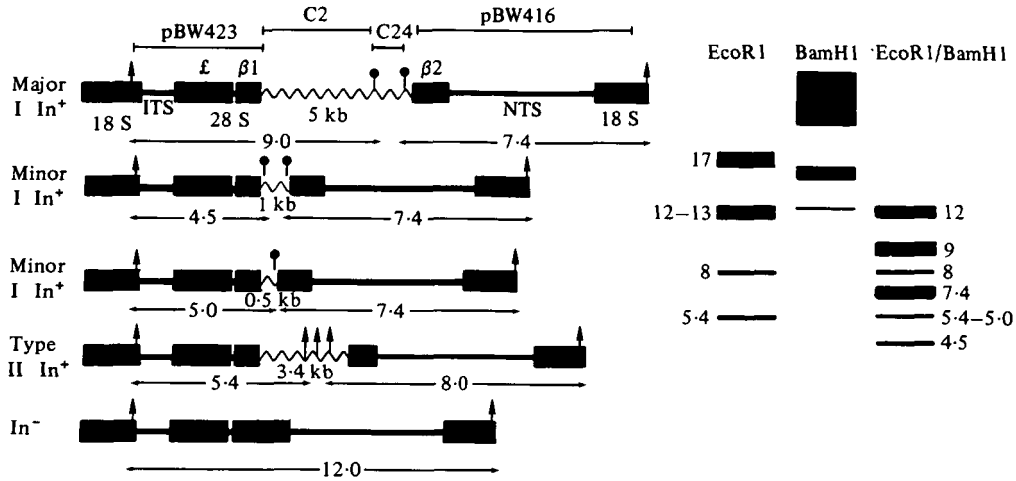

Fig. 1. *D. melanogaster* rDNA repeat unit types. The three length variants of type I insertion repeat units (5·0, 1·0 and 0·50 kb inserts) and type II repeats (3·4 kb) are shown. The inserts are within the 28S coding region. ITS is internal transcribed spacer, NTS is non-transcribed spacer. The sites for the restriction enzymes *EcoRI* (↑) and *BamH1* (🡅) are shown. Fragment lengths (kb) generated by single digestion with *EcoRI* or *BamH1* and double digestion with *EcoRI/BamH1* are illustrated. Hybridization probes are shown above the repeats.

Double digestion of rDNA with *EcoRI* and *BamH1* (Figs. 1 and 2) leaves a 12 kb band composed solely of In^- repeats. The major type I repeats are cleaved into a 5′ end of 9·0 kb and a 7·4 kb 3′ end; the minor type I repeats are cleaved into 7·4 kb 3′ ends and 5′ ends of about 4·5 and 5·0 kb in length. The type II In^+ repeat bands remain at 8·0 and 5·4 kb. The tandemly repeated, sequence and length heterogeneous population of non-nucleolar type I elements are also cleaved by *BamH1* to give fragments between 4 and 5·5 kb in length (Kidd & Glover, 1980). These constituted less than 5% of the hybridizing material with the pBW416 and 423 probes used. The percent of In^- repeats for the various genotypes (Table 1) was determined by scanning the autoradiographs represented by Fig. 2. The total number of rDNA repeats for each *Ybb* mutant was previously determined in two different non-compensating genotypes by saturation hybridization (Gandhi, Sharp & Procunier, 1982). The attached-*X* chromosome *C(1)RM* was used to measure the frequency of the various repeat units. The *C(1)RM* rDNA values are stable under rDNA compensating conditions (Procunier & Tartof, 1978) and individuals with *Y* chromosome *bb* lethal alleles are viable as *C(1)RM/bb*. Estimation of In^- multiplicity of various *Y* chromosomes was done simply by subtracting the *C(1)RM*/O value (% non-insert × total repeat number) from the $C(1)RM/B^S\ Ybby^+$ number (Table 1). *C(1)RM*/O females have about 60 In^- repeats (23% of rDNA total) and are wild type. $C(1)RM/B^S\ Ybb^+y^+$ females showed a substantial increase in In^- repeats (197) due to the presence of the *Y* chromosome. This was expected since a normal *Y* chromosome is predominantly In^- repeats (Wellauer *et al.* 1978). However, the rDNA of this marker *Y* chromosome, $B^S\ Ybb^+y^+$, does contain a few major type I repeats (8%) seen as a 17 kb band in *EcoRI* digests of $sc^4sc^8/B^S\ Ybb^+y^+$ males (unpublished observations). These

https://doi.org/10.1017/S0016672300026586 Published online by Cambridge University Press

presumably are *X*-NO major type I repeats translocated onto the marker *Y* chromosome during its genetic construction. Despite these few foreign type I In^+ repeats, the vast majority (> 90%) of the $B^S\ Ybb^+y^+$ rDNA repeats are of the In^- class (Table 1). The rDNA of the $B^S\ Ybb^+y^+$ chromosome has about 197 In^- repeats and a wild type phenotype in the hemizygous condition ($C(1)DX/B^S\ Ybb^+y^+$ or $sc^4\ sc^8/B^S\ Ybb^+y^+$). About a 50% reduction of *Y* rDNA In^- repeats, to 89–81, gives an extreme *bobbed* adult in the

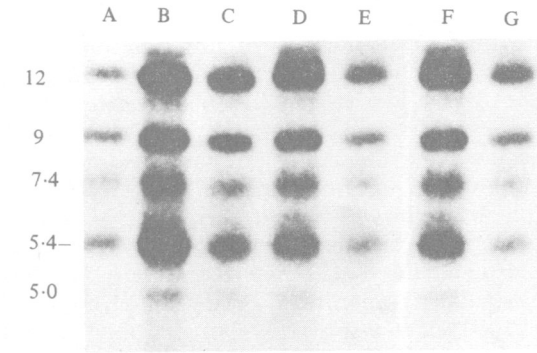

Fig. 2. Autoradiographic patterns of $C(1)RM/B^S\ Ybby^+$ genotypes. DNA from adult females was double digested with *EcoRI/BamH1* and electrophoresed in 0·8% agarose gels for 36–48 h, Southern blotted and hybridized to ^{32}P-pB416 and 423. (A) $C(1)RM/0$ (B) $C(1)RM/B^S\ Ybb^1y^+$ (C) $C(1)RM/B^S\ Ybb^2y^+$ (D) $C(1)RM/B^S\ Ybb^{1-3}y^+$ (E) $C(1)RM/B^S\ Ybb^{1-4}y^+$ (F) $C(1)RM/B^S\ Ybb^{1-5}y^+$ (G) $C(1)RM/B^S\ Ybb^+y^+$. These autoradiographs had a long exposure time to show the less intense bands. Scanning measurements were taken from at least four different autoradiographs in the linear response range and traced using a Schoeffel-Kratos SD 3000 spectrodensitometer equipped with a Hewlett-Packard 3390A Integrator. Numbers indicate size of bands in kilobases.

hemizygous condition ($B^S\ Ybb^1y^+$ and $B^S\ Ybb^2y^+$). Further reduction of In^- repeats, to 60–66, causes death of individuals at the late pupal stage ($B^S\ Ybb^{1-3}y^+$ and $B^S\ Ybb^{1-5}y^+$). These results indicate a strong correlation between a reduction in In^- rDNA and a parallel decrease in development when the *Y* rDNA is the only source of rDNA. This was also shown in *D. hydei:* the lower the number of In^- repeats the greater the severity of the *bobbed* phenotype (Franz & Kunz, 1981).

It is generally accepted that repeats with inserts are transcriptionally inactive and insert free repeats (In^-) are transcribed into functional rRNA. The bb^{1-4} allele, however, showed a significant departure from this observation. The $B^S\ Ybb^{1-4}y^+$ chromosome has a total of 80 rDNA repeats of which about 62 are insert-free. This number of In^- repeats is sufficient to allow development to the late pupal stage (wing and eyes formed), as seen in the bb^{1-3} and bb^{1-5} cases. However, individuals hemizygous for the bb^{1-4} allele ($C(1)DX/B^S\ Ybb^{1-4}\ y^+$ or $sc^4\ sc^8/B^S\ Ybb^{1-4}\ y^+$) did not reach the second instar larval stage. The lack of additivity with another *bb* allele supported the contention that most of the bb^{1-4} In^- repeats are non-functional. The bb^{1-4}/bb^8 individuals have an extreme *bobbed* phenotype identical to that of $bb^8/0$ flies (Table 1). The two other Y-NO *bb* alleles, bb^{1-3} and bb^{1-5}, with the same number of In^- repeats as the bb^{1-4} allele, showed a wild type additivity with the bb^8 allele. Thus the bb^{1-4} allele behaved genetically as a complete rDNA deficiency despite about 63 In^- repeats.

Two *Y* NO mutants which have been extensively studied are the Ybb^- (Tartof, 1973; Endow, 1982) and $Ybb^{SuVar-5}$ chromosomes (Shermoen & Kiefer, 1975; Strausbaugh & Kiefer, 1979). These two deletion mutants have equivalent amounts of rDNA; about 10–40 repeats, however, show unique characteristics. The $Ybb^{SuVar-5}$ NO, when combined with

X NO *bb* mutants, demonstrated a different phenotype and rRNA accumulation pattern than the Ybb^- NO despite their equivalent repeat numbers. The $Xbb/Ybb^{SuVar\text{-}5}$ genotypes had a twofold rate of rRNA synthesis and a wild type phenotype as compared to the *bobbed* Xbb/Ybb^- genotypes. To account for the observed rRNA rate of synthesis differences, one proposal by Shermoen & Kiefer (1975) is that a heterochromatic region adjacent to rDNA affects transcription of rRNA genes. Another possibility, demonstrated by our results, is that the number of active In^- repeats may be different between the two *Y* chromosomes.

A possible explanation for a large number of non-functional In^- repeat units is position-effect variegation (for review see Spofford, 1976). In genotypes having a chromosomal rearrangement which juxtaposes euchromatic genes near heterochromatin or *vice versa*, a fraction of cells exhibit no expression of those genes. The effective distance of variegation can be as much as 67 bands (Hartmann-Goldstein, 1967) or about $2{\cdot}0 \times 10^6$ bp of DNA. The amount of non-functional In^-bb^{1-4} rDNA repeats is well within this range, $0{\cdot}74 \times 10^6$ bp. There is relatively little known about variegation of active genes within heterochromatin. A change in the normal rDNA-*Y* heterochromatic border caused by the bb^{1-4} deletion would place different *Y* heterochromatin near the rRNA repeats. A modifier of variegation, such as temperature, showed no change in the phenotype of the bb^{1-4} mutation (unpublished observation). It is also possible that a substantial portion of *Y* rDNA In^- repeats have a less probability of being transcribed due to their number of repetitive elements in the non transcribed spacer region (Busby & Reeder, 1983). They demonstrated in *X. laevis* that the number of repetitive elements determines the frequency of activation of an rRNA repeat. In *D. melanogaster*, the non-transcribed spacer is comprised in part of 0·24 kb tandem repeats defined by *Alu* I cleavage sites upstream from the promoter site (Kohorn & Rae, 1982). The majority of the 62 $In^- bb^{1-4}$ repeats may consist of repeats with a low number of these repetitive elements. Generalized DNase I sensitivity experiments and cloning of *Y*-NO In^- repeats are currently being done to differentiate between these hypotheses of rDNA suppression.

This study was supported by National Institutes of Health Grant GM 28008. We thank Dr Kathy Beckingham for the gift of pBW416 and pBW423 and Dr John Williamson for isolating the rDNA mutants.

REFERENCES

ASHBURNER, M. (1970). Function and structure of polytene chromosomes during insect development. *Advances in Insect Physiology* **7**, 1–96.

BAKER, W. K. (1971). Evidence for position-effect suppression of the ribosomal RNA cistrons in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* (USA) **68**, 2472–2476.

BUSBY, S. J. & REEDER, R. H. (1983). Spacer sequences regulate transcription of ribosomal gene plasmids injected into *Xenopus* embryos. *Cell* **34**, 989–996.

ENDOW, S. A. & GLOVER, D. (1979). Differential replication of ribosomal gene repeats in polytene nuclei of *Drosophila*. *Cell* **17**, 597–605.

ENDOW, S. A. (1982). Molecular characterization of ribosomal genes on the Ybb^- chromosome of *Drosophila melanogaster*. *Genetics* **102**, 91–99.

FRANZ, G. & KUNZ, W. (1981). Intervening sequences in ribosomal RNA genes and *bobbed* phenotype in *Drosophila hydei*. *Nature* **292**, 638–640.

GANDHI, V. V., SHARP, Z. D. & PROCUNIER, J. D. (1982). Analysis of *Y* chromosome nucleolar organizer mutants in *Drosophila melanogaster*. *Biochemical and Biophysical Research Communications* **104**, 778–784.

HARTMANN-GOLDSTEIN, I. J. (1967). On the relationship between heterochromatinization and variegation in *Drosophila* with special reference to temperature-sensitive periods. *Genetical Research* **10**, 143–159.

JOHNSON, C. L., HARGER, H. & HOLM, D. G. (1979). Maternal effect suppression of lethality

associated with a position-effect inactivation of genes in *X*/O males of *Drosophila melanogaster*. *Genetics* **91**, s54.

KALUMUCK, K. E. & PROCUNIER, J. D. (1984). Non selective amplification of ribosomal DNA repeat units in compensating genotypes of *Drosophila melanogaster*. *Biochemical Genetics* **22**, 453–465.

KIDD, S. J. & GLOVER, D. M. (1980). A DNA segment from *D. melanogaster* which contains five tandemly repeating units homologous to the major rDNA insertion. *Cell* **19**, 103–119.

KIDD, S. J. & GLOVER, D. M. (1981). *Drosophila melanogaster* ribosomal DNA containing type II insertions is variably transcribed in different strains and tissues. *Journal of Molecular Biology* **151**, 645–662.

KOHORN, B. D. & RAE, P. M. (1982). Non transcribed spacer sequences promote *in vitro* transcription of *Drosophila* ribosomal DNA. *Nucleic Acids Research* **10**, 6879–6886.

LINDSLEY, D. L. & GRELL, E. H. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Institution of Washington Publication* 627.

LONG, E. O. & DAWID, I. B. (1979). Expression of ribosomal DNA insertions in *Drosophila melanogaster*. *Cell* **18**, 1185–1196.

LONG, E. O., COLLINS, M., KIEFER, B. & DAWID, I. (1981). Expression of the ribosomal DNA insertions in bobbed mutants of *Drosophila melanogaster*. *Molecular General Genetics* **182**, 377–384.

MARRAKECHI, M. & PRUD'HOMME, N. (1971). A study of bobbed mutants induced by ethyl methanesulfonate in *Drosophila melanogaster*. *Biochemical and Biophysical Research Communications* **43**, 273–277.

NIX, C. E. (1973). Suppression of transcription of the ribosomal RNA cistrons of *Drosophila melanogaster* in a structurally rearranged chromosome. *Biochemical Genetics* **10**, 1–12.

PROCUNIER, J. D. & TARTOF, K. D. (1978). A genetic locus having *trans* and contiguous *cis* functions that control the disproportionate replication of ribosomal RNA genes in *Drosophila melanogaster*. *Genetics* **88**, 67–79.

RITOSSA, F. M., ATWOOD, K. C. & SPIEGELMAN, S. (1966). A molecular explanation of the bobbed mutants of *Drosophila* as a partial deficiency of 'ribosomal' DNA. *Genetics* **54**, 819–834.

RITOSSA, F. M. (1968). Nonoperative DNA complementary to ribosomal RNA. *Genetics* **59**, 1124–1131.

SHARP, Z. D., GANDHI, V. V. & PROCUNIER, J. D. (1983). *X* chromosome nucleolus organizer mutants which alter major type 1 repeat multiplicity in *Drosophila melanogaster*. *Molecular General Genetics* **190**, 438–443.

SHERMOEN, A. W. & KIEFER, B. I. (1975). Regulation in rDNA-deficient *Drosophila melanogaster*. *Cell* **4**, 275–280.

SPOFFORD, J. B. (1976). Position-effect variegation in *Drosophila* pp. 955–1018. In: *The Genetics and Biology of Drosophila, Vol. 1C* (ed. M. Ashburner and E. Novitski). New York: Academic Press.

STRAUSBAUGH, L. & KIEFER, B. (1979). Genetic modulation of RNA metabolism in *Drosophila* III. Requirement for an rDNA deficient *X* chromsome in *Ybb*$^{SuVar\text{-}5}$ mediated increases in RNA synthesis. *Genetics* **93**, 411–422.

TARTOF, K. D. (1973). Regulation of ribosomal gene multiplicity in *Drosophila melanogaster*. *Genetics* **73**, 57–71.

WELLAUER, P. K., DAWID, I. B. & TARTOF, K. D. (1978). *X* and *Y* chromosomal ribosomal DNA of *Drosophila*. Comparison of spacers and insertions. *Cell* **14**, 269–278.