Biochem. J. (1971) **123**, 227–233
Printed in Great Britain

The Number and Location of Genes for 5S Ribonucleic Acid within the Genome of *Drosophila melanogaster*

By R. V. QUINCEY*
Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03755, U.S.A. and Department of Chemical Pathology, King's College Hospital Medical School, London S.E.5, U.K.

(*Received* 17 *December* 1970)

DNA was prepared from wild-type and two mutant stocks of *Drosophila melanogaster* that differed in their dosage of the nucleolar organizer region. The relative amounts of DNA from the nucleolar organizer region in these preparations of DNA were determined by hybridization with ^{3}H-labelled 28S rRNA. As expected, the amount of ^{3}H-labelled 28S rRNA that hybridized was directly related to the dosage of nucleolar organizer region. No positive correlation was observed between the amount of ^{3}H-labelled 5S RNA that hybridized and the dosage of nucleolar organizer region. Thus genes for 5S RNA are located primarily, if not exclusively, outside the nucleolar organizer region. The haploid genome of the wild-type *D. melanogaster* used in this work has 106 genes for 28S rRNA and 96–105 genes for 5S RNA.

Ribosomes from all sources contain two high-molecular-weight RNA species that vary in size according to the organism. All ribosomes contain one molecule of each of these ribonucleic acids (23S and 16S rRNA in bacteria; 28S and 18S rRNA in animal cells) and one molecule of 5S RNA. The synthesis of ribosomes thus requires equimolar amounts of these ribonucleic acids and might be expected to involve the use of equal numbers of genes for each RNA.

In bacteria there are approximately equal numbers of genes for 23S rRNA, 16S rRNA and 5S RNA, sets of which are clustered together, if not joined (Smith, Dubnau, Morell & Marmur, 1968; Zehavi-Willner & Comb, 1966; Colli & Oishi, 1969). In eukaryotic organisms 28S rRNA and 18S rRNA are produced by fragmentation of a common precursor rRNA molecule whose genes are located in the nucleolar organizer region of the genome (Darnell, 1968). Less is known of the genes for 5S RNA. In *Xenopus laevis* genes for 5S RNA are apparently not located in the nucleolar organizer region, and in somatic cells are at least 60 times as abundant as precursor rRNA genes (Brown & Weber, 1968). In rat liver cells, however, there are no more than five 5S RNA genes per precursor rRNA gene (Quincey & Wilson, 1969). Information on the relative location and numbers of genes for the rRNA precursor and 5S RNA in other species is required for an understanding of the control of the synthesis of the ribonucleic acids of ribosomes in somatic cells, and in those oocytes in which there is considerable amplification of the precursor rRNA genes.

Drosophila melanogaster was investigated primarily because the availability of mutants differing in dosage of the nucleolar organizer region made it possible to determine if the genes for 5S RNA are intermingled with those for the precursor rRNA in this organism. Also, oogenesis in many insects, as in the Amphibia, involves considerable amplification of the precursor rRNA genes (Gall, 1969).

During preparation of this manuscript the results of a similar study were reported by Tartoff & Perry (1970), and are in accord with those presented here.

MATERIALS AND METHODS

Stocks of D. melanogaster. A stock of wild-type flies was kindly supplied by Dr G. Saul of Middlebury College, Middlebury, Vt., U.S.A. This stock had been obtained in 1954 from the Carolina Biological Supply Company, Burlington, N.C., U.S.A., and is of unknown strain. Wild-type flies (D^+) have a nucleolar organizer region on each X and Y chromosome. Mutant stocks, designated G-21 and G-31A, were obtained from Dr E. Grell of the Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A. Males of the stock G-21 have the constitution *In* (1) $sc^{4L,\ 8R}$ *y* sc^{4+8} *cv v f*/B^s *Y*, and females the constitution *RA y f*/B^s *Y*. Each has one nucleolar organizer region per diploid genome. Males of the stock G-31A have the constitution *In* (1) sc^{SIL} sc^{4R}, *cv v B*/B^s *Y*, and females the

* Present address: Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.

constitution *C* (1) *RM*, y^2 *sc* W^a v/B^sY. Each has three nucleolar organizer regions per diploid genome.

Flies were reared on medium containing 15g of agar, 135ml of molasses, 100g of cornmeal, 8g of dried yeast and 2.25g of Moldex (Turtox Products, General Biological Supply House, Chicago, Ill., U.S.A.) per litre.

To obtain ^{3}H-labelled RNA, flies grown on normal medium were transferred to bottles containing 0.5mCi of [5-^{3}H]orotic acid/ml. After 3 days the flies were transferred to bottles containing unlabelled medium and left for 24h before being harvested.

Preparation of ribosomes. Adult *D. melanogaster* were homogenized in 7vol. of Gardner & Hoagland's (1968) medium XN[25mM-KCl–8mM-$MgCl_2$–0.10M-tris–HCl buffer (pH7.2 at 22°C)–0.5% (w/v) naphthalene-1,5-disulphonate] containing 0.15M-sucrose. Ribosomes were isolated from the 15000*g* supernatant by treatment with 1% (w/v) sodium deoxycholate and sedimentation through medium XN–1.0M-sucrose at 165000*g* for 3h at 4°C.

Yeast was ground into a paste with 3 parts by weight of fine acid-washed sand. Then 2vol. of medium B[25mM-KCl–5mM-$MgCl_2$–0.10M-tris–HCl buffer (pH7.2, at 22°C)] containing 0.35M-sucrose was added and the resulting slurry was centrifuged at 15000*g* for 10min. Triton X-100 was added to the supernatant to a final concentration of 1% (w/v) and ribosomes were sedimented through medium B–2.0M-sucrose by centrifugation for 3h at 165000*g* at 4°C.

Preparation of RNA. RNA was extracted from ribosomes with phenol as described by Wilson & Quincey (1969). 5S RNA was obtained from whole rRNA by treatment with 1.0M-NaCl for 16h at 0°C, followed by ascending chromatography of the soluble fraction on Sephadex G-200. Elution from the column (100cm×2.5cm internal diam.) was with 0.15M-sodium acetate buffer, pH5.1. An upward flow of about 10ml/h was maintained by using a peristaltic pump. After pooling of the fractions containing 5S RNA and dialysis against 0.2M-NaCl–50mM-tris–HCl buffer, pH7.2 at 24°C, the 5S RNA was applied to a column (10cm×1.5cm internal diam.) of methylated albumin on kieselguhr and recovered by gradient elution with NaCl in 50mM-tris–HCl buffer, pH7.2 at 24°C (Comb, Brown & Katz, 1964). The fractions containing 5S RNA were pooled, made 1% (w/v) with respect to sodium dodecyl sulphate and extracted with phenol to ensure the absence of methylated albumin. The 5S RNA was concentrated by precipitating with 2vol. of ethanol at −20°C and redissolved in about 5ml of 0.01×SSC medium (SSC medium is 15mM-trisodium citrate–0.15M-NaCl, pH7.0).

The 28S rRNA was obtained from RNA extracted from ribosomes by sedimentation for 4h at 5°C through a 5–20% (w/v) isokinetic sucrose gradient in 0.15M-sodium acetate buffer (pH5.1) in a Beckman SW 41 rotor at 40000rev./min. The leading portion of the 28S rRNA peak was subjected to chromatography on methylated albumin on kieselguhr and extracted with phenol after addition of 1% (w/v) sodium dodecyl sulphate. RNA of high molecular weight from ribosomes was obtained free of 5S RNA by chromatography on Sephadex G-200.

All RNA was dialysed against 0.01×SSC medium before use.

Preparation of DNA. Adult flies were anaesthetized with ether, suspended in 10vol. of medium B–0.35M-sucrose and homogenized in a motor-driven Potter–Elvehjem homogenizer. The homogenate was centrifuged at 1500*g* for 10min at 0°C and the pellets were suspended in 10vol. of 0.15M-NaCl–0.1M-EDTA (pH8.0)–0.5% (w/v) sodium dodecyl sulphate. After incubation at 60°C for 30min the DNA was extracted with 2vol. of cold phenol–*m*-cresol–water mixture (50:7:7, by vol.) containing 0.1% (w/v) of 8-hydroxyquinoline. After centrifugation the aqueous layer containing most of the DNA was retained, and the interface and phenol–*m*-cresol layer were extracted with 1vol. of 6% (w/v) sodium 4-aminosalicylate (Kirby, 1968). The aqueous layers, containing DNA, were combined and the phenol extraction was repeated. Then 1vol. of ethanol at −20°C was added, and the precipitated DNA was collected. When redissolved in 1.0×SSC medium at a concentration of about 500μg/ml, the DNA was incubated at 37°C for 4h with ribonuclease (150μg/ml) (5×crystallized; Calbiochem, Los Angeles, Calif., U.S.A.) that had previously been heated at 80°C for 10min in 0.15M-NaCl, pH5.0 (Marmur, 1961), and for 2h with α-amylase (50μg/ml) (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). Pronase (100μg/ml) (Calbiochem) that had previously been 'self-digested' by incubation at 37°C for 2h was then added and the incubation was continued for 2h. Sodium dodecyl sulphate was added to a final concentration of 1% (w/v) and the DNA extracted with phenol–*m*-cresol–water as before. The combined aqueous extract was then shaken with chloroform–3-methylbutan-1-ol (24:1, v/v) and centrifuged. This procedure was repeated until protein was absent from the interface. Potassium acetate [0.1vol. of a 20% (w/v) solution at pH5.1] and 1vol. of ethanol at −20°C were added and the DNA was spooled, to be dissolved finally in 0.02×SSC medium. The purity of the DNA was ascertained by spectral analysis and by the absence of ribonuclease or other protein.

DNA was extracted similarly from mouse liver nuclei prepared as described by Quincey & Wilson (1969).

DNA in 0.02×SSC medium was denatured by treatment with 0.1M-NaOH for 1h at 24°C and was applied to Sartorius nitrocellulose filters (0.45μm, 25mm) after neutralization with 1M-HCl and the salt concentration had been increased to 6×SSC medium. After application of the DNA to the filters, the filters were washed on both sides with 100ml of 6×SSC medium and dried at 80°C for 4h *in vacuo*. The amount of DNA retained on the filters (30–40μg) was determined from the release of acid-soluble nucleotide after treatment with perchloric acid, and corresponded to 74–90% of the DNA applied. Losses of DNA from the filters during the hybridization reaction amounted to 0–9% of the amount originally retained, and were corrected for in the results.

Hybridization. The method of Gillespie & Spiegelman (1965) was used. Each tube, containing two filters of each DNA and appropriate amounts of RNA from *D. melanogaster* in 3.0ml of 4×SSC medium, was incubated at 68°C for 20h to ensure that equilibrium was reached. Heterologous RNA from yeast ribosomes (40μg/tube) was added to minimize non-specific adsorption to DNA of ^{3}H-labelled RNA from *D. melanogaster*. The filters were washed with 4×SSC medium and treated with ribonuclease (20μg/ml) for 90min at 28°C. Damp filters were dissolved in 10ml of Bray's (1960) scintillation fluid modified to

contain 60g of naphthalene/l. Radioactivity was measured in a Packard Tri-Carb Scintillation Spectrometer at an efficiency of about 30% as determined by automatic external standardization. Background was about 20c.p.m. The amount of hybrid was determined after subtraction of the amount of radioactivity bound to filters containing mouse DNA.

The percentage of DNA complementary to 28S rRNA was calculated from results plotted in the form of $(A)/r$ against (A) according to the equation (Klotz, 1953):

$$\frac{(A)}{r} = \frac{(A)}{n} + \frac{K_d}{n}$$

where (A) is the concentration of unbound RNA (μg/ml), r is the RNA bound at equilibrium ($100 \times \mu g/\mu g$ of DNA), n is the concentration of RNA binding sites on the DNA ($100 \times \mu g/\mu g$ of DNA), and K_d is the intrinsic binding constant. Expressed in this way, n is equivalent to the percentage of DNA complementary to the RNA in question, and is equal to the reciprocal of the slope of the line obtained by plotting $(A)/r$ against (A).

The plot of $(A)/r$ against (A) was preferred to the plot of $1/r$ against $1/(A)$ used by Paul & Gilmour (1968) and Shearer & McCarthy (1967) because there is less variation in the statistical weight of each point (Wilkinson, 1961), and an appropriate distribution of experimental points is more readily obtained.

Analytical methods. DNA was determined by measuring the extinction at 268nm ($E^{1\%}_{1cm}$ =262 at 268nm) after hydrolysis in 1M-perchloric acid at 70°C for 30min (Tsanev & Markov, 1960). RNA in 0.01×SSC medium was determined by measuring the extinction at 260nm ($E^{1\%}_{1cm}$ =250 at 260nm). The absence of protein from the preparations of DNA was demonstrated by their failure to react when analysed for protein by the method of Lowry, Rosebrough, Farr & Randall (1951). Ribonuclease was measured by the appearance of acid-soluble radioactivity after incubation of the sample in 1×SSC medium with ^{3}H-labelled rRNA for 3h at 37°C.

RESULTS

The relative amounts of DNA from the nucleolar organizer region in the preparations of DNA from the mutants (G-21 and G-31A) and wild-type (D^+) strains of *D. melanogaster* may be determined by hybridization with precursor rRNA, or one of its products, since these genes are located exclusively within the nucleolar organizer region. In this work the 28S-rRNA component was used because of its abundance in the cell and the ease, by virtue of its large size, with which it may be prepared free of degradation products of other RNA species. The presence or absence of genes for 5S RNA in the nucleolar organizer region was then ascertained by comparing the relative amounts of 5S RNA and

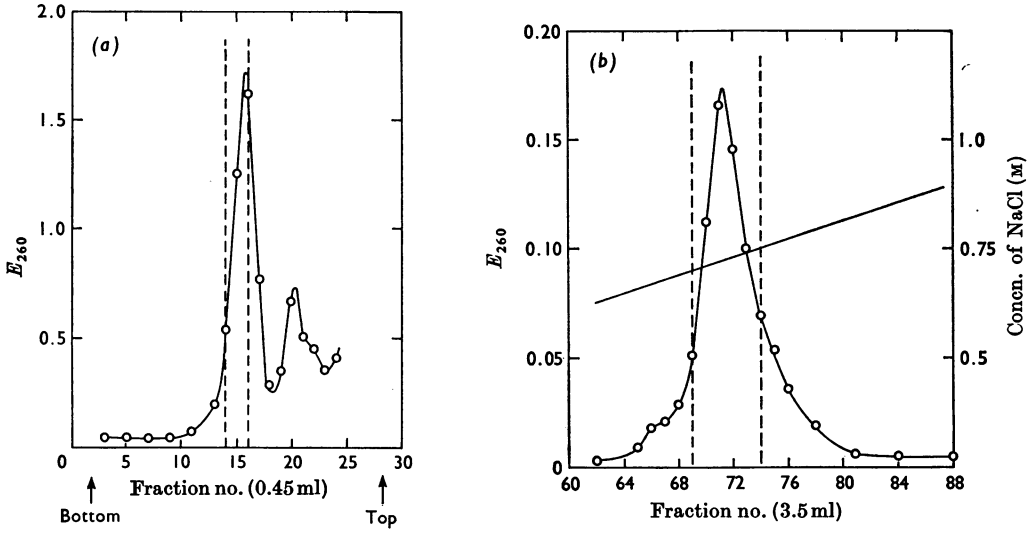

Fig. 1. Isolation of ^{3}H-labelled 28S rRNA from wild-type (D^+) *D. melanogaster*. (*a*) Separation of ^{3}H-labelled 28S rRNA from bulk ^{3}H-labelled rRNA by sucrose-density-gradient centrifugation. About 200μg of RNA in 0.1ml of 0.15M-sodium acetate buffer (pH5.1) was layered on top of a 12ml of 5–20% (w/v) isokinetic sucrose gradient in 0.15M-sodium acetate buffer (pH5.1) and centrifuged for 4h at 5°C in a Beckman SW 41 rotor at 40000rev./min. Fractions from four such gradients were obtained through a needle inserted in the bottom of the centrifuge tube. (*b*) Chromatography on methylated albumin on kieselguhr of ^{3}H-labelled 28S rRNA obtained by sucrose-gradient centrifugation of bulk ^{3}H-labelled rRNA. About 180μg of ^{3}H-labelled 28S rRNA was applied to a column (10cm×1.5cm internal diam.) containing methylated albumin on kieselguhr and was recovered by gradient elution with NaCl (0.4M–1.15M) (——) in 50mM-tris-HCl buffer, pH7.2 at 24°C. Fractions were pooled as indicated (---). ○, E_{260}.

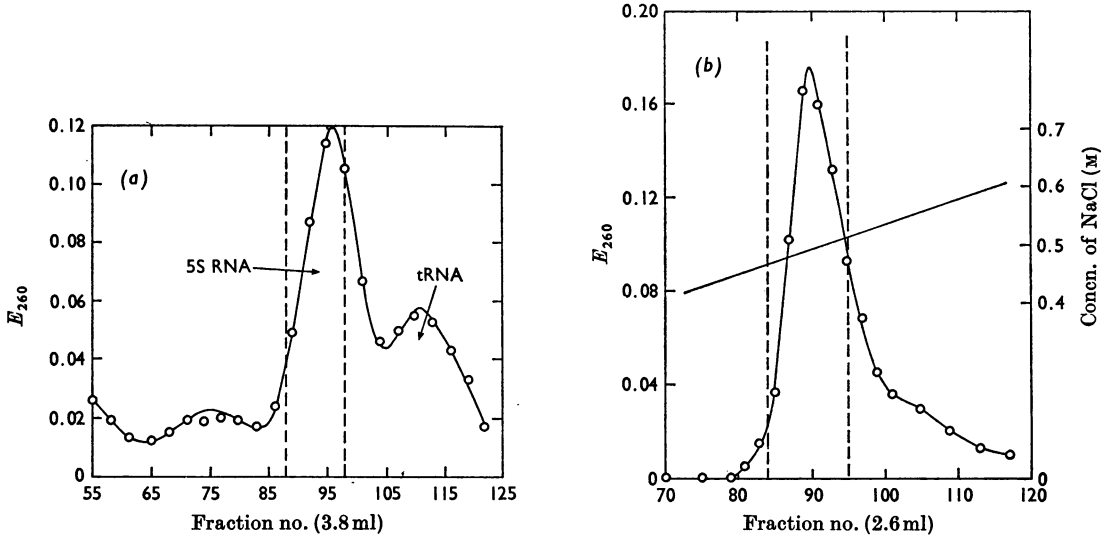

Fig. 2. Isolation of ^{3}H-labelled 5S RNA from wild-type (D^+) *D. melanogaster*. (*a*) Separation of ^{3}H-labelled 5S RNA from ^{3}H-labelled RNA from ribosomes soluble in 1.0M-NaCl by ascending chromatography on Sephadex G-200. About 1.2mg of ^{3}H-labelled RNA was applied to the column (100cm × 2.5cm internal diam.) in 3ml of 0.15M-sodium acetate buffer (pH5.1) and was eluted with the same buffer. An upward flow of 10ml/h was maintained by using a peristaltic pump. (*b*) Chromatography on methylated albumin on kieselguhr of ^{3}H-labelled 5S RNA obtained after chromatography on Sephadex G-200 of ^{3}H-labelled RNA from ribosomes soluble in 1.0M-NaCl. About 300μg of ^{3}H-labelled 5S RNA in 50ml of 0.2M-NaCl–50mM-tris–HCl buffer (pH7.2, at 24°C) was applied to the column and was recovered by gradient elution with NaCl (0.2M–0.8M) (——) in 50mM-tris–HCl buffer, pH7.2, at 24°C. Fractions were pooled as indicated (---). ○, E_{260}.

28S rRNA that hybridized to the three preparations of DNA. By chromatographic criteria the ^{3}H-labelled 28S rRNA and ^{3}H-labelled 5S RNA used in these experiments appeared to be pure (Figs. 1 and 2). The specific radioactivity of the ^{3}H-labelled 28S rRNA was 1.13×10^5d.p.m./μg and of ^{3}H-labelled 5S RNA was 0.876×10^5d.p.m./μg.

Hybridization of ^{3}H-*labelled* 28S *rRNA*. To conserve ^{3}H-labelled rRNA no attempt was made to attain a saturation plateau, and the amount of each DNA complementary to 28S rRNA was calculated from a reciprocal plot of the results. The percentage of DNA complementary to 28S rRNA in the DNA from strains G-21, D^+ and G-31A was 0.095, 0.124 and 0.240% respectively (Fig. 3), and was directly related to the dosage of nucleolar organizer region. The values of 0.095 and 0.240% for the amount of DNA complementary to 28S rRNA in mutants G-21 and G-31A respectively are about two-thirds of those obtained by Ritossa & Spiegelman (1965) for the hybridization of 28S + 18S rRNA to DNA from similar mutants, which was as expected. The percentage of DNA from wild-type D^+ complementary to 28S rRNA was less than has been observed in Oregon-R and Standard Urbana strains of wild-type *D. melanogaster* (Ritossa & Spiegelman, 1965; Tartoff & Perry, 1970).

Hybridization of ^{3}H-*labelled* 5S *RNA*. The 5S RNA obtained from ribosomes is likely to be contaminated by degradation products of RNA of higher molecular weight that may contribute significantly to the hybrid even when present in trace amounts. Elimination of this unwanted hybrid is achieved by dilution of the tritiated contaminants with unlabelled high-molecular-weight RNA from ribosomes. In the present instance addition of a 100-fold excess of unlabelled high-molecular-weight RNA sufficed to eliminate the radioactivity due to the contaminants from the hybrid (Fig. 4).

The percentage of each DNA complementary to 5S RNA was determined by hybridization with various amounts of ^{3}H-labelled 5S RNA to which 135 times the amount of the unlabelled high-molecular-weight RNA had been added (Fig. 5). The amount of DNA from strains G-21, D^+ and G-31A that was complementary to 5S RNA was similar and varied between 0.0027 and 0.0038%. No positive correlation was observed between the amount of 5S RNA hybridized and the dosage of

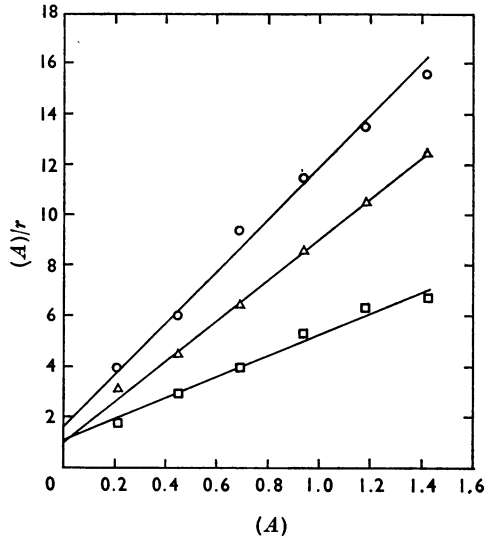

Fig. 3. Hybridization of ^{3}H-labelled 28S rRNA to DNA from strains G-21, D$^+$ and G-31A. Values for the percentage of DNA complementary to 28S rRNA were calculated from the reciprocal of the slope (see the text) and were 0.095, 0.124 and 0.240% for DNA from strains G-21 (○), D$^+$ (△) and G-31A (□) respectively. Hybridization in 3.0 ml of 4×SSC medium was for 20 h at 68°C. (*A*), μg of unbound ^{3}H-labelled 28S rRNA/ml; *r*, 100×μg of ^{3}H-labelled 28S rRNA hybridized/μg of DNA. Each point is the mean of three determinations.

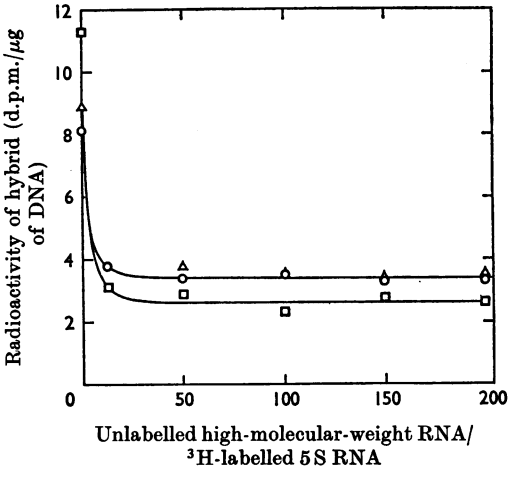

Fig. 4. Effect of addition of unlabelled high-molecular-weight RNA from ribosomes on the hybridization of ^{3}H-labelled 5S RNA to DNA from strains G-21 (○), D$^+$ (△) and G-31A (□). The concentration of ^{3}H-labelled 5S RNA in all tubes was 1.06 μg/ml. Hybridization in 3.0 ml of 4×SSC medium was for 20 h at 68°C. Each point represents a single determination.

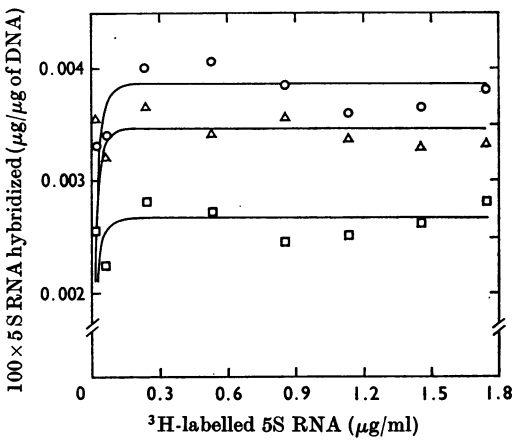

Fig. 5. Hybridization of ^{3}H-labelled 5S RNA to DNA from strains G-21 (○), D$^+$ (△) and G-31A (□) in the presence of a 135-fold excess of unlabelled high-molecular-weight RNA from ribosomes. Hybridization in 3.0 ml of 4×SSC medium was for 20 h at 68°C. Each point is the mean of two determinations.

nucleolar organizer region as determined by hybridization with ^{3}H-labelled 28S rRNA. Genes for 5S RNA are therefore located primarily, if not exclusively, outside the nucleolar organizer region of the genome.

To assess the specificity of the hybridization of 5S RNA, the radioactivity of hybrid formed when various amounts of unlabelled 5S RNA were added to tubes containing saturating amounts of ^{3}H-labelled 5S RNA and 135 times that amount of unlabelled high-molecular-weight RNA was observed (Fig. 6). By comparison of the results with theoretical curves based on isotopic dilution it was concluded that about 10% of the hybrid formed in the absence of unlabelled 5S RNA was due to components other than 5S RNA.

The number of genes for 28S RNA (and hence precursor rRNA genes) and 5S RNA per haploid genome was readily calculated from knowledge of the molecular weights of the RNA and of the weight in daltons of the haploid genome (Table 1). In the wild-type strain of *D. melanogaster* used in this work there are about 100 genes for both precursor rRNA and 5S RNA.

DISCUSSION

Co-ordinate synthesis of equimolar amounts of the RNA species of ribosomes would seem to confer advantages of economy to cells.

RNA–DNA hybridization experiments have shown that in bacteria genes for 23S rRNA, 16S rRNA and 5S RNA are close to each other (Smith *et al.* 1968;

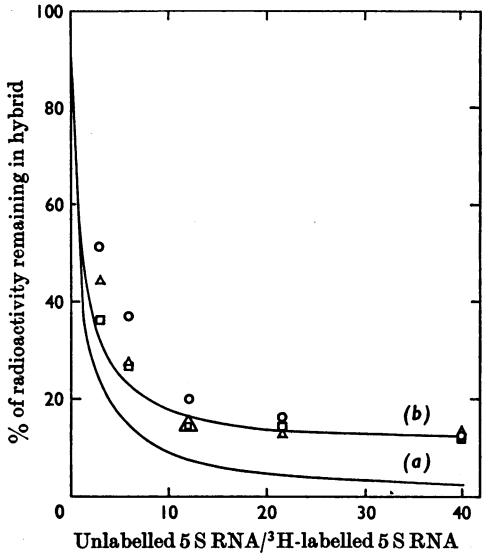

Fig. 6. Effect of addition of unlabelled 5S RNA on the hybrid formed by a 1:135 mixture of ^{3}H-labelled 5S RNA and unlabelled high-molecular-weight RNA from ribosomes to DNA from strains G-21 (○), D$^+$ (△) and G-31A (□). (*a*) and (*b*) are theoretical isotopic-dilution curves. Curve (*a*) would be obtained if all the radioactivity in hybrid was due to 5S RNA, and curve (*b*) would be obtained if 90% was due to 5S RNA and the remainder to other components. Hybridization in 3.0 ml of 4×SSC medium was for 20 h at 68°C. The concentration of ^{3}H-labelled 5S RNA in each tube was 0.203 μg/ml. Each point represents a single determination.

Table 1. *Number of genes for* 5S *RNA and* 28S *rRNA in wild-type* D. melanogaster

Molecular weights of 1.4×10^6 and 4.0×10^4 for 28S rRNA and 5S RNA respectively (Tartoff & Perry, 1970), and 1.2×10^{11} for the haploid genome of wild-type *D. melanogaster* (Ritossa & Spiegelman, 1965) are assumed for the calculation of genes per haploid genome.

RNA	DNA hybridized (%)	Genes per haploid genome
28S rRNA	0.124	106
5S RNA	0.0032–0.0035	96–105

Colli & Oishi, 1969). Evidence from the kinetics of appearance of these RNA species indicates that 5S RNA may well be derived by post-transcriptional modification of the precursor to either 23S rRNA or 16S rRNA (Hecht, Bleyman & Woese, 1968; Doolittle & Pace, 1970). Other evidence suggests that the synthesis of these RNA species might be regulated co-ordinately (Bleyman, Kondo, Hecht & Woese, 1969; Roschenthaler, Devynck, Fromageot & Simon, 1969; Galibert, Eladari, Larson & Boiron, 1970). There would appear to be no less of a need for co-ordinate regulation of synthesis in eukaryotic cells, and one might suppose that the derivation of 28S rRNA and 18S rRNA from a single precursor molecule would be a device for ensuring this. In this context, the finding that the genes for 5S RNA are separated from those for the precursor rRNA in both *D. melanogaster* and *X. laevis* lacks obvious explanation. Much more reasonable is the finding that in *D. melanogaster* the numbers of genes for precursor rRNA and 5S RNA are similar.

During oogenesis in many organisms that produce yolky eggs there is an exceptional demand for ribosome biosynthesis and hence for the provision of precursor rRNA and 5S RNA (Brown, 1966). Extra copies of the genes for these RNA species may be required. Additional copies of genes for precursor rRNA often appear by amplification within the oocyte. Alternatively, transport to the oocyte of RNA synthesized in highly polyploid nurse cells may supplement or supplant the need for gene amplification within the oocyte (Gall, 1969). Whatever the mechanism, equimolar amounts of precursor rRNA and 5S RNA will be required. Amplification of genes for 5S RNA has been investigated only in the amphibian *X. laevis* and apparently does not occur, although those for precursor rRNA are amplified about 2000-fold in that organism (Brown & Weber, 1968; Evans & Birnstiel, 1968). The degree of imbalance that results from such differential gene amplification will depend on the extent of the amplification and on the relative numbers of genes present in the somatic cell. The imbalance caused by amplification of precursor rRNA genes but not 5S-RNA genes in *X. laevis* oocytes is muted because somatic cells contain a large excess of genes for 5S RNA. No such amelioration would be possible in *D. melanogaster*, whose somatic cells contain equal numbers of genes for precursor rRNA and 5S RNA.

I am indebted to Dr Mahlon Hoagland and Professor Charles Gray, in whose Departments this work was begun and completed respectively, for their encouragement and support. The excellent technical assistance of Mr Christopher Davis is gratefully acknowledged.

REFERENCES

Bleyman, M., Kondo, M., Hecht, N. & Woese, G. (1969). *J. Bact.* **99**, 535.

Bray, G. A. (1960). *Analyt. Biochem.* **1**, 279.

Brown, D. D. (1966). *Natn. Cancer Inst. Monogr.* no. 23, p. 297.

Brown, D. D. & Weber, C. S. (1968). *J. molec. Biol.* **34**, 661.

Colli, W. & Oishi, M. (1969). *Proc. natn. Acad. Sci. U.S.A.* **64**, 642.

Comb, D. G., Brown, R. & Katz, S. (1964). *J. molec. Biol.* **8**, 781.

Darnell, J. E. (1968). *Bact. Rev.* **32**, 262.

Doolittle, W. F. & Pace, N. R. (1970). *Nature, Lond.*, **228**, 125.

Evans, D. & Birnstiel, M. L. (1968). *Biochim. biophys. Acta*, **166**, 274.

Galibert, F., Eladari, M. E., Larson, C. J. & Boiron, M. (1970). *Eur. J. Biochem.* **13**, 273.

Gall, J. G. (1969). *Genetics*, **61** (Suppl. no. 1), 121.

Gardner, J. A. A. & Hoagland, M. B. (1968). *J. biol. Chem.* **243**, 10.

Gillespie, D. & Spiegelman, S. (1965). *J. molec. Biol.* **12**, 829.

Hecht, N., Bleyman, M. & Woese, G. (1968). *Proc. natn. Acad. Sci. U.S.A.* **59**, 1278.

Kirby, K. S. (1968). In *Methods in Enzymology*, vol. 12, part B, p. 87. Ed. by Grossman, L. & Moldave, K. New York and London: Academic Press.

Klotz, I. M. (1953). In *The Proteins*, vol. 1, part B, p. 727. Ed. by Neurath, H. & Bailey, K. New York and London: Academic Press.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.

Marmur, J. (1961). *J. molec. Biol.* **3**, 208.

Paul, J. & Gilmour, R. S. (1968). *J. molec. Biol.* **34**, 305.

Quincey, R. V. & Wilson, S. H. (1969). *Proc. natn. Acad. Sci. U.S.A.* **64**, 981.

Ritossa, F. M. & Spiegelman, S. (1965). *Proc. natn. Acad. Sci. U.S.A.* **53**, 737.

Roschenthaler, R., Devynck, M. A., Fromageot, P. & Simon, E. J. (1969). *Biochim. biophys. Acta*, **182**, 481.

Shearer, R. W. & McCarthy, B. J. (1967). *Biochemistry, Easton*, **6**, 283.

Smith, I., Dubnau, D., Morell, P. & Marmur, J. (1968). *J. molec. Biol.* **33**, 123.

Tartoff, K. D. & Perry, R. P. (1970). *J. molec. Biol.* **51**, 171.

Tsanev, R. & Markov, G. G. (1960). *Biochim. biophys. Acta*, **42**, 442.

Wilkinson, G. N. (1961). *Biochem. J.* **80**, 324.

Wilson, S. H. & Quincey, R. V. (1969). *J. biol. Chem.* **244**, 1092.

Zehavi-Willner, T. & Comb, D. G. (1966). *J. molec. Biol.* **16**, 250.