Regulation of ribosomal RNA synthesis of Drosophila melanogaster

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DROSOPHILA MELANOGASTER.

Iowa State University, Ph.D., 1971
Biology-Genetics

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Regulation of ribosomal RNA synthesis
of Drosophila melanogaster

by

Roberto Simon Weinmann

A Dissertation Submitted to the
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1971
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ABBREVIATIONS

4-AS  4-aminosalycilate

bb    bobbed, sex-linked recessive

B^S   Bar of Stone, mutation affecting eye shape

C(1)DX Compound (1) reversed acrocentric, two X chromosomes attached, carries no RTU redon

C(1)RM Compound (1) reversed metacentric, two X chromosomes attached, carrying two RTU redons

cpm   counts per minute

Cy    Curly wing, dominant in the second chromosome

DNA   deoxyribonucleic acid

dimethyl 1,4-Bis-2(4-methyl-5-phenyloxazolyl)-benzene

POPOP

F1, F8 first, eighth filial generation

EDTA  ethylene diaminetetraacetic acid

g     gram

mCi   millicurie

ml    milliliter

mM    millimolar

M     molar

μ     micron

μl    microliter

mRNA  messenger RNA

nm    nanometers

NDS   naphthalene-1,5-disulphonate

3N    triploid

O.D.  optical density
PPO 2,5-diphenyloxazole
RNA ribonucleic acid
rDNA DNA that codes for ribosomal RNA
rRNA ribosomal RNA
RTU ribosomal transcriptional unit, RTU rRNA is the minimum piece (38s in Drosophila) of rDNA transcribed
s sedimentation unit, one Svedberg
sc sex-linked mutation affecting the scutellar bristles
SDS sodium dodecyl sulphate
SSC sodium chloride .15M, sodium citrate .015M
T(2;3) translocation of a piece of the second chromosome into the third (see Figure 4)
C328 (see Figure 4)
TIPNS tri-iso-propylnaphthalenesulphonate
tRNA transfer RNA or 4s RNA
w white eye, sex-linked mutation
X/O males without a Y chromosome, sterile
x g times gravity
y sex-linked mutation giving yellow body
Y Y chromosome
INTRODUCTION

At present, considerable information has been obtained concerning the synthesis of RNA in prokaryotes. However, little is understood concerning RNA synthesis and its regulation in higher organisms. To learn more about the regulation of ribosomal RNA synthesis, *Drosophila melanogaster* is an excellent organism for study. Some of the reasons for choosing *Drosophila* are: (1) It is an eukaryote and genetically very well known (LINDSLEY and GRELL, 1968). (2) The genes for ribosomal RNA (rRNA) and 5s RNA have been localized on the X (RITOSSA and SPIEGELMAN, 1965) and second chromosomes (WIMBER and STEFFENSEN, 1970), respectively. (3) By genetic manipulation, it is possible to construct individuals with increased or decreased doses of the rRNA or 5s RNA genes, in relation to wild type. (4) *Bobbed* mutants are known to exist, which show delayed developmental time, etched abdomen, short bristles, and a reduced amount of ribosomal DNA (rDNA) (RITOSSA, ATWOOD, and SPIEGELMAN, 1966) and are thought to be mutants in the rate of rRNA synthesis (MOHAN and RITOSSA, 1970). Therefore, the fact that rRNA is the direct product of the rDNA genes, as is also true for the 5s RNA, makes these classes of RNA useful for the study of the regulation of transcription.

Since the rRNA and the 5s RNA are found in mature ribosomes in equimolar amounts, the problem of coordination of their transcription in view of the separate loci, is a challenging one. This work will involve a detailed analysis of amounts and rates of RNA synthesis with different doses of rDNA, or with *bobbed* mutants in an effort to get some insight into the regulation of the synthesis of these classes of RNA.
Constitution of Ribosomes

Ribosomes are particles found both in the cytoplasm and the nucleus, sometimes attached to membranes, and are involved in protein synthesis. They are made up of RNA and many different proteins. The dimensions of the ribosomes of eukaryotes are 340 x 240 x 240 Å and they have a molecular weight of 4.1-4.7 x 10^7 daltons (SPIRIN and GAVRILOVA, 1969). This unit, which has a sedimentation coefficient of 80s, can be dissociated into a 60s and a 40s subunit. The large subunit (60s) contains a 28s rRNA and, depending on the species, (Review by ATTARDI and AMALDI, 1970) has a molecular weight of 1.3-1.6 x 10^6 daltons. The 60s subunit also contains a 5s RNA (approximate molecular weight 4 x 10^4 daltons) and a 7s RNA. The 7s RNA has been found to be hydrogen bonded to the 28s rRNA and derived from the same precursor as the 28s (ATTARDI and AMALDI, 1970). In the smaller 40s ribosomal subunit, an 18s rRNA is found to have a molecular weight of .69-.89 x 10^6 daltons, also varying with the species (ATTARDI and AMALDI, 1970). Some 50 different proteins are associated with the RNA to form the complete ribosome. Although little is known about these basic proteins, they have been partially characterized for Drosophila by acrylamide gel electrophoresis (LAMBERTSON, RASMUSON, and BLOOM, 1970).

Synthesis of High Molecular Weight Ribosomal RNA

The synthesis of ribosomal RNA in higher organisms involves mainly two steps: First, one of transcription, and second, one of posttranscriptional modification.
Figure 1. A schematic diagram of the processing of ribosomal RNA in *Drosophila melanogaster*. The nonconserved RNA is shown with a zig-zag line. Base-paired regions of the 7s rRNA are shown. Methylation and folding, probably involved in processing, are not shown. Data used in the diagram are from: LOENING, 1968; PERRY, CHENG, FREED, GREENBERG, KELLEY, and TARTOF, 1970. Data were confirmed by our own results (see Figures 6, 7, 8, and 9).
The two high molecular weight rRNAs are derived from just one precursor molecule (38s), which is later cleaved by a specific enzyme. A diagram of the events is shown in Figure 1. This large stretch of RNA, which is the minimum piece of ribosomal information transcribed, has been called ribosomal transcriptional unit (RTU) by PERRY and others (PERRY et al., 1970). Two molecules are derived from the RTU, one is the 18s rRNA (sedimentation values are given only for the purpose of identification, since they are not exact figures - HASTINGS and KIRBY, 1966) and the 30s rRNA. The 30s has a base-paired area. Further cleavage of the 30s RNA in the base-paired region gives a "28s" rRNA and the 7s RNA, which remains base-paired. Specific enzymes do the cleavage and probably ribosomal proteins attach simultaneously with maturation and subsequent methylation of the rRNA.

Localization of RTU Genes

The localization of the site of synthesis of the RTUs within the cell has been made possible by use of autoradiography coupled with low doses of actinomycin D. Low doses of actinomycin D inhibit the incorporation of radioactive uridine into nucleolar RNA and, consequently, formation of the RTUs (PERRY and KELLEY, 1968, 1970). Precise fractionation of nucleoli has allowed an unequivocal assignment of RTU synthesis to the nucleolus (PENMAN, SMITH, HOLZMAN, and GREENBERG, 1966). Additional evidence comes from the experiments of RITOSSA and SPIEGELMAN (1965); RITOSSA, ATWOOD, LINDSLEY, and SPIEGELMAN (1966) with Drosophila melanogaster, and BIRNSTIEL, WALLACE, SIRLIN, and FISCHBERG (1966) with Xenopus laevis. They showed that the amount of DNA hybridizable in vitro to rRNA was
proportional to the number of nucleolar organizers. This was interpreted to mean that the genes for the RTU are localized at or very close to the nucleolar organizer. Finally, in situ RNA-DNA hybridization followed by autoradiography in salivary gland chromosomes of Drosophila has allowed the identification of the rRNA sites with the nucleolar organizer regions (PARDUE, GERBI, ECKHARDT, and GALL, 1970) of the X and Y chromosomes.

**Origin of the 28s and 18s rRNA**

Evidence that the two high-molecular-weight components are derived from the RTU, (see Figure 1) comes from pulse labeling experiments, combined with actinomycin D. If HeLa cells are left in labeled uridine, and, a short time after, rRNA (RTU) synthesis is inhibited with a low dose of actinomycin, the label is first found in the RTUs. After further incubation in the presence of actinomycin D, the RTUs are split, and the label is found in 28s and 18s rRNAs (PENMAN, SMITH, HOLTZMAN, and GREENBERG, 1966). Further evidence to support a mechanism of this kind is provided by studies of JEANTEUR and ATTARDI (1969). Using hybridization competition, they showed that the DNA sites for the RTUs can be hybridized with both 28s and 18s at the same time, which suggests that there are independent DNA sites for 28s rRNA and for 18s rRNA. However, RTU RNA competed with one or the other of the 28s and 18s RNAs, and in the proportion expected if they were transcribed from the same stretch of DNA.

As can be seen from a comparison of the molecular weights of the RTU RNA and from the 28s and 18s rRNA, the process of maturation is not conservative. Part of the ribosomal precursor is degraded during maturation. Base composition analysis also confirms this fact (TARTOF and PERRY, 1970).
Organization of the Genes for the RTUs

There are several copies of RTU genes per haploid genome (RITOSSA and SPIEGELMAN, 1965). It has been calculated from hybridization studies that *Drosophila* normally has between 130-180* copies of RTU per haploid genome. These are all clustered in the nucleolar organizer region. For convenience, we would like to refer to each cluster of repeated sequences, which is called a redundant region, as a REDON. For example, a diploid normal *Drosophila* has two RTU redons with 130* copies each. It is thought that there are fewer promoters than basic sequences, maybe as few as one promoter per redon (PERRY and KELLEY, 1970; ATWOOD, 1969). Variations in redon size are known to occur. The sex-linked bobbed mutants in *Drosophila* are known to have RTU redons of reduced size (RITOSSA, ATWOOD, and SPIEGELMAN, 1966). These bobbed mutants have short bristles, etched abdomen, and an increased developmental time (LINDSLEY and GRELL, 1968). Different grades of bb are found, from very slight (almost impossible to separate from wild type) up to recessive lethals, the intensity proportional to the extent of the rDNA deletion. The fact that the RTU redon is so large, i.e., has 130 copies, allows partial deletions to survive. It is believed that the RTU redon, the nucleolar organizer region, and the bobbed locus are one and the same thing (RITOSSA, ATWOOD, and SPIEGELMAN, 1966). RITOSSA et al. (1966) proposes that, if less than half the wild type RTUs genes are present, in either one or two redons, a bobbed phenotype occurs. MOHAN and RITOSSA (1970) showed

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*Variations depend on the estimate of DNA per haploid genome and on wild type used.*
that bobbed mutants synthesized the same total amount of rRNA in relation to DNA, but that the rate of rRNA synthesis was reduced. Consequently, we can conclude that the total amount of RNA synthesized does not depend directly on the number of RTU redons (KIEFER, 1968) or on the size of them (MOHAN and RITOSSA, 1970). However, the rate of synthesis is apparently reduced, if the total amount of RTU genes is below 50% of the normal wild-type diploid.

There is a process by which a variation in the redon size can occur (RITOSSA, ATWOOD, and SPIEGELMAN, 1966; RITOSSA, 1968; RITOSSA and SCALA, 1969; TARTOF, 1971). The mechanism by which this occurs is not clear, however, gene magnification or amplification have been suggested as an explanation. One aspect seems to be clear. The increase or decrease in the size of one of the RTU redons depends on the size of the homologous redon. If the redon is smaller than the wild type, they tend to increase to a value corresponding to wild type; however, if larger, they tend to decrease to wild type. This suggests that the level of expression of the RTU genes influences in a certain way this amplification-magnification system.

Low Molecular Weight Ribosomal RNA

There are two kinds of low molecular weight ribosomal RNA: a 7s RNA and a 5s RNA. The 7s component, which is base-paired to the 28s, is transcribed from the RTU-DNA itself. Being derived from the RTU, it accumulates at the same time as the 28s and 18s components (see Figure 1).

The 5s rRNA genes in Drosophila were analyzed by TARTOF and PERRY (1970), using RNA-DNA hybridization. They showed that: (1) the genes
for 5s rRNA form a redon with approximately 190 copies, (2) the 5s redons
have approximately the same number of copies as the RTU redons, since in
the same set of experiments each RTU redon had 180-190 copies of rRNA
cistrons, (3) the 5s redon is not located in the X chromosome as is the
RTU redon. These observations were confirmed by WIMBER and STEFFENSEN
(1970). These authors, using RNA-DNA hybridization on salivary gland pre­
parations (in situ), followed by autoradiography, assigned the 5s RNA
redon to the 56 EF region of the second chromosome. Xenopus is the only
other eukaryote where 5s genes were similarly studied. The results of
BROWN and WEBER (1968) indicate that in Xenopus the 5s RNA genes are not
intermingled with the RTUs. In normal tissue of Xenopus the 5s RNA redon
has 27,000 copies of the 5s cistron and the RTU redon 450 copies of the
rRNA cistron, but both classes of gene product accumulate coordinately.
Also, it was found that in the oocyte of Xenopus where amplification of
RTU redons DNA occurs (up to 30,000 copies), 5s RNA accumulates coor­
dinately with the 28-18s rRNAs. Furthermore, in the anucleolate mutant of
X. laevis, where no RTU synthesis can occur because the genes are not
present, no net synthesis of 5s rRNA is observed. Consequently, in
Xenopus the synthesis of 5s and RTUs seems to be coordinately controlled,
which also appears to be true in prokaryotes (ATTARDI and AMALDI, 1970).

However, different results were obtained with HeLa cells by PERRY
and KELLEY (1968). Low doses of the antibiotic actinomycin D inhibited
RTU transcription but did not affect the synthesis of 5s rRNA. One
molecule of actinomycin D can block all the genes that share a promoter,
since it is envisioned that a polymerase molecule when present on a redon
cannot get off until it reaches the end of that whole redon (MILLER and
BEATTY, 1969). The low number of promoters for the RTU redon would give a high actinomycin sensitivity, i.e., a longer stretch of RNA is a larger target for the drug, requiring a lower actinomycin dose to produce inhibition (PERRY and KELLEY, 1970). ZYLBER and PENMAN (1971) studied the synthesis of 5s rRNA in metaphase-arrested HeLa cells. It was found that at this time ribosomal (RTU) RNA synthesis is inhibited, but transfer and 5s RNA continue to be made.

Development of the Problem

The relationship between RTU RNA synthesis and 5s RNA will be studied. In Drosophila no drugs are necessary to produce variations in ribosomal RNA synthesis. The bobbed mutants (bb) have redons of reduced size (RITOSSA, ATWOOD, and SPIEGELMAN, 1966) and, consequently, a reduced rate of RTU RNA synthesis (MOHAN and RITOSSA, 1970). It would be of interest to determine if concomitantly a reduction in 5s rRNA occurs, thus indicating whether the two forms of RNA (5s and rRNA) are regulated by the same regulator gene. Also, the effect of varying the dose of the RTU redons or of the 5s RNA redons can be determined. The effect of this variation on both rate and amount of all classes of ribosomal RNA synthesized will be studied. Variations in the number of RTU redons were generated by RITOSSA et al. (1966) and will be used. They consist of duplications of the nucleolar organizer region. By genetic manipulation, it is possible to construct individuals with 1, 2, 3, or 4 RTU redons, and also produce triploids with 1, 2, or 3 RTU redons. Also, with a translocation generated by P. ROBERTS (LINDSLEY and GRELL, 1968), it is possible to construct stocks with 2, 3, or 4 5s redons. Since the deficiency in this
stock is not viable, we cannot have the stock with only one 5s redon. Thus, combinations of the different doses of RTU and 5s RNA redons can be obtained, which may give some insight into the control mechanisms for rRNA and 5s RNA synthesis.

In addition to the above described approach, the effect of the compound α-amanitin, which in eukaryotes acts very much like rifampicin in prokaryotes, will be tested. This compound interacts with the nuclear RNA polymerase but not with the nucleolar RNA polymerase (the one that synthesizes RTU-RNA) (BLATTI, INGLES, LINDELL, MORRIS, WEAVER, WEINBERG, and RUTTER, 1970). The interaction of α-amanitin is such that the promoter-specific factor (sigma) cannot attach to RNA polymerase and, consequently, no initiation of transcription occurs (JACOB, SAJDEL, MUECKE, and MUNRO, 1970). After treatment with this compound, RTU-RNA synthesis should continue. However, its effect on 5s rRNA synthesis is unknown and this will be determined.

In order for these approaches to rRNA and 5s RNA regulation to have any validity, a method has to be developed so that rRNA, 5s RNA, and transfer RNA (tRNA) can be measured simultaneously with assurance of accuracy. To this end, a method, using polyacrylamide gel electrophoresis, was developed that allowed simultaneous analysis of 38s, 30s, 28s, 18s, 5s, and 4s RNAs.
MATERIALS AND METHODS

Drosophila stocks were grown on a yeast-cornmeal agar medium. For isotope incorporation experiments, virgin females were collected and kept with X/0 sterile males for 7 days. Males were used to stimulate oviposition. Sterile males were used to avoid production of developing embryos that, if retained by the female, are able to incorporate $^3$H uridine and alter the results with their different genotype. The conditions used produce a "steady state" in the development of the ovary, since egg laying occurs at a constant rate. The ovary contains at this stage 60% of the total fly RNA (MOHAN and RITOSSA, 1970) and 80% of the newly synthesized RNA (MOHAN and RITOSSA, 1970). After 7 days the females were injected with .2 $\mu$l each of .5 mCi/ml $^3$H uridine in Ringer solution (EPHRUSSI and BEADLE, 1936) using a Hamilton microsyringe fitted with a glass needle. In these experiments $^3$H uridine was injected into the abdominal cavity (usually over a 5-minute period, for 25 flies) and incubated at 25°C for a total of 30 minutes. This allowed full recovery of the flies, before freezing them at -20°C.

Stocks

Canton S (wild type) flies were made isogenic for the first, second, and third chromosomes to homogenize the background. All stocks used were prepared by crossing repeatedly against this Canton S isogenic line to reduce variation due to background modifiers. For the analysis of the different bobbed alleles, the scheme shown in Figure 2 was used. Bobbed is a sex-linked, recessive, pleiotropic gene. The phenotype is characterized by short and thin bristles, etched abdomen, and delayed development.
Cleaned of background and adjacent markers by crossing to Canton S repeatedly

One individual hemizygous \( \mathcal{O} \) (\( bb^x/y \ sc^4 sc^8 \))

Stock was derived from a single X chromosome of \( \mathcal{O} \) and inbred for 7-14 generations.

Kept in vials for 7 days, until injection of isotope and biochemical analysis.

Figure 2. Diagram showing the procedure used for producing the \( bb \) stocks analyzed. \( bb \): bobbed, \( y \): yellow body color, \( sc \): scutellar bristles missing, \( w \): white eye, \( B_S \): Bar of Stone, Bar eve.
Each stock was derived from 1 single bobbed chromosome to insure uniformity. The original genetic composition of the stocks and the place where they were obtained from are shown in Table 1. They are ordered in this table according to the degree of intensity of the phenotype.

Bristle length was taken as a quantitative character proportional to the intensity of the phenotype. The two longest posterior scutellar bristles were measured with a graduated ocular micrometer. Several of the _bb_ in the table (bb^3, bb^4, bb^2, and bb^5) behaved as revertants.

Stocks with 2 RTU redons were obtained from the Oak Ridge collection and are the same as the ones used by RITOSSA *et al.* (1966) to show the location of rDNA. These originated by crossing over between two slightly unequal inversions, as shown in Figure 3. Crossover events along the continuous line gave chromosomes with 2 RTU redons while reciprocal crossovers (along the dotted line) gave chromosomes with no RTU redons, like the _y sc^4 sc_8 used in this work. The chromosome with two RTU redons has the left end of inversion _sc_ sc^51 and the right end of _sc^4 (sc_81 sc_8^R_).

For the chromosome with no rDNA, a reciprocal recombinant (for technical reasons with a slightly different inversion) which has the left end of the _sc^4_ inversion and the right end of one similar to _sc_ sc^51, called _sc_8, was used.

Duplications for the 5s redon were obtained from a translocation induced with X rays by P. ROBERTS (LINDSLEY and GRELL, 1968). A piece of the second chromosome containing the 56 EF band was translocated to the third chromosome. Figure 4 shows diagrammatically the constitution of the stock in which the translocation is maintained. This allowed construction of stocks with 2, 3, or 4 5s redons, but it was not possible to
Table 1. Phenotypic characterization of the bb stocks used

<table>
<thead>
<tr>
<th>Name of stock</th>
<th>Origin of stock</th>
<th>Developmental delay for hemizygous females in relation to normal males from the same bottle</th>
<th>Bristle length in hemizygous females (microns)</th>
<th>Additional Information</th>
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<tr>
<td>Canton</td>
<td>Our laboratory</td>
<td>None detected</td>
<td>452 ± 5 (16)</td>
<td>rDNA = 0.370% or 100% of Canton (Quagliarrotti and Ritossa, 1968)</td>
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<td>bb&lt;sup&gt;3&lt;/sup&gt; svr&lt;sup&gt;poi&lt;/sup&gt; dish bb&lt;sup&gt;G3&lt;/sup&gt;</td>
<td>Caltech.</td>
<td>None detected</td>
<td>458 ± 4 (16)</td>
<td></td>
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<tr>
<td>bb&lt;sup&gt;4&lt;/sup&gt; w sn bb</td>
<td>Bowling Green</td>
<td>None detected</td>
<td>457 ± 5 (16)</td>
<td></td>
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<tr>
<td>bb&lt;sup&gt;2&lt;/sup&gt; lh B car bb</td>
<td>Caltech.</td>
<td>None detected</td>
<td>446 ± 5 (19)</td>
<td></td>
</tr>
<tr>
<td>bb&lt;sup&gt;5&lt;/sup&gt; Df(l) y v car bb&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Caltech.</td>
<td>None detected</td>
<td>432 ± 5 (16)</td>
<td></td>
</tr>
<tr>
<td>bb&lt;sup&gt;11&lt;/sup&gt; g&lt;sup&gt;11&lt;/sup&gt; bb</td>
<td>Bowling Green</td>
<td>12 hours</td>
<td>358 ± 7 (29)</td>
<td></td>
</tr>
<tr>
<td>bb&lt;sup&gt;V&lt;/sup&gt;</td>
<td>Dr. Ruby Valencia Madison, Wisconsin</td>
<td>32-48 hours</td>
<td>286 ± 9 (12)</td>
<td>rDNA = 0.168% or 45% of Canton (Quagliarrotti and Ritossa, 1968)</td>
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<tr>
<td>bb&lt;sup&gt;6&lt;/sup&gt; car bb</td>
<td></td>
<td>32-48 hours</td>
<td>243 ± 8 (15)</td>
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<tr>
<td>bb&lt;sup&gt;N&lt;/sup&gt; wi f&lt;sup&gt;3&lt;/sup&gt; bb&lt;sup&gt;N&lt;/sup&gt;</td>
<td>Caltech.</td>
<td>32-48 hours</td>
<td>223 ± 5 (20)</td>
<td></td>
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</table>
Figure 3. Diagram showing the origin of X chromosomes with 2 or no RTU redons per chromosome.

A - Normal X chromosome, (•) centromere, (□) rDNA.
B - Inversion sc, showing the altered position of the rDNA, (§) breakpoints.
C - Inversion sc, showing the unaltered position of the rDNA.

Figure 4. Diagrammatic representation of T(2;3)C328. A piece of the second chromosome, including the 5s redon (□□□□) at 56 EF (position in map of salivary gland chromosome), was translocated to the third. The deficiency is not viable without the duplication, but the duplication can survive with or without the deficiency present. Cy stands for the dominant "Curly" wings, (•) centromeres.
construct a stock with just one 5s redon because the deficiency in this stock is too large to be viable in the hemizygous condition. Triploid stocks were obtained from the California Institute of Technology collection.

**RNA extraction**

RNA extraction was done according to the method of Hastings and Kirby (1966). Twenty-five flies were homogenized in 1 ml of cold 0.5% disodiumnaphthalene 1,5-disulphonate (NDS), 30 mM trishydroxymethylaminomethane (TRIS), .15 M NaCl, and 3 ml of water saturated phenol-cresol-hydroxyquinoline mixture (500 g of redistilled phenol, 70 ml of redistilled cresol, and 0.5 g of 8-hydroxyquinoline). A teflon-glass pestle was used to homogenize the mixture at 0-4°C in centrifuge tubes. After centrifugation for 10 minutes at 3,000 g, the aqueous layer was separated and the phenol layer re-extracted with 1 ml of 1% triisopropynaphthalene sulphonate (TIPNS) containing 5% 4-aminosalycilate (4-AS). After a second centrifugation, the aqueous layers were pooled and washed with ether. Excess ether was evaporated with air, and 2 volumes of cold ethanol-sodium acetate (9 volumes of ethanol 95% and 1 volume of 1M Na acetate pH 5.5) were used to precipitate the nucleic acids at -20°C overnight. The white precipitate was dissolved in SSC (NaCl .15 M, Na citrate .015 M) containing 5% sucrose.

**Electrophoresis**

Electrophoresis was carried out in compound acrylamide gels made of two layers of different concentration. The upper layer contained 2.5% acrylamide and the lower 10% acrylamide. Gels were prepared as described
by LOENING (1967) and BISHOP, CLAYBROOK, and SPIEGELMAN (1967). Acrylamide was recrystallized from chloroform and N,N'-methylenebis-acrylamide from acetone. Ethylenediacrylate cross-linked gels were used at a later stage, when it was realized that radioactivity could be counted more efficiently with them. Also, ethylenediacrylate gels have low enough optical density as to make preswelling unnecessary, which assures greater uniformity and speed. Gels were cast in tubes of 0.6 cm internal diameter by 9 cm, using a dialysis membrane stretched across the bottom for pouring. Once cast, the gels were prerun for 45 minutes in the electrophoresis buffer (30 mM TRIS pH 7.8, 36 mM NaH₂PO₄, 1 mM EDTA, and .2% sodium dodecyl sulphate, SDS). All runs and preruns were done at 5mA/tube and with a voltage gradient of 6-7 volt/cm using a Buchler current regulated power supply and a Buchler Polyanalyst electrophoresis apparatus, at room temperature (20-22°C). Samples were applied in a maximum volume of 50 µl and run for 200 minutes.

After the run, gels were pushed out of the tubes into a 0.5 x 1.5 x 10 cm quartz curvette and scanned at 260 nm in a Gilford 240 with an attached recorder, using a 0.2 mm fixed slit. Amounts of RNA were calculated from the tracings by cutting out and weighing the areas corresponding to the different classes of RNA.

**Measurement of radioactivity**

The method described by WEINBERG and PENMAN (1968) was used for radioactivity measurements. After scanning of the gels at 260 nm, they were frozen and sliced with a manifold of razor blades 1.5 mm apart. Each slice was dropped in a scintillation vial and digested with 0.5 ml of
concentrated NH$_4$OH at 50°C for 2-4 hours. To this, a water miscible scintillant was added which contained 6 parts of toluene based scintillator (4.75 g/l PPO, 0.095 g/l dimethyl POPOP, reagent grade toluene) and 4 parts of ethoxyethanol. Samples were counted either in a Packard 3320 or 3380 liquid scintillation counter. The counting efficiency was 15% using this system.
RESULTS AND DISCUSSION

A quantitative analysis of the presence and synthesis of 38s, 28s, 18s, 5s, and 4s RNAs (tRNA) depends on (1) a complete separation of all components and (2) a reproducible quantitation of each component. Consequently, using polyacrylamide gel electrophoresis, a system has been adapted to meet these requirements (LOENING, 1967; WEINBERG and PENMAN, 1968). A discontinuous polyacrylamide gel was used, made up of 2.5% acrylamide in the upper layer and 10% acrylamide in the lower layer.

To determine the validity of separation of the various RNA species, molecular weights were calculated from their electrophoretic mobilities. A proportionality exists between the electrophoretic mobility and the log of the molecular weight of RNA (BISHOP, CLAYBROOK, and SPIEGELMAN, 1967). *Escherichia coli* RNA (phenol extracted from alumina-ground 30,000 x g supernatant, containing ribosomes, 5s RNA and tRNA, kindly provided by Dr. A. G. Atherly) was used as a standard. The molecular weight values obtained for *Drosophila* RNA (extracted as described in materials and methods) were coincident with the values published by LOENING (1968), as shown in Figure 7. Excellent separation of all classes of RNA was obtained, as can be seen by the 260 nm O.D. scan of Figure 5 for *E. coli* RNA or by the combined O.D., radioactivity profile of *Drosophila* RNA in Figure 6. To further establish the validity of the separations obtained, molecular weight determinations were made on the 38s and the 30s precursors (see Figure 1). The results obtained (1.58 x 10^6 and 1.65 x 10^6 daltons) for the 30s precursor (see Figures 6 and 8) are in excellent agreement with the value of 1.60 x 10^6 daltons published by PERRY, CHENG, FREED,
Figure 5. Scan at 260 nm of acrylamide gel run with *Escherichia coli* RNA. The peak at the gel transition was present even in blank gels. The positions of the 28s and 18s RNAs of *Drosophila* (data from a parallel gel) are indicated by the arrows. Electrophoresis was for 180 minutes at 5mA/tube. Figure is a 0.6 x photographic reduction of original scan. To the left of the gel transition, acrylamide was 2.88%, to the right 10%. Acrylamide was cross-linked with N,N'-methylene bisacrylamide, and gels preswollen for 48 hours to reduce background O.D.
Figure 6. Gel scan (-----) and radioactivity profile (––) of Canton/y sc^4 sc^8 females injected with H\(^3\) uridine and incubated for 30 minutes at 25°C. To the left of the gel transition acrylamide concentration is 2.5%, to the right 10%. Gels were cross-linked with ethylene-diacrylate, and electrophoresis was for 200 minutes at 5mA gel. The position of the 38s, 30s, 28s, 18s, 5s, and 4s molecules are indicated. Using the published values for 28s and 18s as molecular weight standards, in this particular case we obtain for the 38s molecule a value of 2.9 x 10\(^6\) daltons and for the 30s, a value of 1.58 x 10\(^6\) daltons. The original figure has been photographically reduced (0.6x) to fit the page.
O.D. at 260 nm

Incorporation of H^3 uridine (cpm x 10^-2)

Gel transition

38s

Transo 28s

5s

3s

1s

cm migrated

0.5 1.0 1.5 2.0 2.5
Figure 7. Determination of the molecular weight of degraded RNA. Data is based on a run of 2 parallel gels of 2.8% acrylamide, cross-linked with bisacrylamide and preswollen in electrophoresis buffer for 48 hours to remove background O.D. Runs were for 240 minutes at 5mA/tube. Mobilities are expressed relative to 4-5s RNA mobility (4s and 5s are not separated in this conditions).

(△) - Drosophila normal ribosomal RNA (28 and 18s)

(□) - E. coli ribosomal RNA (23s and 16s)

(○) - Drosophila degraded RNA
Figure 8. Determination of the molecular weight of 30s rRNA, data from $^3$H uridine incorporation. Compare the value of $1.65 \times 10^6$ (PERRY, CHENG, FREED, GREENBERG, KELLEY and TARTOF, 1970). Drosophila RNA, (•) data from radioactive incorporation, (A) Drosophila 28 and 18s rRNA, (o) Drosophila degraded 28s RNA. Molecular weights are indicated, Drosophila RNA used as a standard. Gels were 2.8% acrylamide cross-linked with ethylene diacrylate, run for 180 minutes at 5mA/tube.
GREENBERG, KELLEY, and TARTOF (1970). For 38s RTU RNA a value of 2.8-2.9 x 10^6 daltons was obtained (see Figures 6 and 9), which coincides with the values obtained by TARTOF and PERRY (1970) of 2.8 x 10^6 daltons and by MOHAN and RITOSSA (1970) of 2.9 x 10^6 daltons. With reference to the low molecular weight components 5s and 4s RNAs of *E. coli* and *Drosophila* migrated at the same rate in comparable gels. The radioactivity tracing (see Figure 6) in the 5s region of *Drosophila* RNA is not sharply defined and might indicate the existence of a heavier precursor to the 5s molecule. However, further analysis is needed to establish this point.

To calculate the amount present of each class of RNA, the area under the O.D. scan was determined. To estimate baseline levels, blank gels were scanned and the O.D. value of this subtracted from the final reading. Since the residual O.D. is lower for the 2.5% acrylamide section than for the 10% acrylamide, the resulting scans were replotted to take into account this difference. Tracings of each 28s, 18s, 5s, and 4s area were cut out and weighed. For example:

<table>
<thead>
<tr>
<th></th>
<th>28s</th>
<th>18s</th>
<th>5s</th>
<th>4s</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg area</td>
<td>140.3</td>
<td>71.6</td>
<td>5.6</td>
<td>33.9</td>
</tr>
</tbody>
</table>

The areas were then divided by the molecular weight of the respective component, giving the number of molecules in arbitrary units.

<table>
<thead>
<tr>
<th></th>
<th>28s</th>
<th>18s</th>
<th>5s</th>
<th>4s</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg area</td>
<td>140.3</td>
<td>71.6</td>
<td>5.6</td>
<td>33.9</td>
</tr>
<tr>
<td>daltons</td>
<td>1.4 x 10^6</td>
<td>.7 x 10^6</td>
<td>4 x 10^4</td>
<td>2.4 x 10^4</td>
</tr>
<tr>
<td>no. of molecules</td>
<td>1.00 x 10^{-4}</td>
<td>1.02 x 10^{-4}</td>
<td>1.40 x 10^{-4}</td>
<td>14.12 x 10^{-4}</td>
</tr>
</tbody>
</table>
Figure 9. Molecular weight determination of RTU RNA (38s). Combined data from 260 nm (△) scan and H³ uridine incorporation (●; ●). Value for 38s (●, 2.8 x 10⁶) is the same as published by TARTOF and PERRY (1970). Gels as in Figure 8.
From these data, the number of molecules per each 28s molecule (or per ribosome) was calculated

<table>
<thead>
<tr>
<th></th>
<th>28s</th>
<th>18s</th>
<th>5s</th>
<th>4s</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of molecules</td>
<td>1.00 x 10^{-4}</td>
<td>1.02 x 10^{-4}</td>
<td>1.40 x 10^{-4}</td>
<td>14.12 x 10^{-4}</td>
</tr>
<tr>
<td>no. of 28s molecules</td>
<td>1.00 x 10^{-4}</td>
<td>1.00 x 10^{-4}</td>
<td>1.00 x 10^{-4}</td>
<td>1.00 x 10^{-4}</td>
</tr>
<tr>
<td>no. of molecules per ribosome</td>
<td>1</td>
<td>1.02</td>
<td>1.40</td>
<td>14.12</td>
</tr>
</tbody>
</table>

For specific activity calculations of each RNA species, the radioactivity incorporated under each area for an RNA species was considered in relation to the weight of that same area. An example is shown in Figure 6. It can be seen that very little 28s precursor is accumulating under the conditions of this experiment. Doing the experiments in vivo (by injection), we see that rRNA processing is normal, with little accumulation of 38s precursor, contrary to what occurs when organs are incubated in vitro (MOHAN and RITOSSA, 1970; PETRI, FRISTROM, STEWART, and HANLY, 1971; and our own results). The radioactivity in the 38s peak was not taken into consideration when measuring rRNA synthesis. The 30s precursor makes a continuum with the 28s component, and the radioactivity for both components was pooled and considered as 28s rRNA. The separation of radioactivity between the 5s and 4s components is not excellent, so for the point in between, half of the counts were assigned to 5s RNA and half to tRNA. From Figure 6, it can also be seen that the cpm incorporated during a 30-minute incubation are sufficient for reasonable accuracy, the minimum being around 200 cpm for the 5s fraction. Background is very low (up to 7-14 cpm per slice).
The presence of unexplainable peaks was observed in earlier *Drosophila* RNA preparations. Two extra peaks, one between 28s and 18s RNA and one lighter than 18s, were observed (Figure 10). It was believed that these were mitochondrial RNAs, although the quantities observed showed great variability from experiment to experiment. Molecular weight determinations with comparison to published values of mitochondrial RNA showed that the molecular weights of the spurious peaks were quite different from mitochondrial RNA values. Values published by MONTENECOURT, LANGSAM, and DUBIN (1970) for mouse and hamster mitochondrial RNA were \(0.75 - 0.79 \times 10^6\) daltons and \(0.42 - 0.45 \times 10^6\) daltons for the large and small mitochondrial components, respectively. However, the sum of the molecular weights of the two unidentified components give a value corresponding to the molecular weight of the 28s rRNA \(0.97 \times 10^6\) daltons + \(0.43 \times 10^6\) daltons = \(1.40 \times 10^6\) daltons), as can be seen in Figure 7. PETRI, FRISTROM, STEWART, and HANLY (1971) showed that at \(40^\circ C\) a nick is introduced into 28s rRNA that would result in the formation of two lower molecular weight species. Treatment with DEPC (diethylpyrocarbonate, a ribonuclease inhibitor) or with dithiothreitol (DTT) during RNA preparation did not improve the results. However, degradation of the 28s rRNA very likely occurred during homogenization of the cells, which results in an increase in temperature due to friction of the motor driven pestle. Cooling of the pestle, plus a lower speed avoided the problem and the two mysterious peaks completely disappeared, as can be seen in Figure 6.

The following sections will present results obtained with the different genotypes tested. For better understanding, they have been subdivided in: (1) variations in RTU redon size, where the effect of the \(bb\) gene is
Figure 10. RNA extracted from adult flies was run for 120 minutes in 7% acrylamide-bisacrylamide gels. The ratio of 28s to 18s RNA by area is 1.41. Transfer RNA (4s) is not separated from 5s RNA in this gel. The peaks marked are the ones that are thought to be degradation products of 28s rRNA.
analyzed, (2) the effect of redon dosage, where we will consider variations in the number of RTU and 5s RNA redons, and (3) the effect of α-amanitin. A consideration will be made of the effects on the total amounts of each class of RNA, and effects on rate of synthesis of each class of RNA.

The Effect of Redon Size on the Amounts of RNA Synthesized

In order to determine if a regulatory mechanism for 5s RNA and rRNA exists in Drosophila, an analysis of the relative concentrations of the compounds (rRNA and 5s RNA) can be made in bb mutants. Bobbed mutants, that have RTU redons of reduced size, were obtained from various sources for these experiments (listed in Table 1). The preparation of Drosophila melanogaster for chemical analysis of RNA amounts and $^{3}H$ uridine incorporation will be briefly described followed by an analysis of the results obtained.

The criterion for arranging the stocks in Table 1 is bristle length. Since bb is a continuously variable characteristic, the intensity of the phenotype is considered to be proportional to the size of the RTU redon (RITOSSA, ATWOOD, and SPIEGELMAN, 1966). The intensity of the phenotype was quantitated using bristle length measurements. Bristle length is easily measured with a graduated ocular in the microscope, and the measurements in hemizygous females show reasonably small standard errors (Table 1). Bristle length was used earlier to eliminate spontaneous bbs from the wild-type stock. Some of the stocks used here behaved as revertants from bb, which can be deduced by measuring bristle lengths and developmental delays (Table 1).
The data from the analysis of the relative amounts of each RNA species are shown in Table 2. Results are expressed as the number of tRNA or 5s RNA molecules per ribosome (or per 28s rRNA). The results show that the amount of tRNA per ribosome remains constant with variations in RTU redon size from 100% of wild type (Canton) to less than 45% of wild type (bb⁶−bb⁴). MOHAN and RITOSSA (1970), analyzing RNA/DNA ratios, found that the total amount of RNA synthesized was the same whatever the size of the RTU redon, i.e., if they used bb or not. It is possible that the total amount of rRNA is reduced while the amounts of tRNA increases resulting in the same total amount of RNA. However, this is not the case, as the results of Table 2 demonstrate. The number of tRNA molecules per ribosome (approximately 16-18) is similar in bacteria where 13 tRNA molecules per ribosome are found (WATSON, 1965; also see last line in Table 2).

The number of 5s RNA molecules per ribosome is also shown in Table 2. There is more than one 5s RNA molecule per ribosome, although isolated ribosomes only have one 5s RNA molecule per ribosome. This means that some 5s RNA molecules are not attached to ribosomes. Consequently, these unattached molecules form an intracellular pool of 5s RNA, as already shown for other organisms (ATTARDI and AMALDI, 1970). With RTU redons of size varying all the way from wild type to 45% of the wild-type value (bb⁶), the amounts of 5s RNA remain constant per 28s RNA species with some exceptions. The results obtained for three of the genotypes analyzed deserve some additional comment. The results shown for bb⁵/y sc⁴ sc⁸, which behaves like a bb revertant, suggest that the amounts of 5s RNA and tRNA per ribosome are higher than in wild type (167% for 5s RNA and 130% for tRNA). Because a comparison was made between amounts of rRNA
Table 2. Amounts of RNA synthesized with redons of different size

The number of 5s RNA and transfer RNA molecules relative to the number of 28s molecules in bobbed mutants is shown. Data calculated from areas of 260 nm scan. Standard error is shown after values of 3 or more observations. Bristle lengths (from Table 1) are shown to evaluate the intensity of the bb phenotype.

<table>
<thead>
<tr>
<th>Genotype of females</th>
<th>Bristle length (μ)</th>
<th>Number of observations</th>
<th>Number of 5s RNA molecules/28s molecule</th>
<th>Number of tRNA molecules/28s molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton/y sc⁴ sc⁸</td>
<td>452</td>
<td>10</td>
<td>1.53 ± .18</td>
<td>17.8 ± 1.5</td>
</tr>
<tr>
<td>bb³/y sc⁴ sc⁸</td>
<td>458</td>
<td>3</td>
<td>1.39 ± .18</td>
<td>16.4 ± 2.0</td>
</tr>
<tr>
<td>bb²/y sc⁴ sc⁸</td>
<td>446</td>
<td>1</td>
<td>1.42</td>
<td>14.8</td>
</tr>
<tr>
<td>bb⁵/y sc⁴ sc⁸</td>
<td>432</td>
<td>3</td>
<td>2.50 ± .08</td>
<td>24.6 ± 3.4</td>
</tr>
<tr>
<td>bb¹¹/y sc⁴ sc⁸</td>
<td>358</td>
<td>3</td>
<td>1.64 ± .07</td>
<td>17.1 ± 1.2</td>
</tr>
<tr>
<td>bb⁵/y sc⁴ sc⁸</td>
<td>286</td>
<td>9</td>
<td>1.47 ± .04</td>
<td>15.5 ± 1.2</td>
</tr>
<tr>
<td>bb⁶/y sc⁴ sc⁸</td>
<td>243</td>
<td>3</td>
<td>.71 ± .18</td>
<td>12.0 ± .6</td>
</tr>
<tr>
<td>bb⁶/y sc⁴ sc⁸</td>
<td>223</td>
<td>3</td>
<td>2.31 ± 1.08*</td>
<td>24.02 ± 9.22</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>1</td>
<td>2.09</td>
<td>13.4</td>
</tr>
</tbody>
</table>

*Standard error of this set of data is unusually large because of low viability of the stock (it is a very extreme bb, semilethal). The flies for each experiment had to be collected over several days.
relative to the amounts of 5s RNA or tRNA, it is not possible to tell if
the difference found for \( \text{bb}^6/\text{y sc}^4 \text{ sc}^8 \) is due to an increase in the
amounts of 5s RNA and tRNA or to a decrease in the amount of rRNA. In
one particular set of analysis, the amount of tRNA and 5s RNA extracted
per fly in \( \text{bb}^5 \) was double the amount extracted from wild type. So prob­
ably we are in the presence of an increase in 5s RNA and tRNA. However,
the important point is that the amounts of rRNA and 5s RNA seem to vary
in opposite directions, i.e., their control appears to be independent,
or they may be negatively correlated, in this case. The \( \text{bb}^6/\text{y sc}^4 \text{ sc}^8 \) flies show (Table 2) a reduction in the total levels of 5s RNA, while
\( \text{bb}^6/\text{y sc}^4 \text{ sc}^8 \) does not seem to be affected. Since \( \text{bb}^6 \) is a more extreme
phenotype than \( \text{bb}^6 \) (see Table 1), we can presume that its RTU redon is
smaller (i.e., has less copies) and consequently affects rRNA synthesis to
a greater degree. This suggests that when rRNA synthesis is affected
below a certain threshold amount, 5s RNA relative levels are also affect­
ed, and a reduction in the 5s RNA pool occurs. We cannot tell what
happens in \( \text{bb}^N/\text{y sc}^4 \text{ sc}^8 \) (Table 2) due to the huge standard error of these
determinations. This phenotype \( \text{bb}^N \) behaves as a semilethal, and it is
very difficult to collect sufficient flies to make accurate determina­
tions. The age of the flies was not as uniform due to this fact, and the
data cannot be used to support or deny the above conclusions. The slight
reduction in the number of tRNA molecules per ribosome in \( \text{bb}^6/\text{y sc}^4 \text{ sc}^8 \)
is not considered statistically significant.

Summarizing the data from this table, it can be seen that the amounts
of 5s RNA and tRNA relative to rRNA found in genotypes with RTU redons of
different sizes are independent of the RTU redon size, with one exception.
When the RTU redon size is below a certain threshold (50% of the wild-type redon size), the 5s RNA pool is significantly reduced. We can also say that in one other case (bb^5), there was an increase in the levels of 5s RNA and tRNA per ribosome, or that when 5s RNA amounts were increased in relation to wild type, rRNA levels were not affected.

The Effect of Redon Size on the Rates of RNA Synthesis

The results of the analysis of the rates of RNA synthesis in the different genotypes of Drosophila melanogaster will be described. The rate of RNA synthesis was followed by incorporation of radioactive uridine into RNA. Injections of H\(^3\) uridine, as described above, followed by incubation for 30 minutes were used to measure rates of rRNA synthesis. To compensate for varied efficiencies of RNA extraction, the cpm incorporated were considered in relation to the amount of the respective class of RNA isolated in a specific experiment and expressed as specific activities. Since the amounts of each kind of RNA were calculated directly from the weight of traced areas, they could be expressed as cpm incorporated in 30 minutes/mg area. To avoid differences in pool size of the different genotypes, the specific activity of 28s, 18s, or 5s RNA were compared relative to the specific activity of tRNA. Two assumptions are needed to justify this procedure: (1) rRNA, 5s RNA, and tRNA draw their precursors from the same nuclear pool, and (2) the genes for tRNA are not duplicated or reduced in number in the duplications or deficiencies used throughout this work. Some evidence has been presented by WU and SOEIRO (1971) to justify the first assumption. With reference to the second assumption, preliminary evidence is available that tRNA genes are distributed along the whole
genome, and each class of tRNA may correspond to one of the 55 mutants known as Minutes in Drosophila (LINDSLEY and GRELL, 1968). RITOSSA, ATWOOD, LINDSLEY, and SPIEGELMAN (1966) showed that tRNA genes are not included in the rDNA duplication \( \text{sc}^1 \text{sc}^4 \), and it seems improbable that they all are linked to the 5s rDNA, so as to be in the 56 EF duplication. Preliminary evidence presented by STEFFENSEN (private communication) using RNA-DNA hybridization "in situ" followed by autoradiography, supports the idea of a relationship between tRNA-DNA and the Minute loci.

The results obtained from the analysis of the rates of rRNA synthesis in relation to the rate of tRNA synthesis is presented in Table 3. Confirming the results of MOHAN and RITOSSA (1970) it was found that the rate of rRNA synthesis is reduced in \( b^6 \) mutants, i.e., when the RTU redon size is reduced. An interesting circumstance is the fact that the rate of rRNA synthesis seems to be directly proportional to the parameter used to measure the intensity of the phenotype, that is bristle length. A plot of these results is presented in Figure 11. Since the rDNA of \( b^6 \) has been measured (QUAGLIAROTTI and RITOSSA, 1968) and shown to be 45% of the rDNA found in Canton wild type, we can determine (in the upper border of Figure 11) a proportionality between rDNA amounts and bristle length. This would allow a prediction of "equivalent redon sizes". For example, \( b^{11} \) behaves as if its RTU redon was 77% the size of a wild-type RTU redon. Since the actual redon size is not known, this cannot be confirmed as a fact. The threshold, below which rRNA rate of synthesis is not enough for the flies to survive, determines the limit of bristle length. Because \( b^N \) behaves as a semilethal, the known \( b^b \) lethals probably have less than 40% of the rDNA (or RTU redon) of the wild type.
Table 3. Rates of RNA synthesis with redons of different sizes

The number of cpm/mg-area of ribosomal or 5s RNA relative to cpm/mg-area tRNA incorporated in 30 minutes in different \textit{bb} genotypes is indicated. Standard errors follow the values when 3 or more observations were made. Bristle lengths were used to evaluate the intensity of the \textit{bb} phenotype.

<table>
<thead>
<tr>
<th>Genotypes of females</th>
<th>Bristle length ((\mu))</th>
<th>Number of observations</th>
<th>cpm/mg-area of 28s rRNA per cpm/mg-area of tRNA</th>
<th>cpm/mg-area of 18s rRNA per cpm/mg-area of tRNA</th>
<th>cpm/mg-area of 5s RNA per cpm/mg-area of tRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton/(y) sc(^4) sc(^8)</td>
<td>452</td>
<td>7</td>
<td>1.47 ± .39</td>
<td>1.80 ± .49</td>
<td>3.83 ± .31</td>
</tr>
<tr>
<td>(bb^5/y) sc(^4) sc(^8)</td>
<td>432</td>
<td>2</td>
<td>1.44</td>
<td>1.75</td>
<td>3.81</td>
</tr>
<tr>
<td>(bb^11/y) sc(^4) sc(^8)</td>
<td>358</td>
<td>2</td>
<td>1.11</td>
<td>1.23</td>
<td>3.30</td>
</tr>
<tr>
<td>(bb^V/y) sc(^4) sc(^8)</td>
<td>286</td>
<td>5</td>
<td>.71 ± .06</td>
<td>.49 ± .12</td>
<td>3.28 ± .40</td>
</tr>
<tr>
<td>(bb^6/y) sc(^4) sc(^8)</td>
<td>243</td>
<td>3</td>
<td>.54 ± .10</td>
<td>.69 ± .21</td>
<td>4.01 ± .88</td>
</tr>
<tr>
<td>(bb^N/y) sc(^4) sc(^8)</td>
<td>223</td>
<td>1</td>
<td>.41</td>
<td>1.09</td>
<td>2.49</td>
</tr>
</tbody>
</table>
Figure 11. Relation between the relative rate of rRNA synthesis and the intensity of the bobbed phenotype, as measured by bristle length. The genotype of the respective hemizygous females is indicated. Prediction of RTU redon size would be \( \text{bb}^N:39\%; \text{bb}^6\) (QUAGLIARROTTI and RITOSSA, 1968); \(45\%\); \(\text{bb}^V:55\%\); \(\text{bb}^{11}:77\%\); Canton and \(\text{bb}^5:100\%\).
Also, in Table 3, the analysis of the rate of 5s RNA synthesis is presented. The data shows very clearly that even with a large reduction in the rate of rRNA synthesis, as in \( bb^6 \) (30% of controls) the rate of synthesis of 5s RNA is not affected. These data are in agreement with data presented by ZYLBER and PENMAN (1971), which showed that metaphase-arrested cells, which do not make new rRNA, still synthesize 5s RNA at a normal rate. PERRY and KELLEY (1968, 1970) also showed that actinomycin D doses that inhibited rRNA synthesis did not affect 5s RNA synthesis. Thus these data would suggest that the regulation of the rate of 5s RNA synthesis is independent of the rate of rRNA synthesis. However, a lower rate of rRNA synthesis in \( bb^N \) results in what again looks like a threshold effect on the rate of 5s RNA synthesis, which is reduced.

Two mechanisms could be proposed to explain the effect on the extent of 5s RNA synthesis and the rate of 5s RNA synthesis. Either: (1) the rate of 5s RNA is affected when the amount of rDNA is below a threshold level, or (2) the degradation of 5s RNA is speeded up when the rate of rRNA synthesis is very low (i.e., when rDNA is below the threshold). A preference exists for the second alternative since in the case of \( bb^6 \), the rate of 5s RNA does not seem to be affected (Table 3) while the relative level of 5s RNA found is lower (Table 2). If 5s RNA is synthesized in the nucleus and degraded in the cytoplasm, the rates would be unaffected while the amounts found in the cytoplasm would be lower. In the case of \( bb^N \), probably both the rate of synthesis in the nucleus and the total levels (in the cytoplasm) are affected. Consequently, within a range of variations of rates of rRNA synthesis (\( bb^+, bb^{11}, bb^V \)), the synthesis of 5s RNA is not affected, but when rRNA synthesis is below a certain thresh-
old level \((bb^6, bb^N)\), 5s RNA total levels (probably because of degradation as in \(bb^6\)) and 5s RNA rates of synthesis (probably affecting directly the regulation mechanism as in \(bb^N\)) are also altered. In the case of \(bb^N\) and \(bb^6\), the results could also be explained by the pleiotropic effect of \(bb\) on the whole metabolism of the individual, so that even 5s RNA synthesis is lower.

Effect of Varying the Doses of the RTU Redons

The effect of variations in the number of doses of RTU redons on the amount and rate of synthesis of the different classes of RNA will be analyzed. For this purpose, individuals with 1 to 4 doses of RTU redons can be obtained using the duplication \(sc^1 sc^4\) (2 RTU redons) and the deficiency \(sc^4 sc^8\) (no RTU redons). These were obtained by crossing over between two unequal inversions, as shown in Figure 3. Triploids were also used in combination with these duplications and deficiencies to determine if modifiers in the rest of the genome affected RNA synthesis differentially.

The results obtained for the number of 5s RNA and tRNA molecules per rRNA molecule in the different genotypes is indicated in Table 4. These data show that doses of 1, 2, 3, or 4 RTU redons do not affect the relationship between the number of tRNA molecules and the number of ribosomes. As a result, the amount of tRNA molecules synthesized per ribosome is the same, whether we have 1, 2, 3, or 4 RTU redons. This confirms results obtained by KIEFER (1968) measuring a different parameter (KIEFER compared RNA/DNA ratios in flies with 1, 2, and 3 RTU redons). It can be seen that the amount of rRNA is the same whether the two redons are on different
Table 4. Relative amounts of RNA with different doses of RTU redons (results expressed as in Table 2)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of observations</th>
<th>Number of RTU redons</th>
<th>Number of molecules per each 28s molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton/(y \text{ sc}^4 \text{ sc}^8)</td>
<td>10</td>
<td>1</td>
<td>1.53 ± 0.18</td>
</tr>
<tr>
<td>Canton homozygous</td>
<td>2</td>
<td>2</td>
<td>1.33</td>
</tr>
<tr>
<td>(\text{sc}^1 \text{ sc}^4 / y \text{ sc}^4 \text{ sc}^8)</td>
<td>2</td>
<td>2</td>
<td>1.38</td>
</tr>
<tr>
<td>(\text{sc}^1 \text{ sc}^4 ) homozygous F1</td>
<td>2</td>
<td>4</td>
<td>1.04</td>
</tr>
<tr>
<td>(\text{sc}^1 \text{ sc}^4 ) homozygous F8</td>
<td>2</td>
<td>4</td>
<td>1.12</td>
</tr>
<tr>
<td>(y \text{ sc}^4 \text{ sc}^8 / C(1)RM) triploid</td>
<td>1</td>
<td>2</td>
<td>1.55</td>
</tr>
<tr>
<td>+/-C(1)RM triploid</td>
<td>1</td>
<td>3</td>
<td>1.59</td>
</tr>
<tr>
<td>(\text{sc}^1 \text{ sc}^4 ) B/C(1)RM triploid</td>
<td>1</td>
<td>4</td>
<td>1.47</td>
</tr>
</tbody>
</table>

\(^a\)Data from Table 2.
chromosomes (as in Canton homozygous female) or on the same chromosome (as in $sc^1 sc^{4/2} y sc^4 sc^9$). Additional results were obtained in relation to the amount of 5s RNA demonstrating that it remains at a constant level, independent of the number of RTU redons. One exception is the case with 4 RTU redons, where the 5s RNA amount is slightly reduced. A look at Table 5 which shows the rates of rRNA and 5s RNA synthesis will suggest a similarity with the case of $bb^6$ previously discussed. There is a reduction in the rate of rRNA synthesis and to explain the low level of 5s RNA, a cytoplasmic degradation of 5s RNA is proposed, as occurred in $bb^6$. The important point is that with 4 RTU redons, the amounts of 5s RNA synthesized is not increased, and the effect on it may just be due to the reduction of rRNA synthesis.

Table 4 shows results obtained with triploid individuals. When 2, 3, or 4 RTU redons are present in triploid individuals, the amount of rRNA in relation to tRNA remains constant. The same can be said of the amounts of 5s RNA in relation to rRNA. Consequently, even in triploids, where instead of 2 we have 3 doses of all genes for tRNA, 5s RNA, ribosomal proteins, regulator genes, etc., a constant relationship is maintained between the relative amounts of the RNAs synthesized.

The rates of synthesis of rRNA and 5s RNA relative to tRNA were analyzed as described for $bbs$ and the results are shown in Table 5. Flies with 1 or 2 doses of RTU redons were found to have the same rate of synthesis of rRNA or 5s RNA in relation to tRNA synthesis. Again, a comparison can be made between the case where 2 RTU redons that are on the same or on different chromosomes. The results (Table 5) show that the rates are identical, thus indicating the absence of position effect. Conse-
Table 5. Rates of RNA synthesis with varying doses of RTU redons. Incorporation of $^{3}H$ uridine in 30 minutes (results expressed as in Table 3)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of observations</th>
<th>Number of RTU redons</th>
<th>cpm/mg-area of 28s rRNA per mg-area of tRNA</th>
<th>cpm/mg-area of 5s RNA per mg-area of tRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton/y sc$^4$ sc$^8$</td>
<td>7</td>
<td>1</td>
<td>$1.47 \pm .39$</td>
<td>$3.83 \pm .31$</td>
</tr>
<tr>
<td>Canton homozygous</td>
<td>1</td>
<td>2</td>
<td>1.48</td>
<td>3.53</td>
</tr>
<tr>
<td>sc$^1$ sc$^4$ sc$^4$ /y sc$^4$ sc$^8$</td>
<td>2</td>
<td>2</td>
<td>1.21</td>
<td>3.45</td>
</tr>
<tr>
<td>sc$^1$ sc$^4$ homozygous F1</td>
<td>2</td>
<td>4</td>
<td>.82</td>
<td>4.31</td>
</tr>
<tr>
<td>sc$^1$ sc$^4$ homozygous F8</td>
<td>2</td>
<td>4</td>
<td>.48</td>
<td>4.66</td>
</tr>
<tr>
<td>y sc$^4$ sc$^8$/C(1)RM triploid</td>
<td>2</td>
<td>2</td>
<td>1.24</td>
<td>3.66</td>
</tr>
<tr>
<td>+/-C(1)RM triploid</td>
<td>1</td>
<td>3</td>
<td>.55</td>
<td>3.76</td>
</tr>
<tr>
<td>sc$^1$ sc$^4$ B/C(1)RM triploid</td>
<td>1</td>
<td>4</td>
<td>.51</td>
<td>2.79</td>
</tr>
</tbody>
</table>

$^a$Data from Table 3.
quently, the same synthesizing potential is present in the two RTU redons of the \( \text{sc}^1 \text{ sc}^4 \) chromosome as found for Canton homozygous females (Table 5), where the two RTU redons are on different chromosomes. However, when we look at the rate of rRNA synthesis of flies with 4 RTU redons (Table 5, genotype, line 4) the results are unexpected. There is a reduction in the rate of rRNA synthesis to a level characteristic of \( \text{bb} \) flies. The flies for this experiment were obtained from the cross:

\[
\frac{\text{sc}^1 \text{ sc}^4}{y \text{ sc}^4 \text{ sc}^8} \times \frac{\text{sc}^1 \text{ sc}^4}{y}
\]

so this was the first generation that 4 RTU redons were in the same cells. A similar result was obtained using autoradiography on salivary gland nucleoli by KRIDER (1971). He compared the rate of \( ^3 \)H uridine incorporation into RNA in the nucleoli of salivary gland preparations of flies with 4 RTU redons for the first generation to wild-type flies. Using the hypothesis that the RTU redon and the nucleolar organizer are equivalent structures, he found only 2 nucleolar organizers in ganglia of genotypes with 4 RTU redons. KRIDER (1971) thus concluded that of the 4 RTU redons, only 2 are transcribed, but with less than normal activity. After 5-7 generations of inbreeding this stock with 4 RTU redons, KRIDER found 4 nucleolar organizers in ganglia and normal rate of \( ^3 \)H uridine incorporation into RNA of salivary gland nucleoli. For comparison, a stock where the \( \text{sc}^1 \text{ sc}^4 \) chromosome was maintained in homozygous condition during 8 consecutive generations was analyzed for rates of rRNA synthesis and the results are shown in Table 5 (genotype, line 5). It is seen that the rate of rRNA synthesis does not return to normal levels in ovaries after
8 generations of inbreeding. However, in salivary glands, there seemed to be a normalization of RTU redon expression (KRIDER, 1971). Simultaneously with this phenomenon, a reduction in the size of the redons has occurred, as shown by RITOSSA, ATWOOD, LINDSLEY, and SPIEGELMAN (1966). These authors showed that after inbreeding a stock with 4 RTU redons for several generations, the size of the RTU redons decreased and the total amount of rDNA tended to decrease to the value equivalent to that found in wild-type flies, possessing 2 RTU redons. Apparently after the first generation of inbreeding, repression of RTU redon transcription occurs when we have 4 RTU redons per cell. This occurs in spite of the fact that each one of the RTU redons of the $sc_1.sc^4$ chromosome can be as active as a normal RTU redon, as shown above. After the total amount of rDNA is reduced, the expression of the RTU redons does not return to normal in ovaries. This is related to the mechanism of regulation of redundancy level in a way not yet understood.

With reference to the rate of 5s RNA synthesis, it is not affected by the variations in the number of RTU redons even after 1 or 8 generations of inbreeding (Table 5). Consequently, the repression mechanism for rRNA synthesis does not affect the rate of 5s RNA synthesis. The reduced rate of rRNA synthesis of the flies with 4 RTU redons is low enough to activate the mechanism of reduction of 5s RNA levels, as proposed for bbs.

When the rates of rRNA and 5s RNA synthesis were analyzed in triploids, results showed a reduced rate of rRNA synthesis, especially in the case of 3 or 4 RTU redons. This is probably a similar effect to the one that occurs in diploids with 4 RTU redons. The rates of 5s RNA synthesis were not affected.
Summarizing, we can say that the dose of the rRNA redons does not affect the amounts of rRNA or 5s RNA synthesis except in the case of flies with 4 RTU redons, where a very low level of rRNA synthesis triggers the mechanism for regulation of 5s RNA levels. Triploids also have a reduced rate of rRNA synthesis, especially when more than 2 RTU redons are present. However, 5s RNA is normal, both in rate and amount, in this case.

Effect of Variations in the Dose of 5s RNA Redons

Different doses of the 5s redons (2 or 3) in combination with varying numbers of RTU redons (1, 2, or 3) were analyzed. In addition, triploids were used to test the effect of modifiers in the rest of the genome.

To obtain variations in the dose of the 5s redon, the translocation stock T(2;3)C328 described in Figure 4 was used. This stock, crossed for example to males with 1 RTU redon:

\[ \varphi +/+; Cy/Df; Dp/Dp \times \sigma^4 Y sc^4 sc^8 /Y; +/+; +; + \]

would give two classes of female progeny:

\[ +/y sc^4 sc^8; Cy/+; Dp/+ and +/y sc^4 sc^8; Df/+; Dp/+ \]

The first kind of females has two 5s redons in the second chromosome and a third 5s redon in the duplication, while the second kind carries one 5s redon in the second chromosome and one in the third chromosome duplication. Both have only one RTU redon in the wild-type X chromosome. Because the deficiency is not viable, flies with only 1 5s redon could not be obtained. Other variations of the RTU redons can be obtained using other males.

Analysis of the relative amounts of tRNA and 5s RNA per each 28s molecule were used as before, and results are presented in Table 6.
Table 6. Relative amounts of RNA present with varying doses of 5s redon

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of 5s redons</th>
<th>Number of RTU redons</th>
<th>Relative number of molecules per 28s molecules 5s</th>
<th>4s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton homozygous</td>
<td>2</td>
<td>2</td>
<td>1.59</td>
<td>20.7</td>
</tr>
<tr>
<td>±/±; Cy/+; Dp/+</td>
<td>3</td>
<td>2</td>
<td>.93</td>
<td>13.0</td>
</tr>
<tr>
<td>±/±; Df/+; Dp/+</td>
<td>2</td>
<td>2</td>
<td>1.87</td>
<td>18.4</td>
</tr>
<tr>
<td>y sc⁴ sc⁸/+; Cy/+; Dp/+</td>
<td>3</td>
<td>1</td>
<td>1.26</td>
<td>14.2</td>
</tr>
<tr>
<td>sc¹ sc⁴ B/+; Cy/+; Dp/+</td>
<td>3</td>
<td>3</td>
<td>1.94</td>
<td>17.9</td>
</tr>
<tr>
<td>sc¹ sc⁴ B/+; Df/+; Dp/+</td>
<td>2</td>
<td>3</td>
<td>1.35</td>
<td>15.6</td>
</tr>
<tr>
<td>±/C(1)RM; ±/+/+; ±/+/+</td>
<td>3</td>
<td>3</td>
<td>1.59</td>
<td>15.4</td>
</tr>
<tr>
<td>±/C(1)RM; ±/+/Cy; ±/+/+/Dp/+</td>
<td>4</td>
<td>3</td>
<td>.79</td>
<td>10.57</td>
</tr>
</tbody>
</table>

aData from Table 4.
First, we can compare lines 1 and 3 of Table 6. The identical result obtained when the two 5s redons are on homologous chromosomes, as in wild type, or when they are on different chromosomes, as in the translocation, indicates that the translocation in itself has no position effect. We can say that the expression of the genes for 5s RNA is the same whether they are on homologous chromosomes or not. Also, no effect on tRNA levels relative to 28s RNA is seen.

Lines 2, 4, and 5 of Table 6 show the results obtained with an increased number of 5s redons (3) with varying doses of RTU redons. We can see that with one or two RTU redons, the effect of increasing the 5s redon dosage seems to be a reduction of the relative amounts of 5s RNA present. However, when 3 RTU redons are present (a 1:1 redon relationship) the relative amounts of 5s RNA return to a normal level. The amounts of tRNA per ribosome seem to be slightly lower, but not significantly in lines 2 and 4. They are equal to wild type for line 5 (3 RTU redons, 3 5s redons). The case with 3 RTU redons and 2 5s redons in the translocation (line 6) behaves normally, or nearly so, with the slight reduction in 5s RNA making it more similar to the case of 4 RTU redons in Table 4.

A triploid was used because it provided an opportunity to compare the case of 3 RTU redons, 3 5s redons (line 5) with 3 whole genomes (line 7). There is no significant difference between the two, which suggests that all the elements that control the relative levels of 5s RNA and tRNA in relation to 28s RNA are contained in the duplications used in this experiment. It can also be interpreted as if the regulatory mechanism for the levels of these classes of RNA was not constitutive, and was regulated by elements like tRNA, ribosomal protein, or other genes.
A triploid with 4 doses of the 5s redon (line 8) was analyzed. It was found that a drastic reduction of the relative amounts of 5s RNA and tRNA per ribosome occurred. The reason for this will be analyzed when the results of the rates of rRNA synthesis are discussed.

The rates of synthesis of rRNA and 5s RNA relative to tRNA were analyzed as already described. The results are shown in Table 7.

Again, we can compare the effect of the translocation T(2;3)C328, without duplication, on the relative rates of rRNA and 5s RNA synthesis, with the wild type (lines 1 and 3). Here we can see that there is an effect of the translocation on the rate of rRNA synthesis and no effect on 5s RNA rate of synthesis. The translocation, besides of the position effect for the genes translocated, produced three breakpoints, two in the second chromosome and one in the third. The effect produced would be a position effect for the 5s redon or any other genes included in the translocation, or it could also be a result of altering specific genes at the breakpoints.

Flies with 3 5s RNA redons and 1, 2, and 3 RTU redons were analyzed (lines 2, 4, and 5). A reduced rate of rRNA synthesis is found in lines 2 and 4, that is with 1 or 2 RTU redons. There also seems to be an increase in the specific activity of the 5s RNA but it is thought to be an artifact due to the decrease in the relative amounts of 5s RNA. One fact seems to be clear, and that is that there is a decrease in the amounts of 5s RNA in these two cases. However, when we have 3 5s RNA redons and 3 RTU redons (1:1 relationship) there is a recovery of the balance and rates of rRNA and 5s RNA (as well as amounts) return to near normal. So when a 1:1 relationship exists between RTU redons and 5s RNA redons, the
Table 7. Rates of rRNA and 5s RNA synthesis relative to tRNA synthesis, with varying doses of the 5s redon

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of 5s redons</th>
<th>Number of RTU redons</th>
<th>cpm/mg-area of 28s RNA per cpm/mg-area of tRNA</th>
<th>cpm/mg-area of 5s RNA per cpm/mg-area of tRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton homozygous</td>
<td>2</td>
<td>2</td>
<td>1.28</td>
<td>3.78</td>
</tr>
<tr>
<td>±/±; Cy/±; Dp/±</td>
<td>3</td>
<td>2</td>
<td>.56</td>
<td>5.52</td>
</tr>
<tr>
<td>±/±; DF/±; Dp/±</td>
<td>2</td>
<td>2</td>
<td>.80</td>
<td>3.83</td>
</tr>
<tr>
<td>y sc^4 sc^8/±; Cy/±; Dp/±</td>
<td>3</td>
<td>1</td>
<td>1.03</td>
<td>4.49</td>
</tr>
<tr>
<td>sc^4 sc^1 B/±; Cy/±; Dp/±</td>
<td>3</td>
<td>3</td>
<td>1.17</td>
<td>2.90</td>
</tr>
<tr>
<td>sc^1 sc^4 B/±; DF/±; Dp/±</td>
<td>2</td>
<td>3</td>
<td>1.02</td>
<td>4.17</td>
</tr>
<tr>
<td>±/C(1)RM; ±/±/±; ±/±/±^a triploid</td>
<td>3</td>
<td>3</td>
<td>.55</td>
<td>3.76</td>
</tr>
<tr>
<td>±/C(1)RM; ±/±/Cy; ±/±/Dp triploid</td>
<td>4</td>
<td>3</td>
<td>.27</td>
<td>2.85</td>
</tr>
</tbody>
</table>

^aData from Table 5.
rate of rRNA synthesis increases above the threshold and the trigger for 5s RNA degradation is not activated. Since the translocation in itself places the rate of rRNA synthesis close to the threshold, any additional imbalance, which may be due to the duplication or to the presence of Cy, triggers the 5s RNA degradation mechanism.

Triploids were analyzed in lines 7 and 8 of Table 7. Triploids were found to have a reduced rate of rRNA synthesis (Table 5), but it seems that the threshold for triggering the 5s RNA degradation system is lower than in diploids. Rates of synthesis as found in line 7 do not affect the levels of 5s RNA, although the rate of rRNA synthesis is as low as in bb⁶ (Table 3). Also, a level of rRNA synthesis as found for the genotype in line 8 would probably be lethal in diploids, while it affects both rates and levels of all RNAs in the triploid but is not lethal.

Summarizing, we can conclude that the effect found varying the dose of the 5s redons is probably not a direct one, and seems to be due to the fact that rRNA synthesis is reduced by the translocation itself too near to the threshold value. Any additional effect is obscured by the fact that a slight influence on the rate of rRNA synthesis activates the 5s RNA degradation mechanism. A translocation involving a smaller piece of the second chromosome including 56 EF (5s DNA) has to be generated that does not influence rRNA synthesis and hopefully viable as a deficiency, to further continue this line of work.
The Effect of α-amanitin

This toxic cyclic peptide interacts specifically with eukaryotic RNA polymerase II or mRNA polymerase (BLATTI, INGLES, LINDELL, MORRIS, WEAVER, WEINBERG, and RUTTER, 1970). It does not affect rRNA polymerase (RNA polymerase I) in vitro, but in vivo it has a transitory effect (JACOB, SAJDEL, MUECKE, and MUNRO, 1970; NIESSING, SCHNIEDERS, KUNZ, SEIFART, and SEKERIS, 1970). These authors showed that C\(^{14}\) orotic acid (a nucleic acid pyrimidine precursor) incorporation into rRNA is inhibited when α-amanitin is administered in vivo. The effect wears off, however, after several hours. It is not clear if the effect of α-amanitin on rRNA polymerase is due to a specific effect on mRNA coding for a regulatory protein or to a direct inhibitory effect on the polymerase due to some kind of modification of α-amanitin in vivo. The regulatory protein hypothesis was preferred because of an analogous effect of cycloheximide (JACOB et al., 1970). To investigate the effect of α-amanitin on rRNA and also on 5s RNA synthesis in Drosophila melanogaster, flies were injected various doses of α-amanitin and RNA synthesis measured as described in Materials and Methods. The doses of α-amanitin ranged from \(4 \times 10^{-9}\) μg/fly up to \(4 \times 10^{-2}\) μg/fly. The immediate effect of α-amanitin on \(^3\)H uridine incorporation was very slight, probably because the drug did not have time to enter the nuclei of the cells when administered together with the isotope. However, pre-incubation with α-amanitin (injections of \(2 \times 10^{-1}\) μg/fly of α-amanitin) for 3 hours followed by \(^3\)H uridine injection and further incubation for 30 minutes showed the results presented in Table 8. Control experiments were made in the absence of α-amanitin and compared to the results obtained in its presence. From
Table 8. The effect of α-amanitin on RNA synthesis

Flies were injected with $2 \times 10^{-1}$ μg/fly of α-amanitin and 3 hours later followed by an injection of .2 μl H$_2$ uridine per fly, with further incubation at 25°C, results are the average of 2 determinations

<table>
<thead>
<tr>
<th></th>
<th>Specific activities (cpm/mg area)</th>
<th>Ratio of specific activities in relation to tRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28s</td>
<td>18s</td>
</tr>
<tr>
<td>Control</td>
<td>10.49</td>
<td>13.03</td>
</tr>
<tr>
<td>Treated with α-amanitin</td>
<td>2.91</td>
<td>5.06</td>
</tr>
</tbody>
</table>
the specific activities shown, there seems to be an inhibition of rRNA synthesis and a slight enhancement of tRNA synthesis with this kind of treatment. The specific activities of 5s RNA do not appear to be significantly affected. However, in an analysis of the ratios of the specific activities of rRNA in relation to tRNA, rRNA seems to be greatly affected, while 5s RNA in relation to tRNA is only slightly affected. At this concentration of \( \alpha \)-amanitin, rRNA synthesis seems to be inhibited below the level of \( \frac{b}{b} \) (if we consider the ratio of rRNA in relation to tRNA synthesis) and consequently it would be expected that the rate of 5s RNA synthesis in relation to tRNA would be slightly affected. In this situation, a reduction in the relative rate of 5s RNA synthesis occurred because rRNA synthesis was below the threshold value. However, the total levels of 5s RNA were not affected due to the short time that the organism was treated with the drug. It would appear, therefore, that the effect of \( \alpha \)-amanitin on 5s RNA synthesis is not by the same mechanism by which rRNA synthesis is affected. It may be an indirect effect, as a result of the effect of \( \alpha \)-amanitin on rRNA synthesis itself. If the effect of \( \alpha \)-amanitin is on the transcription of the mRNA coding for the rRNA polymerase regulatory protein, then it is possible that transcription of 5s RNA is by a different polymerase. However, the first point needs further confirmation before any support is given to this second conclusion.
CONCLUSIONS AND SUMMARY

Flies with rDNA regions of different sizes (bbs) were analyzed for the amounts of 5s RNA and tRNA per rRNA and, these were found to be unrelated to the size of the rDNA deletion, down to a threshold value. The rates of rRNA and 5s RNA synthesis relative to tRNA synthesis were studied and the relative rate of rRNA synthesis was found to be proportional to the intensity of the phenotype as measured by bristle length. The rate of 5s RNA synthesis and total levels were unaltered when the rRNA rate of synthesis varied through a wide range, but when it reached a certain low threshold value, 5s RNA synthesis was affected both in rate and amount. This effect, however, was not directly proportional to the rRNA rate of synthesis, and, consequently suggests some kind of indirect control.

The cyclic peptide, α-amanitin gave a similar kind of response. When inhibition of 5s RNA synthesis occurred, it was found never to be as intense as the inhibition on rRNA synthesis. Because α-amanitin acts on mRNA polymerase, it is thought that the inhibitory effect on rRNA polymerase by α-amanitin in vivo is due to the inhibition of synthesis of a regulatory protein. However, this protein probably does not act on the enzyme(s) that transcribe the 5s redon.

When different doses of the RTU redons were tested, it was found that the amounts of rRNA and 5s RNA were constant and similar to that observed in the wild type. However, when rates were analyzed it was found that in flies, which have 4 RTU redons, the rate of rRNA synthesis is reduced. This is somehow coupled to the mechanism of regulation of redon sizes in a way that is not understood. However, the rate of 5s RNA syn-
thesis was not affected in this manner. It appears, therefore, that regulation of 5s RNA amounts and rate of synthesis is independent of the rates of rRNA synthesis down to a threshold level. Below this threshold level, there is a direct coupling of the two regulatory mechanisms.

Different doses of the 5s redon were going to be tested. However, it was found that there was an effect of the translocation itself on rates of rRNA synthesis. Since the translocation placed the level of rRNA synthesis near the threshold of the 5s coupling mechanism, no conclusions could be drawn from this experiment. It was found that in triploids the level of rRNA synthesis that allows survival of flies is lower than in diploids, and so is the threshold for the coupling mechanism between rRNA and 5s RNA rates and levels.

The data of BROWN and WEBER (1968) supports this model because anucleolate mutants of *Xenopus* (no rDNA) synthesize no 5s RNA. However, when actinomycin D is used (PERRY and KELLEY, 1968-1970) or metaphase-arrested cells are used (ZYLBER and PENMAN, 1971) the rate of rRNA synthesis is stopped only for short times. Consequently, there is no scarcity of rRNA in the cell, rRNA synthesis is stopped but 5s RNA synthesis continues at a normal rate. However, in the case of bbs, below the threshold rate of rRNA synthesis, there is a developmental delay until the amount of rRNA necessary for growth is made. The fact that there is always a deficit of rRNA triggers the mechanism for reduction of 5s RNA rates of synthesis (affecting transcription) and 5s RNA levels (affecting degradation).
LITERATURE CITED


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