

NUCLEOLAR-DERIVED RIBONUCLEIC ACID  
IN CHROMOSOMES, NUCLEAR SAP,  
AND CYTOPLASM OF *CHIRONOMUS TENTANS*  
SALIVARY GLAND CELLS

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ABSTRACT

The distribution of monodisperse high molecular weight RNA (38, 30, 28, 23, and 18S RNA) was studied in the salivary gland cells of *Chironomus tentans*. RNA labeled in vitro and in vivo with tritiated cytidine and uridine was isolated from microdissected nucleoli, chromosomes, nuclear sap, and cytoplasm and analyzed by electrophoresis on agarose-acrylamide composite gels. As shown earlier, the nucleoli contain labeled 38, 30, and 23S RNA. In the chromosomes, labeled 18S RNA was found in addition to the 30 and 23S RNA previously reported. The nuclear sap contains labeled 30 and 18S RNA, and the cytoplasm labeled 28 and 18S RNA. On the basis of the present and earlier analyses, it was concluded that the chromosomal monodisperse high molecular weight RNA fractions (*a*) show a genuine chromosomal localization and are not due to unspecific contamination, (*b*) are not artefacts caused by in vitro conditions, but are present also in vivo, and (*c*) are very likely related to nucleolar and cytoplasmic (pre)ribosomal RNA. The 30 and 23S RNA components are likely to be precursors to 28 and 18S ribosomal RNA. The order of appearance of the monodisperse high molecular weight RNA fractions in the nucleus is in turn and order: (*a*) nucleolus, (*b*) chromosomes, and (*c*) nuclear sap. Since both 23 and 18S RNA are present in the chromosomes, the conversion to 18S RNA may take place there. On the other hand, 30S RNA is only found in the nucleus while 28S RNA can only be detected in the cytoplasm, suggesting that this conversion takes place in connection with the exit of the molecule from the nucleus.

INTRODUCTION

Ribosomal RNA in mammalian cells is synthesized in the nucleolus as a 45 S precursor from which 18 and 32 S RNA are formed, the latter a precursor to 28 S ribosomal RNA. The finished 28 and 18 S ribosomal RNA molecules are supposed to form in the nucleolus (for review see Darnell, 1968). Previous work on nucleolar RNA synthesis in the salivary gland cells of *Chironomus tentans* showed that a 38 S RNA molecule is first synthesized and later converted to 30 and 23 S RNA (Edström and

Daneholt, 1967; Ringborg, Daneholt, Edström, Egyházi, and Lambert, 1970 *a*). After 30 and 23 S RNA fractions had formed in the nucleolus, first 23 S and later also 30 S RNA appeared in the chromosomal RNA (Ringborg, Daneholt, Edström, Egyházi, and Rydlander, 1970 *b*). This occurred before any labeled ribosomal or pre-ribosomal RNA was observed in the nuclear sap or in the cytoplasm.

The salivary gland material is, so far, the only

one in which preribosomal-like RNA components have been detected on the chromosomes (Pelling, personal communication, has observed in the same material, labeled in vivo, a monodisperse peak in the chromosomes, slightly heavier than the finished 28 S RNA component). Since the association of (pre)ribosomal RNA with chromosomes may be of central importance in gene expression in higher cells also, it was considered desirable to reinforce earlier data as regards the question of whether these fractions show a genuine chromosomal localization. It was also desirable to ascertain that the components were not artefacts of in vitro labeling. In the course of this work, 18S RNA was also observed in chromosomal RNA, and 30 and 18S RNA were recovered in the nuclear sap. The kinetic studies and inhibition experiments indicate that the components are not formed in the chromosomes and that therefore the nucleolus is the source and the molecules are (pre)ribosomal in nature.

## MATERIAL AND METHODS

### *Biological Material and Labeling Conditions*

Larvae of *Chironomus tentans* (late fourth instar) were cultured as described by Beermann (1952). For in vivo labeling, tritiated cytidine was injected into the body cavity of the larva under stereomicroscopic control with a glass syringe, about 10  $\mu$  at the tip, handled with a de Fonbrune micromanipulator. 20  $\mu$ Ci in 1  $\mu$ l of salt solution (0.67% NaCl) was administered to each animal. The incorporation time was 180 min at 18°C. Glands were also incubated in vitro in Cannon's modified insect medium (four glands in 25  $\mu$ l) (Ringborg et al., 1970 *b*) containing tritiated cytidine and uridine, 100  $\mu$ Ci of each. Streptomycin, earlier included in the medium, was omitted and the pH was adjusted with NaOH instead of KOH. The incubation times in vitro varied between 45 and 180 min at 18°C. When 5,6-dichloro-1( $\beta$ -D-ribofuranosyl)benzimidazole (DRB) was used as an inhibitor of chromosomal H RNA synthesis (Egyházi, Danholt, Edström, Lambert, and Ring-

borg, 1970), glands were preincubated for 60 min in Cannon's modified medium containing 20  $\mu$ g of DRB per ml. After preincubation, the glands were transferred to an equal volume of the same solution containing tritiated cytidine and uridine, 100  $\mu$ Ci of each. The radioactive precursors were obtained from the Radiochemical Centre (Amersham, England) at a specific activity of 24–28 c/mmole, and the DRB from Merck, Sharp & Dohme, West Point, Pa.

### *Preparation of Cannon's Modified*

#### *Insect Medium*

The composition of the medium (mg/300 ml) is as follows:

INORGANIC SALTS: Na<sub>2</sub>HPO<sub>4</sub>, 356; MgCl<sub>2</sub>·6H<sub>2</sub>O, 93.5; KCl, 82; NaCl, 420; Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O, 2000; CaCl<sub>2</sub>, 42.

SUGARS: glucose, 140; fructose, 80; sucrose, 80; trehalose, 1000.

ORGANIC ACIDS: malic, 134;  $\alpha$ -ketoglutaric, 74; succinic, 12; fumaric, 11.

AMINO ACIDS: L-arginine-HCl, 140; DL-lysine-HCl, 250; L-histidine, 500; L-aspartic acid, 70; L-asparagine, 70; L-glutamic acid, 120; L-glutamine, 120; glycine, 130; DL-serine, 220; L-alanine, 45; L-proline, 70; L-tyrosine, 10; DL-threonine, 70; DL-methionine, 180; L-phenylalanine, 30; DL-valine, 40; DL-isoleucine, 20; DL-leucine, 30; L-tryptophan, 20; L-cystine, 5; L-cysteine-HCl, 16.

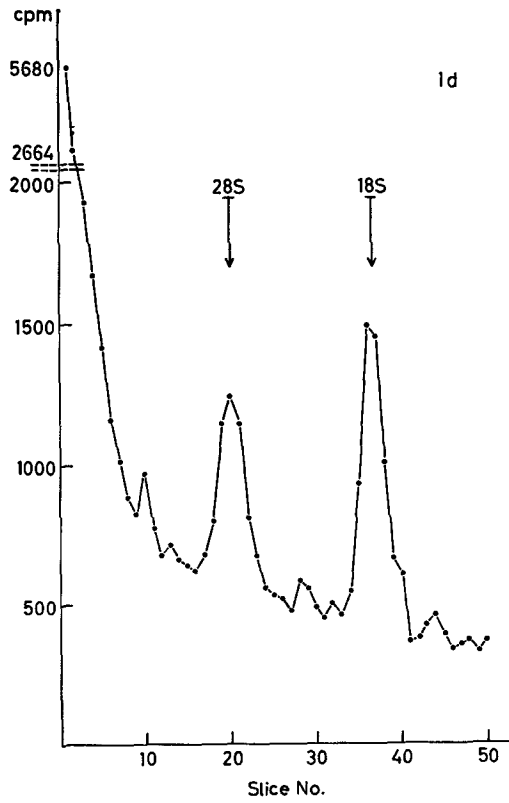
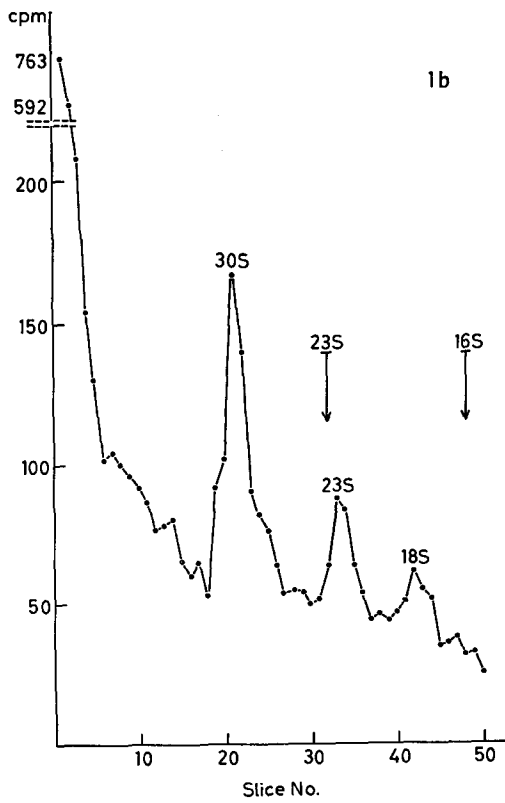
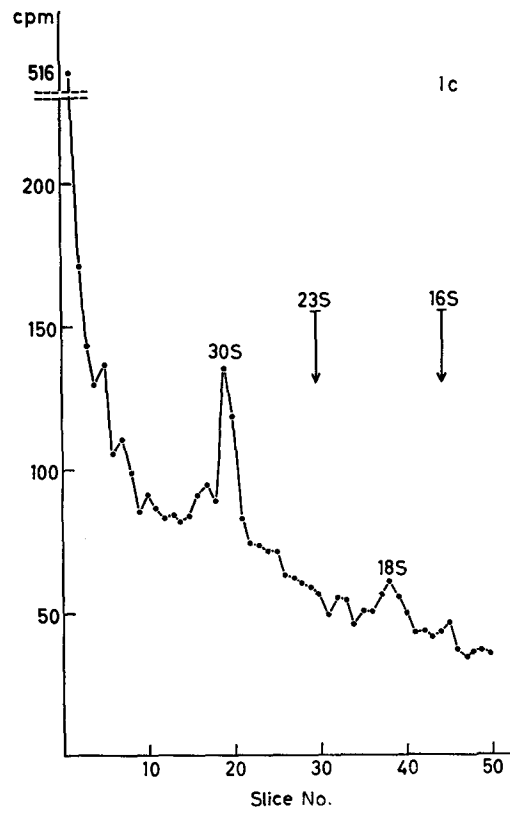
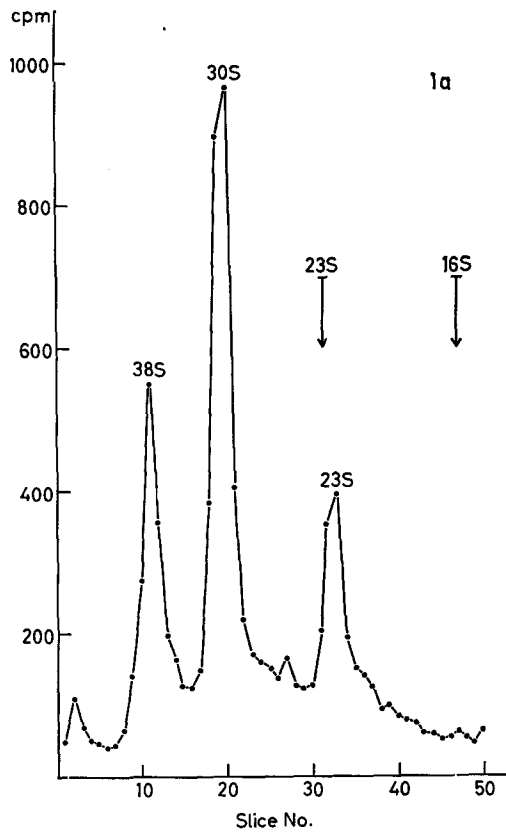
VITAMIN B COMPLEX: thiamin-HCl, riboflavin, nicotinic acid, pantothenic acid, biotin, folic acid, inositol, choline, each 0.004.

OTHER COMPOUNDS: cholesterol, 6; penicillin, 12; phenol red, 20.

The solutes are dissolved in redistilled water. Inorganic salts, excepting CaCl<sub>2</sub>, are dissolved in 60 ml; CaCl<sub>2</sub> in 14.4 ml; sugars and organic acids in 30 ml; amino acids in 80 ml; cholesterol, penicillin, phenol red, and vitamins in 9 ml. The solutions are mixed with CaCl<sub>2</sub>, being added last. The pH is adjusted to 7.2 by addition of NaOH and water is added to bring the total volume to 300 ml. The solution is filtered through a Millipore filter (Millipore Corporation, Bedford, Mass.) and stored frozen.

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FIGURE 1 Separation of: Fig. 1 *a*, nucleolar; Fig. 1 *b*, chromosomal; Fig. 1 *c*, nuclear sap; and Fig. 1 *d*, cytoplasmic RNA after 180 min of labeling. Glands were incubated with 100  $\mu$ Ci of cytidine-<sup>3</sup>H and 100  $\mu$ Ci of uridine-<sup>3</sup>H in 25  $\mu$ l of Cannon's modified insect medium for 180 min at 18°C. After fixation, nucleoli, chromosomes, nuclear sap, and cytoplasm were collected by micromanipulation from 25 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. *E. coli* and salivary gland RNA were used as markers. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel. The positions of 28, 23, 18, and 16S are indicated by arrows. For other data see Material and Methods.



## Isolation of RNA

The glands were fixed in acid alcoholic formaldehyde (Egyházi et al., 1969). Nucleoli, chromosomes, nuclear sap, and cytoplasm were isolated by micro-manipulation and digested for 3 hr at 37°C in 0.1–1  $\mu$ l volumes of a 0.05 M Tris buffer containing 0.5% sodium dodecyl sulfate, 0.1 M NaCl, and 1 mg of pronase (Calbiochem, Los Angeles, Calif., B grade) per ml in an oil chamber. The pronase solution was predigested for 30 min at 37°C. The dissolved sample was then transferred by means of a small piece of glass filter paper to E buffer (0.02 M Tris-buffer, pH 8.0, 0.02 M NaCl and 0.002 M EDTA) with 0.2% sodium dodecyl sulfate for electrophoresis as described earlier (Ringborg et al., 1970 a). Marker RNA (*E. coli* and salivary gland RNA) was obtained by sodium dodecyl sulfate-phenol extraction at 4°C.

## Electrophoresis and Measurement of Radioactivity

Slabs of 0.5% agarose–2.25% acrylamide composite gel were used for the electrophoretic analyses (Ringborg et al., 1970 a). Gels were prepared according to Peacock and Dingman (1968), with the exception that 10% ammonium persulfate (0.33 ml/g acrylamide) and *N,N,N',N'*-tetramethylethylenediamine (0.033 ml/g acrylamide) were used (Loening, 1967). After electrophoresis, the gel was sliced into 1.1-mm slices. The carrier bands were localized by ultraviolet light. The slices were transferred to scintillation vials containing 10 ml of toluene with 5 g of 2,5-diphenyloxazole (PPO), 0.5 g of dimethyl *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) and 30 ml of NCS (Nuclear-Chicago, Des Plaines, Ill.) or 50 ml of Soluene (Packard Instrument Co., Inc., Downers Grove, Ill.) per liter, incubated overnight at 37°C, and counted in a Packard liquid scintillation spectrometer at an efficiency of around 33%.

## RESULTS

### *Distribution of Monodisperse High Molecular Weight RNA in Nucleoli, Chromosomes, Nuclear Sap, and Cytoplasm after In Vitro Labeling*

After 180 min of incorporation time in vitro, the nucleolar RNA profile shows three monodisperse RNA fractions at 38, 30, and 23S positions (Fig. 1 a). In chromosomal RNA (Fig. 1 b), monodisperse 30, 23, and 18S RNA fractions are observed on a background of H RNA. The nuclear sap analyses (Fig. 1 c) shows two minor monodisperse 30 and 18S fractions on the slope of H RNA, and

the cytoplasm (Fig. 1 d) contains 28 and 18S RNA. The chromosomal 18S RNA is labeled with a delay similar to the 23S RNA described earlier (Ringborg et al., 1970 b): it is absent after 45 min of incorporation time (as is 30S RNA) when label is found only in H RNA and low molecular weight RNA on the chromosomes (not shown here). It can, however, be demonstrated after 90 min when the nucleolus contains label in 30 and 23S RNA. In Fig. 2, the different cellular compartments have been analyzed after 90 min of labeling and the nucleolar RNA (Fig. 2 a) shows the 38, 30, and 23S RNA components, the 38S fraction now being the dominating one. In Fig. 2 b, chromosomal RNA contains monodisperse 18S RNA. At this time, no labeled monodisperse RNA fractions are present in the nuclear sap or cytoplasm (Fig. 2 c).

With the earlier used in vitro incubation media (hemolymph from larvae and modified Cannon's insect medium), cytoplasmic 28 and 18S RNA was irregularly labeled after 180 min of incorporation time. Minor changes of the in vitro procedure such as exclusion of streptomycin from the incubation medium and a changed sodium-potassium ratio together with greater experience in handling the glands to avoid exposure to air and mechanical damage of the cells may be responsible for the apparent improvement of the incubation system. Cytoplasmic ribosomal RNA is labeled more reproducibly after 180 min of incubation, and this improvement is correlated with the appearance of 18 instead of 23S RNA in chromosomal RNA after 90 min of labeling.

### *The Intranuclear Distribution of High Molecular Weight Monodisperse RNA Is Not an In Vitro Artefact*

In order to ascertain that monodisperse high molecular weight RNA fractions observed in vitro are not artefacts due to the incubation conditions, glands were labeled in vivo as described in Material and Methods. After 180 min, the nucleoli contain 38, 30, and 23S RNA (Fig. 3 a), the chromosomes 30, 23, and 18S RNA fractions on the slope of H RNA (Fig. 3 b), and the nuclear sap 30 and 18S RNA fractions (Fig. 3 c). In a special study, nuclear sap and cytoplasmic RNA were compared. Larvae were each injected at time 0, 2, and 4 hr and analyzed after 6 hr with salivary gland RNA as a marker in order to establish the difference in migration between nuclear sap 30S and cytoplasmic 28S RNA. Fig. 4 a shows that the

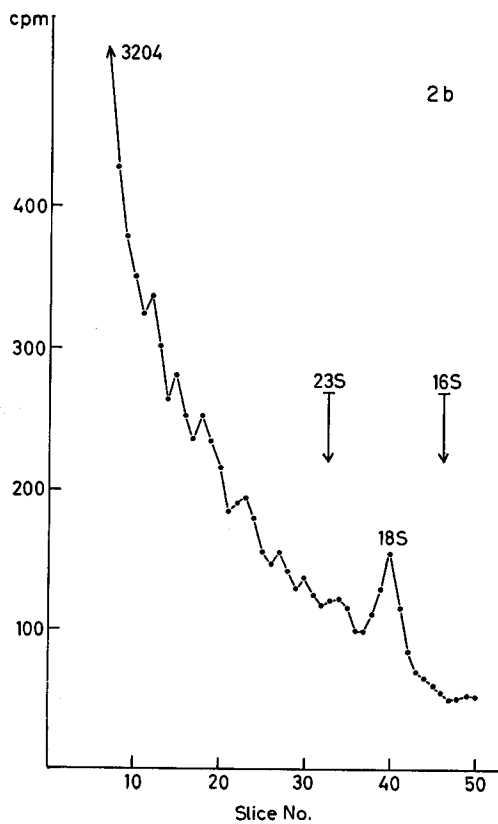
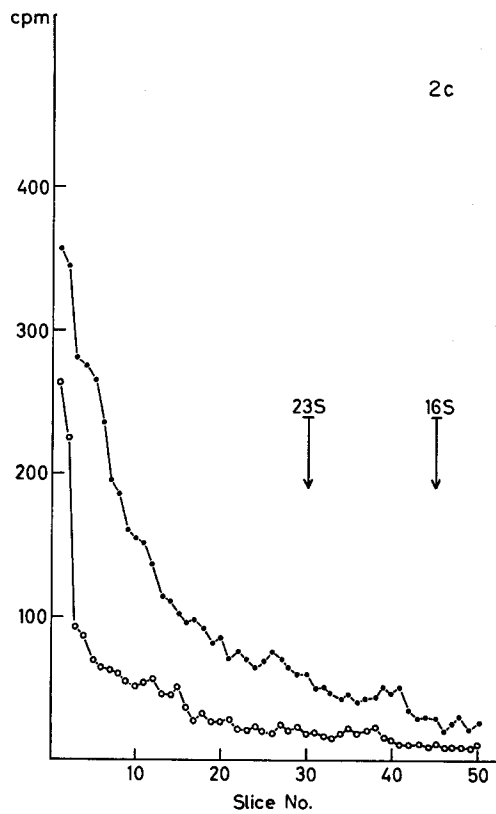
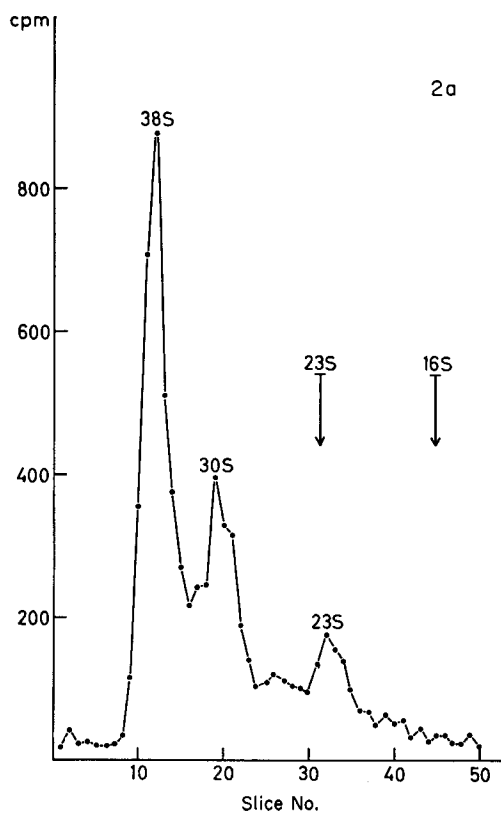


FIGURE 2 Separation of Fig. 2 a, nucleolar; Fig. 2 b, chromosomal; and Fig. 2 c, nuclear sap and cytoplasmic RNA after 90 min of labeling. Glands were incubated with 100  $\mu$ Ci of cytidine- $^3$ H and 100  $\mu$ Ci of uridine- $^3$ H in 25  $\mu$ l of Cannon's modified insect medium for 90 min at 18°C. After fixation, nucleoli, chromosomes, nuclear sap, and cytoplasm were collected by micromanipulation from 25 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. *E. coli* RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel. Fig. 2 c: open circles = nuclear sap, closed circles = cytoplasm.

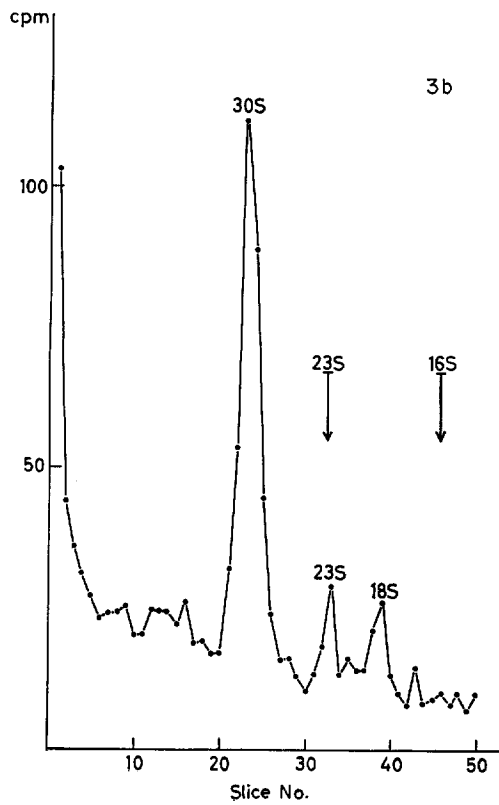
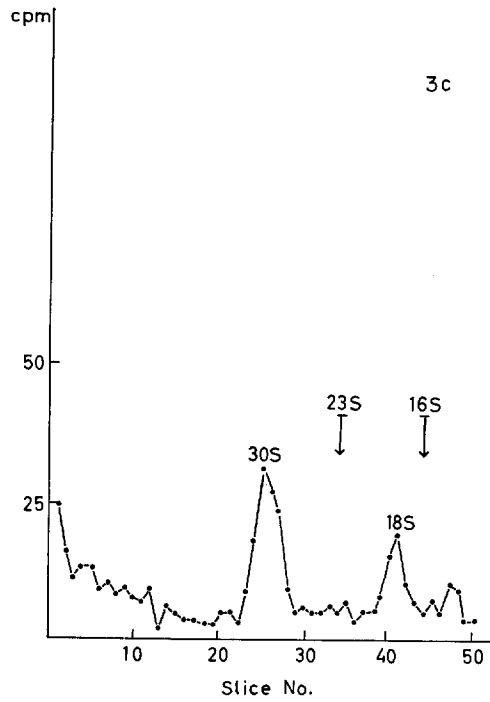
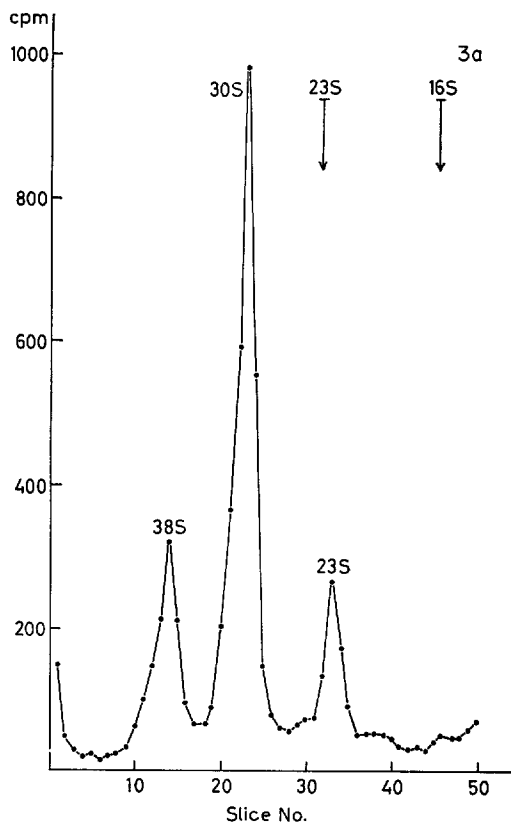


FIGURE 3 Separation of: Fig. 3 a, nuclear; Fig. 3 b, chromosomal; and Fig. 3 c, nuclear sap RNA after 180 min of in vivo labeling. 20  $\mu$ Ci of cytidine- $^3$ H was injected into the body cavity of the larvae for incorporation for 180 min at 18°C. After fixation, nucleoli, chromosomes, and nuclear sap were collected by micro-manipulation from 25 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. *E. coli* RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel.

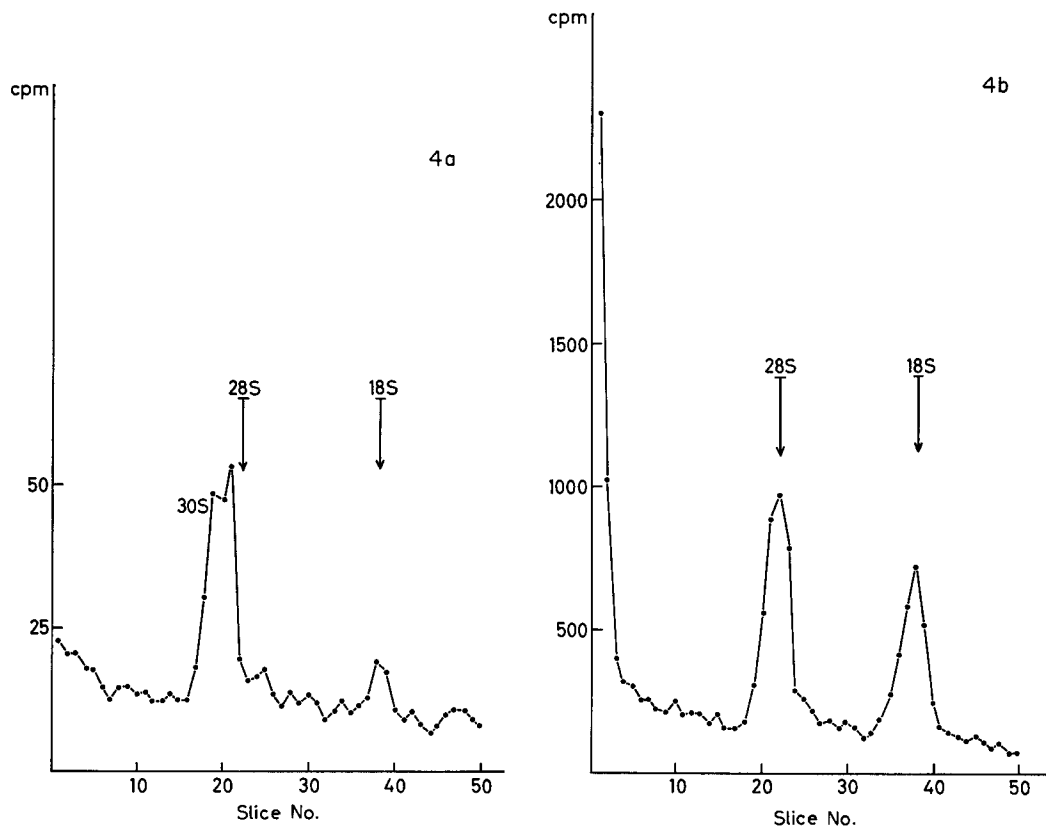


FIGURE 4 Separation of: Fig. 4 *a*, nuclear sap and Fig. 4 *b*, cytoplasmic RNA after 6 hr of in vivo labeling. 20  $\mu$ Ci of cytidine- $^3$ H was injected at times 0, 2, and 4 hr with a total incorporation time of 6 hr at 18°C. After fixation, nuclear sap and cytoplasm were collected by micromanipulation from 25 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. Salivary gland RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel.

nuclear sap 30S RNA migrates slower than the carrier 28S RNA, while the cytoplasmic 28S fraction coincides with the carrier ribosomal RNA (Fig. 4 *b*). The 28S fraction is not detectable in the sap, and the 30S fraction not in the cytoplasm. The low amount of label in nuclear sap H RNA in vivo relative to in vitro was a constant finding. Thus, the monodisperse high molecular weight RNA fractions demonstrated in vitro (Fig. 1) exist also in vivo.

#### *Intrachromosomal Distribution of 18S RNA*

The salivary gland cells contain four chromosomes, three large ones containing small puffs and the fourth small chromosome containing three giant puffs, named Balbiani rings. The Balbiani rings are considered to be the genetic loci responsible for the salivary polypeptide synthesis

(Beermann, 1966; Grossbach, 1969), and their RNA-synthesizing capacity is relatively high when compared to the total chromosomal RNA synthesis (Pelling, 1964; Dancholet et al., 1969). In order to investigate whether the 18S RNA found on the chromosomes after 90 min of labeling is related to the Balbiani rings or distributed according to chromosomal length, we isolated and analyzed RNA separately from the large chromosomes I-III and the chromosome IV. We found that about the same amount of monodisperse 18S RNA is located in the chromosomes I-III (Fig. 5 *a*) as in the chromosome IV (Fig. 5 *b*). As the chromosome IV constitutes about 10% of the total chromosomal length or DNA content (Dancholet and Edström, 1967), 18S RNA is enriched on the chromosome IV relative to the other chromosomes. The chromosomal 23S RNA also showed an enrichment on the chromosome IV of the same magnitude as the

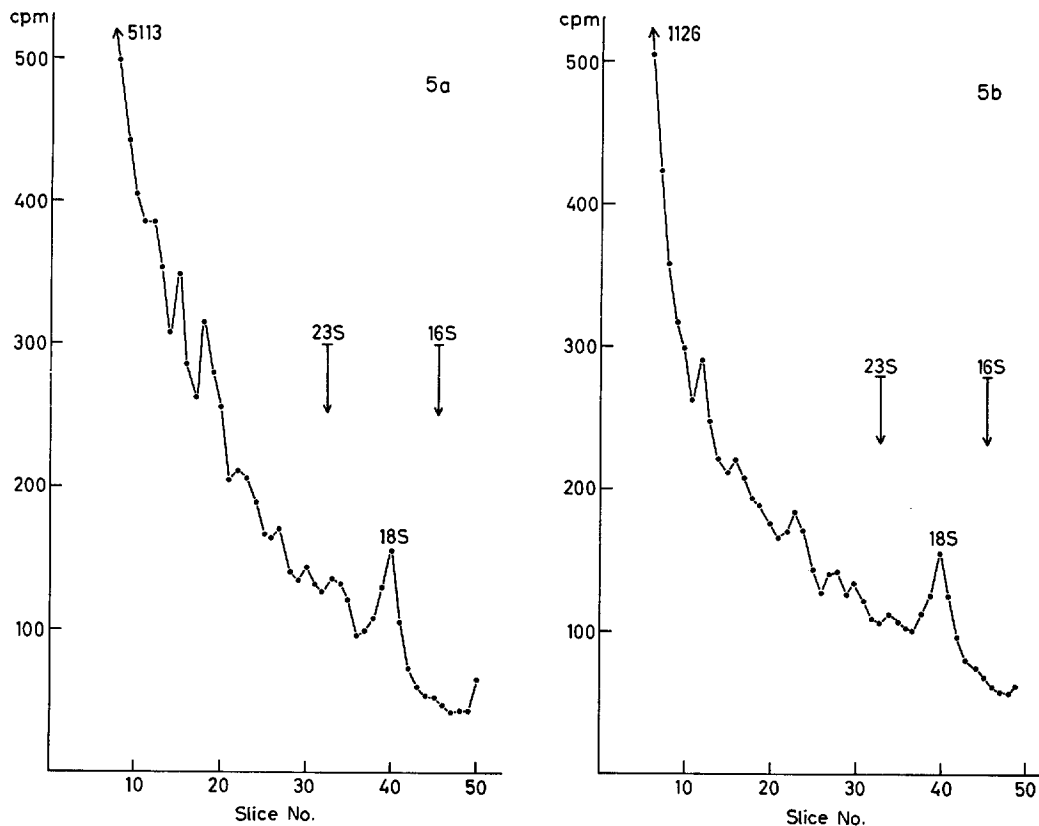


FIGURE 5 Separation of chromosomal RNA from Fig. 5 a, chromosomes I-III and Fig. 5 b, chromosome IV analyzed separately after 90 min of labeling. Glands were incubated with 100  $\mu$ Ci of cytidine- $^3$ H and 100  $\mu$ Ci of uridine- $^3$ H in 25  $\mu$ l of Cannon's modified insect medium for 90 min at 18°C. After fixation, chromosomes were collected by micromanipulation from 45 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. *E. coli* RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel.

18S RNA (not shown). When the 30S RNA was studied in an analogous way, it was present in amounts proportional to chromosomal length (not shown). Such results are less conclusive with regard to the question of whether 30S RNA shows any characteristic association with certain chromosome regions and are therefore not shown here. This may be because the Balbiani rings regress during the time it takes for 30S RNA to enter the chromosomes, and this could influence its distribution.

*Distribution of Monodisperse High Molecular Weight RNA during Selective Inhibition of Chromosomal H RNA Synthesis with DRB*

It was demonstrated previously that first 23S RNA and somewhat later also 30S RNA appeared

in chromosomal RNA at a time when fractions with corresponding electrophoretic mobility had formed in the nucleolus (Ringborg et al., 1970 b), and it was suggested that these fractions were transported from the nucleolus to the chromosomes. In the present study, 18S RNA was also observed and it, too, labeled with a time lag. Like 30 and 23S RNA, it could be either a product of nucleolar RNA or, alternatively, of the chromosomal H RNA. The former alternative is more reasonable, if the chromosomal 18S RNA is supposed to be ribosomal in type, as it has been shown that the genes for ribosomal RNA are located in the nucleolus (Brown and Gurdon, 1964; Ritossa and Spiegelman, 1965).

The chemical agent 5,6-dichloro-1( $\beta$ -D-ribofuranosyl)benzimidazole (DRB) was earlier shown to selectively inhibit chromosomal H RNA labeling, leaving the low molecular weight and nucle-



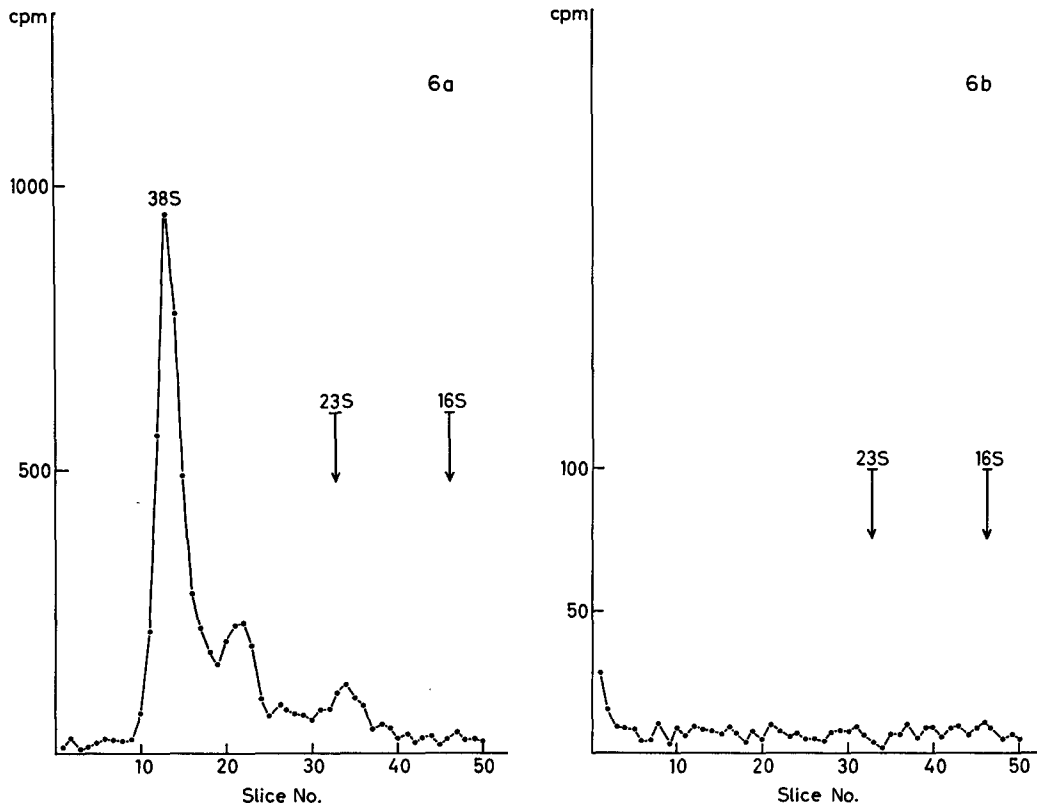


FIGURE 6 Separation of Fig. 6 *a*, nucleolar and Fig. 6 *b*, chromosomal-nuclear sap RNA after 45 min of labeling in presence of DRB. Glands were preincubated for 60 min at 18°C in 25  $\mu$ l of Cannon's modified insect medium containing 20  $\mu$ g of DRB per ml. They were then transferred to another 25  $\mu$ l of the same medium containing 100  $\mu$ Ci of cytidine- $^3$ H and 100  $\mu$ Ci of uridine- $^3$ H and incubated for 45 min at 18°C. Nucleoli and chromosomes-nuclear sap were collected by micromanipulation from 25 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. *E. coli* RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel.

olar RNA labeling relatively unaffected (Egyházi et al., 1970). As the processing of nucleolar 38S to 30 and 23S RNA components was shown to take place in the presence of DRB, this drug could be used to study whether high molecular weight monodisperse RNA label would appear in the chromosomes in absence of H RNA labeling. Glands were preincubated with DRB and incubated for a further 45 min with labeled nucleosides in the presence of DRB. Nucleolar RNA shows 38S RNA, and minor 30 and 23S peaks (in Fig. 6 *a*). The chromosomal and nuclear sap RNA (analyzed together) are almost free from radioactivity (Fig. 6 *b*), indicating an effective inhibition of the H RNA labeling. After 180 min, the amounts of nucleolar 30 and 23S RNA have increased considerably relative to 38S RNA, confirming that the conversion of 38S RNA to 30 and

23S RNA takes place in presence of DRB (Fig. 7 *a*). A 30S RNA fraction was observed in chromosomal and nuclear sap RNA (Figs. 7 *b* and 7 *c*). The delayed labeling of 30S RNA indicates its nucleolar origin. No 23S RNA was found outside the nucleolus, and 18S RNA did not appear on the chromosomes or in the nuclear sap during DRB treatment. A 28S RNA fraction appears in the cytoplasm, but 18S RNA could not be demonstrated (Fig. 8 *a*). Fig. 8 *b* is a cytoplasmic control without DRB.

With DRB inhibition, strong evidence was thus obtained for a nucleolar origin of the chromosomal and nuclear sap 30S RNA. Likewise, the inhibition of the labeling of 23 and 18S RNA in the chromosomes is correlated with the absence of 18S RNA in the nuclear sap and the cytoplasm, supporting the notion that the chromosomal 23 and 18S

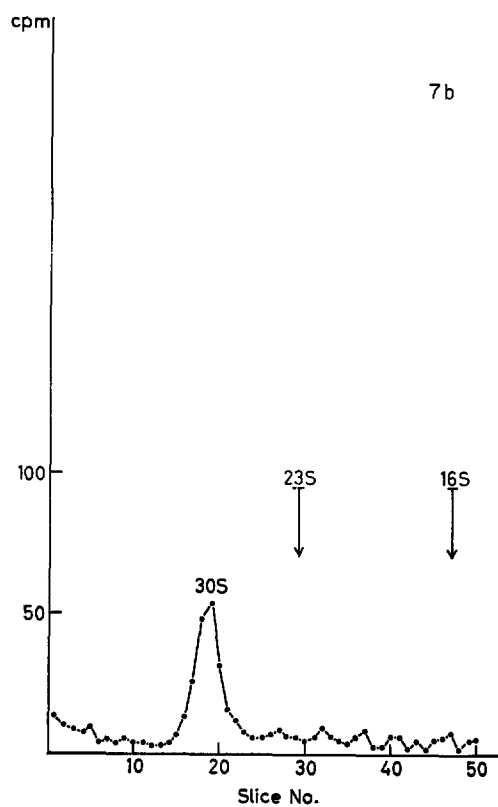
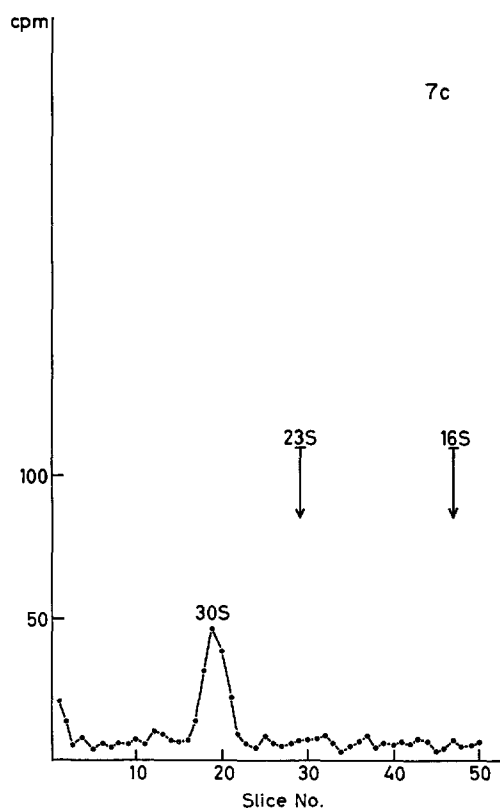
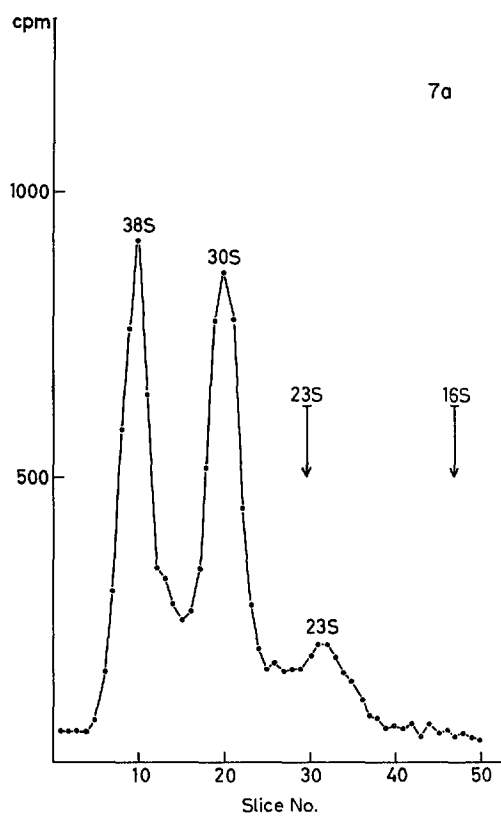


FIGURE 7 Separation of: Fig. 7 a, nucleolar; Fig. 7 b, chromosomal; and Fig. 7 c, nuclear sap RNA after 180 min of labeling in presence of DRB. Glands were pre-incubated for 60 min at 18°C in 25  $\mu$ l of Cannon's modified insect medium containing 20  $\mu$ g of DRB per ml. They were then transferred to another 25  $\mu$ l of the same medium containing 100  $\mu$ Ci of cytidine- $^3$ H and 100  $\mu$ Ci of uridine- $^3$ H and incubated for 180 min at 18°C. Nucleoli, chromosomes, and nuclear sap were isolated by micromanipulation from 25 cells. RNA was isolated by pronase-sodium dodecyl sulfate digestion. *E. coli* RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel.

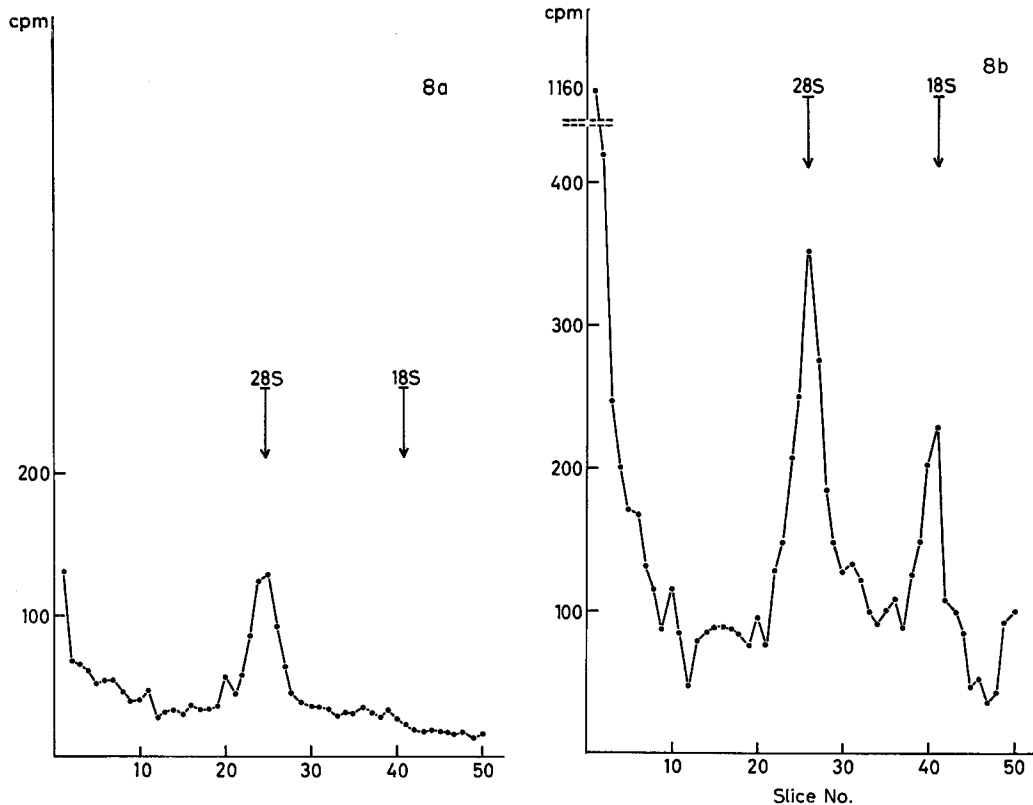


FIGURE 8 Separation of cytoplasmic RNA after 180 min of labeling in presence of DRB. Glands were preincubated for 60 min at 18°C in 25  $\mu$ l of Cannon's modified insect medium containing 20  $\mu$ g of DRB per ml. They were then transferred to another 25  $\mu$ l of the same medium containing 100  $\mu$ Ci of cytidine- $^3$ H and 100  $\mu$ Ci of uridine- $^3$ H and incubated for 180 min at 18°C. Control glands were incubated in the same way but with DRB excluded from the medium. Cytoplasm was collected by micromanipulation, and RNA was isolated by pronase-sodium dodecyl sulfate digestion. Salivary gland RNA was used as a marker. Electrophoresis was carried out on 0.5% agarose-2.25% acrylamide composite gel. Fig. 8 a, cytoplasmic RNA after DRB treatment and Fig. 8 b, cytoplasmic control.

fractions are related to the light ribosomal RNA component.

#### *Summary of the Distribution of High Molecular Weight Monodisperse RNA*

Our findings on the distribution of monodisperse high molecular weight RNA in the different cellular compartments of salivary gland cells are shown in Table I.

#### DISCUSSION

Nuclei with polytene chromosomes offer exceptional opportunities for study of the intranuclear processing of preribosomal RNA since chromosomes, nucleoli, and nuclear sap can be isolated

and analyzed. The present investigation gives a picture which, although not yet complete, shows many new traits.

Earlier data showed the formation of a 38S precursor molecule and its conversion to 30 and 23S molecules in the nucleus in the salivary gland cells of *Chironomus tentans* (Ringborg et al., 1970 a). Mature 28 or 18S RNA was not detected within the nucleolus; instead first 23S and later also 30S RNA appeared on the chromosomes after such fractions were formed in the nucleolus (Ringborg et al., 1970 b). In addition to 30 and 23S RNA, we have now also found labeled 18S RNA on the chromosomes. In the nuclear sap 30 and 18S RNA were found, and 28 and 18S RNA in the cytoplasm.

TABLE I  
Distribution of Monodisperse High Molecular Weight RNA in Different Cellular Compartments of the Salivary Gland Cell

Cellular component	RNA fraction				
Nucleolus	38S	30S	23S		
Chromosomes		30S	23S	18S	
Nuclear sap		30S		18S	
Cytoplasm			28S		18S
Molecular weights $\times 10^6$ *	3.3	2.0	1.75	1.1	0.75

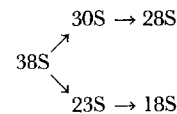
\* Obtained from Ringborg et al., 1970 a.

The present results allow us to conclude that the chromosomal RNA components are genuine and not due to contamination from the nuclear sap and/or nucleoli. Considering first nucleolar contamination, it should be pointed out that the components are isolated by micromanipulation, permitting clear identification and separation of nucleoli and chromosomes. Absence of 38S RNA in chromosomal RNA and, during DRB-inhibition, of 23S RNA in the chromosomes, in spite of the presence of 23S RNA in the nucleoli, strengthens these conclusions. Considering, secondly, the possibility that nuclear sap may contaminate chromosomes, it should be pointed out that 30S RNA as well as 18S RNA may be present in chromosomal RNA before they have appeared in the sap. A further argument, indicating specificity in distribution, is the characteristic localization of 18S RNA in the chromosomes.

Our in vivo experiments also rule out the possibility that the distribution of monodisperse high molecular weight RNA among the nuclear components is an in vitro artefact. It should be pointed out, however, that these findings do not exclude differences between the in vitro and in vivo systems in, e.g., the rates of processing and the efficiency of transport to the cytoplasm.

DNA-RNA hybridization studies have shown that the genes for ribosomal RNA molecules are located in the nucleolus (Ritossa and Spiegelman, 1965), and the two ribosomal RNA molecules are located in a common precursor molecule (Brown and Weber, 1968; Quagliariotti and Ritossa, 1968; Birnstiel et al., 1968; Jeanteur and Attardi, 1969). If the *Chironomus tentans* system is analogous to mammalian cell systems (for review see Darnell, 1968), the 30S nucleolar component should be a precursor to 28S ribosomal RNA. The 23S RNA, on the other hand, is a most likely precursor to 18S RNA, because, according to the molecular

weights of the nucleolar components (38S =  $3.3 \times 10^6$ , 30S =  $2.0 \times 10^6$ , and 23S =  $1.1 \times 10^6$  [Ringborg et al., 1970 a]), there is no place in the 38S molecule for an 18S RNA molecule in addition to 30 and 23S molecules. The 23S RNA fraction, like 30S RNA, methylates, and this is a further argument that 23S RNA is a precursor to ribosomal RNA (Ringborg et al., 1970 a). Thus, the scheme for processing of preribosomal RNA in *Chironomus tentans* should be:



In the present and previous work, we have found three different combinations of 23 and 18S RNA in the chromosomes after in vitro as well as in vivo labeling. In some analyses, 23S RNA is found, in others 18S RNA, and in a third group both 23 and 18S RNA. The ratio between the label in chromosomal 18S RNA and its presumed precursor 23S shows variations unrelated to the time of labeling, and there are obviously factors which are unknown to us regulating their amount.

It could be argued that the 23 and 18S components are artefactual degradation products of the 30S RNA. There is, however, good evidence that this cannot be the case. 23S RNA (Ringborg et al., 1970 b) as well as 18S RNA may appear on the chromosomes before 30S RNA is measurable; 30S RNA, on the other hand, dominates after long incubation times. During DRB treatment, 30S RNA is the only chromosomal monodisperse high molecular weight component.

We consider our data highly suggestive for an identity of the chromosomal 23 and 30S RNA with preribosomal RNA. This evidence is best for the 30S component which can be shown to have a nucleolar origin by DRB inhibition experiments. A

fortuitous electrophoretic migration of both 23 and 18S RNA on the chromosomes with the nucleolar 23S RNA and the ribosomal 18S RNA in the cytoplasm would seem almost unbelievable, and we, therefore, consider the evidence rather suggestive that also these fractions are (pre)ribosomal.

When considering the intranuclear route for (pre)ribosomal RNA, one has to evaluate whether the (pre)ribosomal RNA enters the chromosomes before the sap or *vice versa*. One also has to judge whether there is evidence for one common route or for more than one. It was previously shown that 30 and 23S label is present on the chromosomes before it becomes detectable in the nuclear sap. The parallel result has been obtained with labeled 18S RNA; it can be detected on the chromosomes earlier than in the nuclear sap. It still may be possible, however, that there is more than one transport route for (pre)ribosomal RNA in the nucleus.

The presence of preribosomal RNA outside the nucleolus indicates that the maturation to finished ribosomal RNA molecules is an extranucleolar process. Since the chromosomes are the only place in which both 23 and 18S RNA can be found, we suggest that the conversion 23-18S RNA takes place there. The distribution of 18S on the chromosomes with the enrichment in chromosome IV, which contains the Balbiani rings, further indicates that the conversion takes place on certain places on the chromosomes, probably in the puffs. The 30S RNA fraction, on the other hand, is present in all nuclear compartments but absent in the cytoplasm, where only 28S RNA can be detected. This indicates that the conversion of 30-28S RNA takes place in connection with the transport to the cytoplasm. If the conversions are nonconservative, as they seem to be in HeLa cells (Willems et al., 1968; Granboulan and Scherrer, 1969),  $0.25 \times 10^6$  daltons are lost during the 30-28S conversion, and  $0.35 \times 10^6$  during the 23-18S conversion.

The concentration of 18S RNA on the chromosome containing the Balbiani rings may have further functional significance, since the Balbiani rings seem to be the carriers for the genetic information for the salivary polypeptides, the main synthetic products of the gland (Beermann, 1966; Grossbach, 1969). A similar distribution was found for 23S RNA but not for 30S RNA (unpublished data). The different behavior of 30 and 23S/18S RNA during DRB treatment indicates different functions for the two different types of (pre)ribo-

somal RNA on the chromosomes. The correlated inhibition of H RNA and 18S RNA production may reflect a mutual function of some type on chromosomes. A selective degradation of the newly synthesized 18S RNA has been described during protein synthesis inhibition (Ennis, 1967; Soeiro et al., 1968) and amino acid starvation (Vaughan et al., 1967), and it is possible that the relatively high sensitivity of 18S to exogenous disruptions is related to its special role on the chromosomes.

Association of (pre)ribosomal RNA with interphase chromosomes has, so far, not been described in other eukaryotic cells. It is, however, doubtful whether such negative evidence has any bearing on the question of whether such an association is a regular phenomenon in eukaryotic cells. It seems likely that in many other types of cells investigated it is not methodologically possible to elucidate the question. In the case of mitotic chromosomes, the RNA which can be isolated is ribosomal in type (Huberman and Attardi, 1966; Maio and Schildkraut, 1967). The migration of ribosomal RNA to the chromosomes takes place in bacteria, where the transcription-translation processes are coupled (for references see Geiduschek and Haselkorn, 1969). Possibly some function of the ribosome in the prokaryote chromosome has been conserved and perhaps further evolved in the eukaryotes.

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