LARGE-SIZED POLYSOMES IN *CHIRONOMUS TENTANS* SALIVARY GLANDS AND THEIR RELATION TO BALBIANI RING 75S RNA

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ABSTRACT

Polysomes from the salivary glands of *Chironomus tentans* were investigated to determine whether Balbiani ring 75S RNA is incorporated into polysomal structures, and thus probably acts as messenger RNA. A new extraction technique for obtaining ribonucleoproteins was applied that gives a high yield of polysomes with only moderate degradation of the cytoplasmic, high molecular weight RNA. The polysomes sedimented in a broad region (200–2,000S) with a peak value of about 700S, which suggested that they were partly of very large sizes. This was confirmed by visualization of the polysomes in the electron microscope: 400S polysomes contained mainly 11–16 ribosomes, and 1,500S polysomes about 60 ribosomes per polysome. However, polysomes containing 100 or more ribosomes were also observed. It was further established that most of the cytoplasmic 75S RNA was located in polysomes, preferentially in the most rapidly sedimenting ones. From the available information on Balbiani ring RNA in cytoplasm and the present demonstration of 75S RNA molecules in polysomes, it was concluded that at least some Balbiani ring RNA, generated as 75S RNA within the Balbiani rings, eventually enters polysomes without being measurably changed in size. The present information on the potential amino acid coding sequences in 75S RNA is discussed in relation to the large size of the polysomes observed.

Salivary glands of the dipteran *Chironomus tentans* have proved useful in investigations of gene expression in eukaryotic cells. In previous studies of this system it was shown that RNA molecules can be followed from their site of synthesis on the chromosomes into the nuclear sap and further into cytoplasm (5, 7, 8). Most attention has been paid to the RNA made in two segments, Balbiani rings (BR) 1 and 2, of chromosome IV (for review, see reference 4). These products, 75S RNA in size, are released from the BRs and delivered into the cytoplasm without a concomitant substantial reduction in size (5, 16). Although a messenger function seems most plausible, the role of 75S RNA in the cytoplasm has not been demonstrated (3). In further analysis of the significance of the cytoplasmic 75S RNA, it is therefore logical to isolate and characterize polysomes from the salivary gland cells.

It has been established earlier that *C. tentans* salivary gland polysomes can be obtained in a high yield by homogenization of the glands in a detergent mixture at high ionic strength (14). This technique, however, resulted in considerable degradation of the highest molecular weight RNA, and so it therefore became desirable to work out

an improved method for polysome isolation. In the present report we describe a new technical approach which greatly reduced the degradation of RNA during the polysome extraction. Polysomes, partly of very large sizes, were obtained and their RNA was characterized. The results suggest that at least some 75S RNA molecules from the BRs enter polysomes without being measurably changed in size. Furthermore, the large size of polysomes containing 75S RNA indicates that there are amino acid coding regions in 75S RNA corresponding in size to those required for polypeptides of exceptional sizes.

MATERIALS AND METHODS

Culturing and Labeling Conditions

The dipteran *C. tentans* was cultured as earlier described (15). About 10 fourth instar larvae (4–6 wk-old) were incubated at 18°C for 3 days in 20 ml of ordinary culture medium supplied with 400 μCi of tritiated cytidine (sp act, 25.6 Ci/mmol) and 400 μCi of tritiated uridine (sp act, 49 Ci/mmol).

Extraction of Polysomes

For each extract five salivary glands were isolated and immediately transferred to 400 μl of extraction medium, consisting of 0.5% Tween 80, 0.5% sodium deoxycholate (DOC), 0.1% β-mercaptoethanol in TEAKM (0.1 M KCl, 0.003 M $MgCl_2$, 0.02 M triethanolamine-HCl, pH 7.6). (Stock solutions of 1% β-mercaptoethanol dissolved in TEAKM, of 10% Tween 80 in TEAKM and of 10% DOC in 0.02 M triethanolamine-HCl (pH 7.6) were freshly made. Adequate volumes from these stock solutions were then added to TEAKM to make up the final extraction medium.) The extraction medium was kept in an incubation cup on a cooled plate (2–4°C), which was connected to a Colora Klein-Kryostat (Colora Messtechnik GmBH., Lorch, West Germany). The extraction was monitored under a Wild stereomicroscope and allowed to continue for 10–15 min. To facilitate the process, the glands were torn open with two siliconized steel needles. After completion of the extraction, the sticky, nonsolubilized portions of the gland including the nuclei were removed to the side of the incubation cup with the two needles, and the polysome extract was pipetted onto a cold sucrose gradient. The remainder of the glands was solubilized in 500 μl of a Sarkosyl-pronase solution (0.5% Sarkosyl, 1 mg/ml pronase) for 60 min at room temperature, and the TCA-precipitable activity was measured as described below.

Sucrose Gradient Centrifugation

The sample was layered on top of a 4.8-ml 15–60% (wt/wt) sucrose gradient made up in TEAKM. The centrifugation was carried out at 2°C for 30 min at 40,000 rpm (130,000 g_{av}) in a Spinco L2B ultracentrifuge (SW 65 rotor) (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The gradient was collected into about 40 fractions. The linearity of the gradient was checked by refractive index measurements. Fractions in which radioactivity content was to be determined were diluted with 250 μl of TEAKM, and TCA and bovine serum albumin were added to final concentrations of 10% and 0.5 mg/ml, respectively. The samples were shaken vigorously, kept in the cold for 30 min, and plated on glass fiber disks (Whatman GF/C). When dry, the disks were placed in 10 ml of Soluene scintillator (0.3 ml of Soluene 100, 0.2 ml of methoxyethanol, 50 mg of Permablend III, 9.5 ml of toluene), kept at room temperature for 60 min, and then counted in a Packard Tri-Carb liquid scintillation spectrometer (Model 3380; Packard Instrument Co., Inc., Downers Grove, Ill.).

Release of RNA and Electrophoresis of Labeled RNA

Certain fractions from the sucrose gradients were pooled as indicated in the figure legends. Subsequently, an equal volume of TEAKM, 25 μg of *Escherichia coli* RNA and 0.1 vol of a preincubated Sarkosyl-pronase solution (5% and 10 mg/ml, respectively) were added. The samples were carefully shaken for 5 min in the cold, 2 vol of cold absolute ethanol were added, and the RNA was allowed to precipitate overnight at −20°C. The precipitates were collected by centrifugation, dissolved in an SDS-containing electrophoretic buffer (0.2% SDS, 0.02 M NaCl, 0.002 M EDTA in 0.02 M Tris-HCl, pH 8.0), and then analyzed in 1% agarose gels as earlier described (2, 5).

Electron Microscopy of Polysome Fractions

25-μl droplets from defined sucrose gradient fractions were placed on a cooled Teflon surface (4°C). One carbon-stabilized, parlodion-coated grid was placed inside each droplet for 5 min. The grids were then immersed in 4% formaldehyde (in TEAKM) at room temperature for 15 min. The grids then were treated for 10 s in 0.4% Photo-Flo (Kodak) in TEAKM, blotted dry and stained in a freshly prepared 1% phosphotungstic acid (PTA) solution (in 70% ethanol) for 30 s, rinsed in 95% ethanol, and air dried (18). The samples were examined and photographed in a Philips EM 300 electron microscope.

RESULTS

Extraction of Polysomes

To obtain a high yield of polysomes from *C. tentans* salivary glands, it was found essential to solubilize the secretion efficiently (cf. reference 14). This was accomplished when both a nonionic (0.5% Tween 80) and an ionic detergent (0.5% DOC) were present in the extraction medium.

The addition of β-mercaptoethanol (0.1%) further promoted solubilization of the secretion. The ionic conditions (0.1 M KCl and 0.003 M $MgCl_2$) were chosen so that the polysomes remained intact and the size and morphology of the polytene chromosomes were conserved during the extraction.

Salivary glands were isolated and transferred into the extraction medium, and the polysomes were extracted for 10–15 min in the cold as described in Materials and Methods. The extraction process was monitored under a stereomicroscope. Before the extraction, the salivary gland cells were readily observed (Fig. 1*a*), as well as the nuclei containing the polytene chromosomes (Fig. 1*c*). After completion of the extraction, the cells were almost transparent (Fig. 1*b*), while the nuclei with the chromosomes could still be recognized (Fig. 1*d*). The undissolved portions of the glands (Fig. 1*b*) were removed from the solution, and the extracts were then directly available for further analysis.

During the extraction of the polysomes, it is important that the high molecular weight RNA remain intact. Therefore, the integrity of the RNA in the polysomal extract was compared to that of RNA directly released from sister glands by Sarkosyl-pronase treatment. It was found that the RNA from the polysome extract (Fig. 2*a*) had a similar size distribution when compared with that released directly from glands with Sarkosyl-pronase (Fig. 2*b*). The main difference was found in the highest molecular weight region: the 75S RNA peak was less prominent and some additional label instead appeared in the 30–75S region. Consequently, while the degradation of RNA had to a large extent been restricted during the present extraction conditions, there was still some degradation of 75S RNA. The radioactivity in the nonsolubilized portion of the glands (about 10–25% of the total activity) did not show any preferential enrichment of either 75S RNA or the ribosomal and transfer RNA species when analyzed in agarose gels (not shown). It can be concluded therefore that the RNA in the polysomal extract is kept essentially undegraded and constitutes a large, representative portion of the total labeled RNA of the gland.

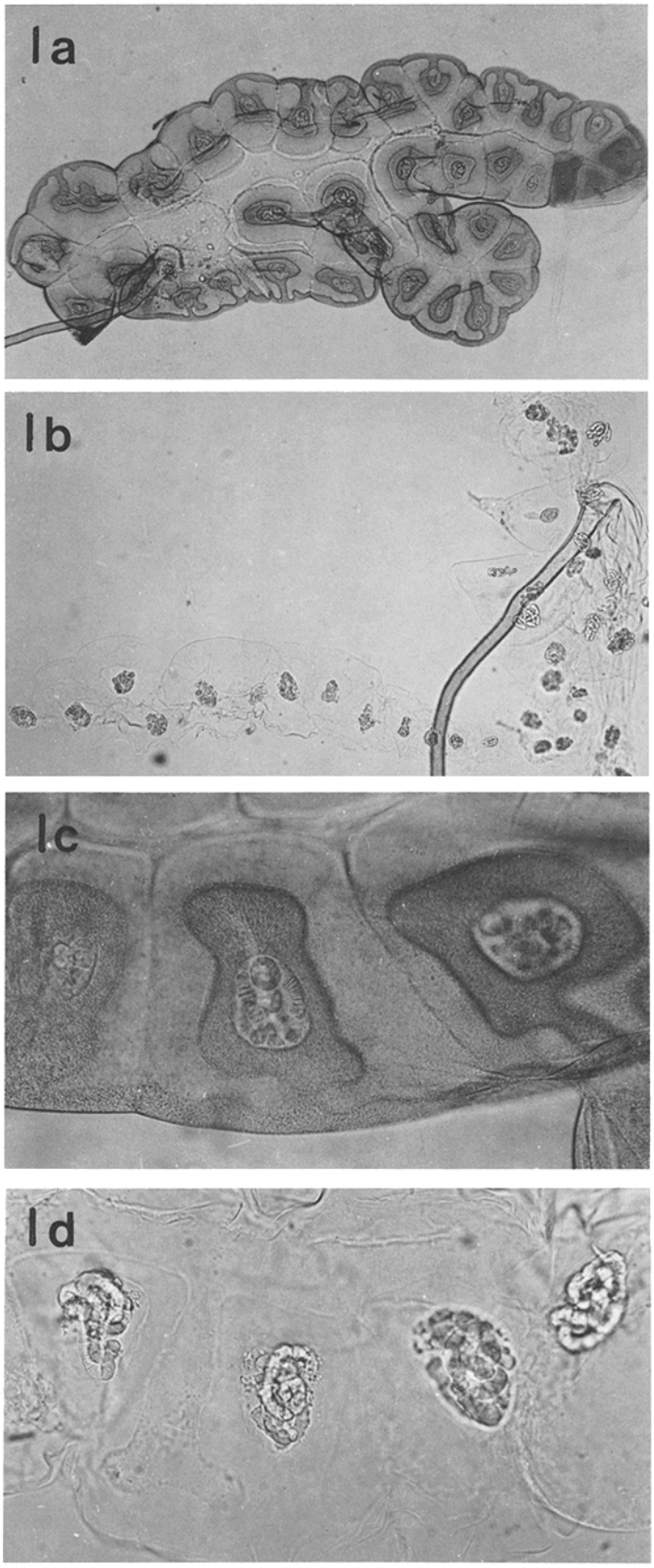
Sucrose Gradient Sedimentation of Polysomes

The ribonucleoprotein particles in the polysomal extract were characterized by sucrose gradient sedimentation. A typical profile is presented in Fig. 3. A sharp monosome peak (~75S according to reference 14) is recorded, as well as a broad distribution of more rapidly sedimenting ribonucleoproteins (200–2,000S) with an average peak value of about 700S. The polysomal nature of the rapidly sedimenting material in the 200–2,000S range was inferred from the following observations. When the polysomes in the extract were dissociated by EDTA (0.02 M), essentially all the radioactivity was shifted from the polysome region towards the top of the gradient (Fig. 3). Moreover, polysomes from corresponding sucrose gradient fractions were directly visualized in the electron microscope (see below).

Electron Microscopy of Fractionated Polysomes

Material from defined sucrose gradient fractions was collected on grids and visualized in the electron microscope as described in Materials and Methods. Fraction 7 from the heavy polysome region and fraction 19 from the light polysome region (Fig. 3) were selected for a detailed analysis. Grids from both these fractions contained a sparse distribution of polysomes, but these structures were present in all grid squares. There was no evidence for major amounts of other cellular material cosedimenting with the polysomes. Photographs of polysomes from the two gradient fractions are presented in Fig. 4. Large-sized polysomes, characteristic of polysomes from fraction 7, are displayed in Fig. 4*a–c*, while smaller ones typical for fraction 19 are shown in Fig. 4*d–f*. Most polysomes appeared reasonably clean, although sometimes minor amounts of attached material were observed. Regardless of the sucrose gradient fraction used as the source of the polysomes, the ribosomes were typically densely clustered as shown in Fig. 4*a* and *d*. Sometimes more extended polysomes were seen (Fig. 4*b* and *e*), and in favorable cases a strand between the adjacent ribosomes was also visible (Fig. 4*c* and *f*). The most extended polysomes in both fractions clearly suggested that the structures observed corresponded to individual polysomes rather than to polysomal aggregates. This inference was further strengthened by the observation that even in the most extended polysomes more than two free ends were never recognized.

To determine the size distribution of polysomes in the two sucrose gradient fractions, the number of ribosomes per polysome was determined from four independent experiments. 200 random poly-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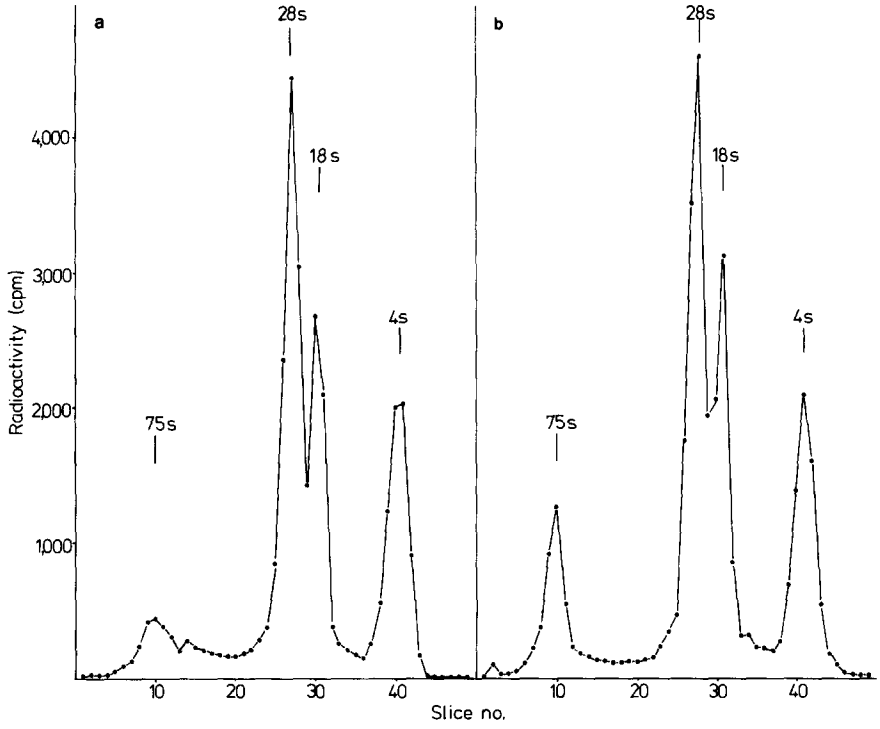


FIGURE 2 Electrophoretic analysis of long-term labeled RNA in a polysome extract and in a Sarkosyl-pronase extract from *C. tentans* salivary glands. Larvae were kept for 3 days in 20 ml of culture medium supplied with tritiated cytidine and uridine. Polysomes from three salivary glands were extracted in 400 μl of Sarkosyl-pronase. *E. coli* RNA was present during the extractions and later on served as carrier RNA. The RNA of the two samples was precipitated in ethanol, redissolved, and analyzed in parallel in a 1% agarose gel. *a:* polysome extract, *b:* Sarkosyl-pronase extract.

somes from gradient fractions 7 and 19 were studied in each experiment, and the number of ribosomes counted in each polysome. The results of these experiments are summarized in the histogram in Fig. 5. It can be observed that the average polysome in fraction 7 (corresponding to approximately 1500S) contains about 60 ribosomes, though some polysomes contain 100 ribosomes or more. The smallest polysomes in fraction 7 might be real but, alternatively, they may also represent degraded structures. In fraction 19 (corresponding to 400S), polysomes harboring 11–16 (ribosomes are dominant, but again the size range is considerable. The results from one of the four experiments have been included in Fig. 5 (dotted areas) to demonstrate that the size variations within fractions 7 and 19 were not the result of a variation between experiments but rather reflect a variation within each sucrose gradient fraction in a given experiment.

Electrophoretic Analysis of Polysomal RNA

The following experiment was carried out to determine whether cytoplasmic 75S RNA is lo-

FIGURE 1 Salivary glands from *C. tentans* before and after extraction of polysomes. Salivary glands were extracted in a Tween-DOC solution as described in Materials and Methods. During the treatment the glands were torn open with dissection needles. In *a* and *c*, a salivary gland is shown before, and in *b* and *d* after the extraction. The cytoplasmic material is almost completely solubilized, while the nuclei containing the polytene chromosomes can still be observed. The gland duct can be discerned in *a* and *b*. The diameter of a cell nucleus is about 75 μm.

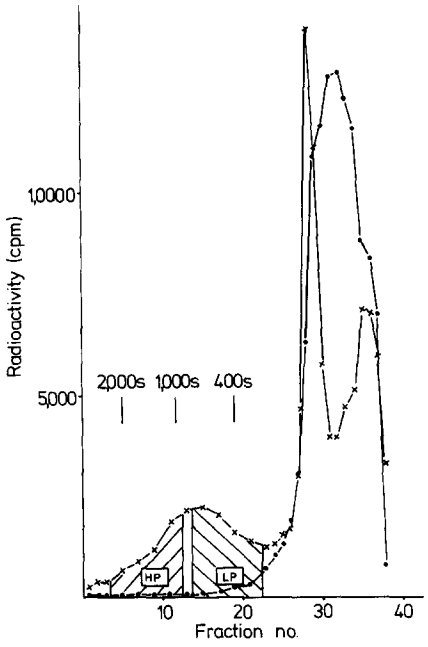


FIGURE 3 Sedimentation analysis of a polysome extract from *C. tentans* salivary glands with and without EDTA. Larvae were labeled for 3 days in culture medium with tritiated cytidine and uridine. Two polysome extracts were prepared as described in Materials and Methods, mixed, and then split into two equal portions. To one of them, 0.1 vol of 0.2 M EDTA was added. Both samples were kept for 5 min at 2–4°C, layered onto 15–60% sucrose gradients, and spun as described in Materials and Methods. The gradients were fractionated and the TCA-precipitable activity was determined in each fraction, unless some of the fractions were saved for further analysis. The sedimentation values along the gradient were estimated according to Noll and Stutz (19), using a preparation of *C. tentans* ribosomes (S-value ~75S as determined in reference 14). Non-EDTA-treated sample: (×), EDTA-treated: (●).

cated in salivary gland polysomes, and if so, to what extent. A polysome extract was split into two equal parts, one of which was EDTA treated. The two samples were run in parallel sucrose gradients. To study the distribution of 75S RNA within the two gradients, fractions from the polysomal as well as the post-polysomal region of each gradient were saved (see legend to Fig. 6), and the RNA from these fractions was extracted and analyzed by electrophoresis in 1% agarose gels. The electrophoretic profiles of the RNA from the polysomal regions are presented in Fig. 6*a* and those of the RNA from the post-polysomal regions in Fig. 6*b*. During the experiment, some 75S RNA was degraded to a certain extent, but it was still mainly recorded in the 30–75S range (cf. Fig. 2). Since native and partially degraded 75S RNA constitute most of the 30–75S RNA, we will study 75S RNA alone as well as RNA from the 30–75S range.

Before the analysis of the effect of EDTA treatment on the distribution of 30–75S RNA along the gradient, it was important to establish whether equal amounts of 30–75S RNA were recovered in the two gradients, and whether considerable amounts of this RNA appeared in the gradient pellets. It was calculated that the activity in 30–75S (polysomal + postpolysomal region) in the non-EDTA-treated sample (● in Fig. 6*a* and *b*) did amount to about the same value as in the EDTA-treated one (○) (3,774 and 3,821 cpm, respectively). Furthermore, the pelleted activity was very low in both gradients (less than 5% of the total recovered activity), and ribosomal and transfer RNA constituted the major RNA species in the pelleted RNA (not shown). There was no evidence for an enrichment of 30–75S RNA in the pellets.

It is evident from Fig. 6*a* and *b* that EDTA changed the distribution of RNA along the gradient. When the polysomes were kept intact, considerably more labeled 30–75S RNA was recorded in the polysomal than in the post-polysomal region (3,155 vs. 619 cpm). However, when the polysomes were dissociated, most radioactive 30–75S RNA was detected in the post-polysomal region (2,930 vs. 891 cpm). It was concluded that more than 60% of total 30–75S RNA shifted from a polysomal to a post-polysomal position upon EDTA treatment, and therefore was likely to be present in polysomal structures. Moreover, it can be noted that RNA of 75S size was almost exclusively located in the polysome region and that at least 40% of this RNA appeared in the post-polysomal region after disruption of the polysomes. These figures for the proportion of EDTA-sensitive RNA should be regarded as minimum estimates, because some 30–75S RNA might have been released from polysomes while still, as part of large ribonucleoprotein particles, sediment in the polysomal region (cf. review on ribonucleoproteins by Spirin, 25). The fact that the material remaining in the polysomal region after EDTA treatment is located mainly in the slowly sedimenting portion of that region (Fig. 3) might, in fact, support such an idea. Although it is evidently difficult to arrive at a more precise estimate of the amount of polysome-

bound 75S RNA, it seems reasonable to conclude that cytoplasmic 75S RNA is predominantly located in polysomes.

Further analysis of polysomal RNA was carried out by a separate extraction of RNA from the heavy polysome (HP) as well as the light polysome (LP) region of the sucrose gradient (Fig. 3). In both samples, RNA in the range of 10–75S was observed (Fig. 7), but there was relatively more RNA of the highest molecular weights in the HP than in the LP fraction. Evidently, 75S RNA is preferentially present in polysomes of larger sizes.

DISCUSSION

Extraction of Salivary Gland Polysomes

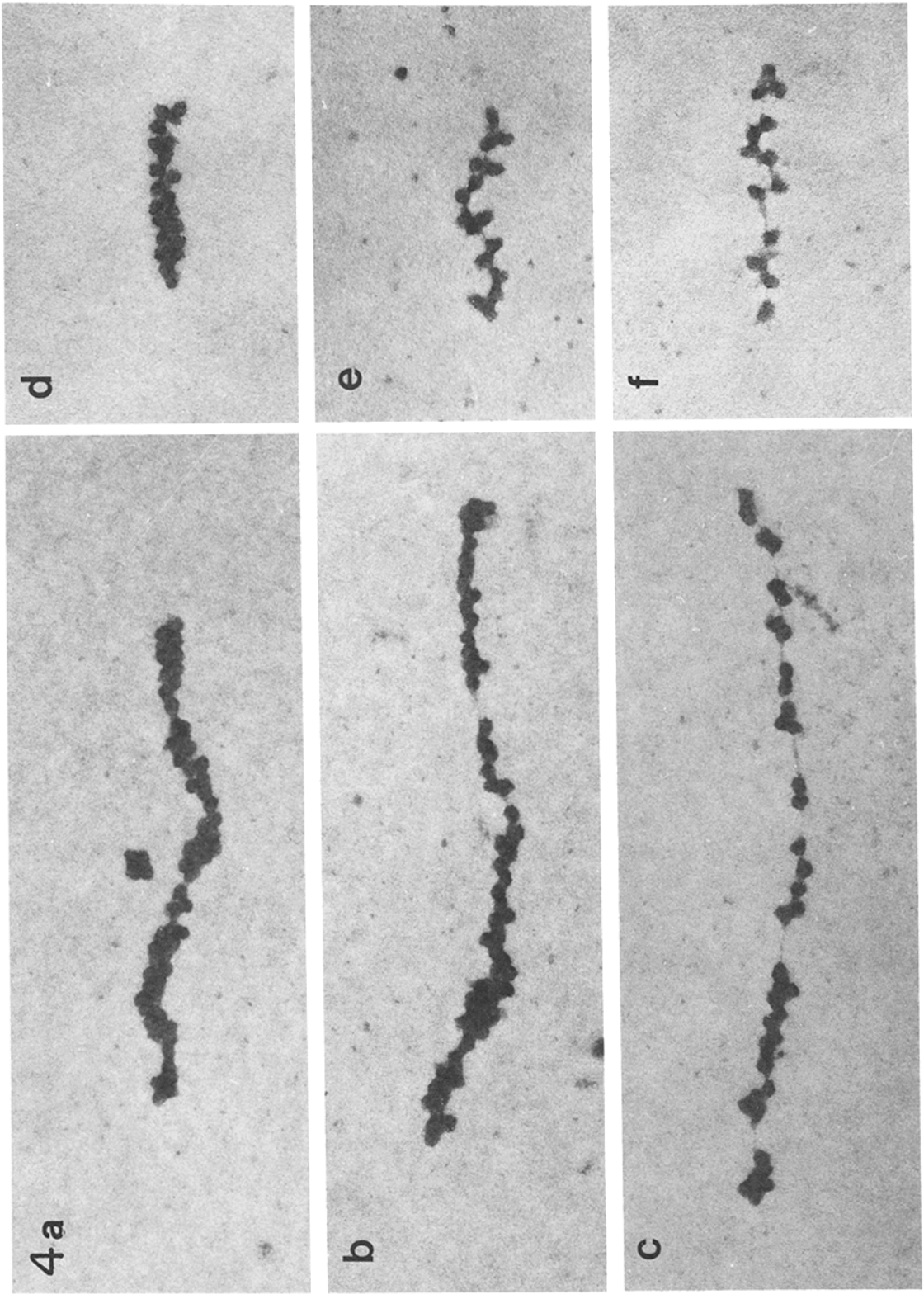
It was earlier shown that polysomes could be efficiently released from *C. tentans* salivary glands by a conventional homogenization technique, provided the salivary secretion was efficiently solubilized in a high salt medium containing a nonionic and an ionic detergent (14). However, this approach caused a considerable degradation of the highest molecular weight RNA and, therefore, a new technique was developed and applied in the present investigation. The homogenization step was replaced by a mild, free-hand dissection procedure carried out under a stereomicroscope. The extraction process was followed in the microscope and interrupted when proper solubilization had been obtained. Moreover, the nondissolved residue including the nuclei was directly removed by dissection needles, thereby alleviating a precentrifugation step. This procedure proved to be rapid, mild, and well controlled. When the RNA of the extract was characterized, it was found that it contained RNA of very high molecular weights, including 75S RNA (Fig. 2*b*). Although some degradation of 75S RNA still took place, it was evident that the present technique preserved the highest molecular weight RNA to a larger extent than the homogenization method (cf. Fig 1 A in Hosick and Daneholt, 14). It should also be noted that the polysomes extracted with the new technique sedimented more rapidly (peak at about 700S) than those obtained with the old one (peak at 300–400S).

Large-Sized Polysomes in the Salivary Glands of C. tentans

The most remarkable property of the polysomes from *C. tentans* salivary glands is their rapid sedimentation in sucrose gradients: the peak corresponded to about 700S and the range covered S-values from 200 up to 2,000. This result suggested that polysomes of large sizes exist in the salivary glands. It is, however, important to consider other explanations before such a conclusion is drawn. The EDTA-shift experiment certainly indicated that we are dealing with polysomal material (see also result of mild RNase treatment in reference 14), but it did not exclude the possibility that the rapid sedimentation was mainly due to contaminating material or, alternatively, to an aggregation of polysomes. The visualization of the polysomal material in the electron microscope showed that the polysomes were free from contaminants such as secretion and membranous material (Fig. 4). The rapid sedimentation therefore cannot be attributed to material adhering to the polysomes. In fact, the number of ribosomes per polysome in fractions 7 and 19 (Fig. 5) seems to agree reasonably well with the number that can be approximately predicted from the corresponding sedimentation values (21). Furthermore, the electron microscope results directly suggested that the rapidly sedimenting material corresponds to single, large-sized polysomes rather than to aggregates of smaller polysomes. Well-extended polysomes showed a linear arrangement of ribosomes with a thin strand in between (Fig. 4*c*). It should be noted that these polysomes never displayed more than two free ends, which should at least occasionally have been observed if two or more polysomes were randomly aggregated.

Two other observations also suggested that we are dealing with large-sized polysomes. First, if some degradation of RNA did occur during preparation of the polysomes, the most rapidly sedimenting material was not observed (cf. results presented by Hosick and Daneholt [14] with the homogenization technique). It seemed, therefore, as if the most rapidly sedimenting material is dependent upon intact RNA of very high molecular weight. Furthermore, RNA extracted from the heavy polysomes contained relatively more giant-sized RNA (50–75S) than RNA from the light polysomes (Fig. 7). Since these observations are not compatible with a random type of aggregation, again the most likely interpretation of the polysome profile observed is that it represents individual polysomes, some of very large size.

It can be argued, on the other hand, that the observed polysomes represent partly degraded ones and that polysomes in the salivary glands are,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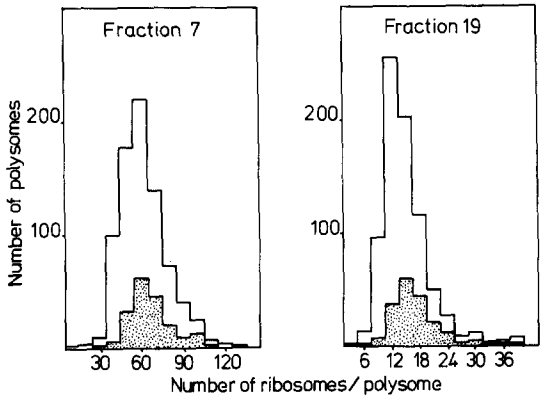

FIGURE 5 Size determination in the electron microscope of heavy and light polysomes from *C. tentans* salivary glands. Heavy and light polysomes were prepared for electron microscopy as described in the legend to Fig. 4. In fractions 7 and 19 the number of ribosomes per polysome was determined from 200 consecutive, analyzable polysomes. About each second polysome had to be discarded because the ribosomes were too closely associated to permit accurate counting of the ribosomes. The plotted data were obtained from four separate experiments. The results of one of the four experiments are also given in the figure (dotted areas).

in fact, larger. When essentially undegraded salivary gland RNA (Fig. 2*b*) was compared to that obtained immediately after the extraction of the polysomal material (Fig. 2*a*) or to that released from polysomes fractionated in sucrose gradients (Fig. 6), it was evident that the highest molecular weight RNA, in particular 75S RNA, was degraded to some extent during the experiment. It would therefore not be surprising if many of the polysomal structures visualized in the electron microscope only represent fragments of huge polysomes present *in situ* in the salivary gland cells. The observation that some polysomes harbor 100 or more ribosomes (Fig. 5) is compatible with such a view.

Polysomal 75S RNA and Its Significance

It was concluded from the EDTA-shift experiment (Fig. 6) that most of the cytoplasmic 75S RNA is present in polysomes. Since most cytoplasmic 75S RNA probably originates from the BRs (3), it is likely that at least some 75S RNA recorded in the polysomes is BR RNA. Such a conclusion is further supported by a recent *in situ* hybridization study (27) showing that polysomal RNA contains BR 1 and BR 2 sequences. The process of transfer of 75S RNA from the BRs via nuclear sap into cytoplasm was studied in detail earlier (5, 16) and the conclusion was drawn that this transfer occurs without a major change in the size of the primary transcripts (75S RNA). From the present investigation, it can furthermore be stated that at least some 75S RNA is also incorporated into polysomes without being changed in size.

The finding that 75S RNA from the BRs enters polysomes makes it likely that this fraction acts as messenger RNA. On the basis of the available polysome data, it is not possible to predict precisely the length of the coding segments in 75S RNA, but some information can be obtained. It was found that RNA of 75S size was enriched in the heavy polysome region. The most frequent polysome size in this region (fraction 7 analyzed) was 60 ribosomes per polysome, but polysomes of still larger sizes (100 or more ribosomes) were observed. This implies that these polysomes are in the size range of those known to produce polypeptides of very large sizes. Examples of such large-sized polysomes are those making fibroin (polypeptide 350,000 daltons in mol wt [26]; 50–80 ribosomes per polysome [17]), myosin (200,000 daltons [6]; 50–60 ribosomes [13]), and vitellogenin (170,000 daltons [23]; 30–40 ribosomes [22]). By comparison, it would be expected therefore that the coding regions in 75S RNA probably be of considerable length and correspond in size to those required for polypeptides of about 200,000 daltons or more.

It is interesting to relate the possible presence of long coding segments in BR 75S RNA to the information available on the sizes of the salivary polypeptides, because cytogenetic studies (1, 9,

FIGURE 4 Visualization in the electron microscope of rapidly and slowly sedimenting polysomes from *C. tentans* salivary glands. Polysomes were isolated and sedimented in sucrose gradients as described in Materials and Methods. One sample from the heavy polysome region (fraction 7) and one from the light polysome region (fraction 19) were saved for electron microscopy. 25-μl droplets were made and a grid was inserted in each of them. The polysomal material was allowed to attach to the grid for 5 min in the cold and was then fixed in formaldehyde, rinsed in Photo-Flo, dried, stained in phosphotungstic acid, and finally examined in the electron microscope. (*a–c*): heavy polysomes, (*d–f*): light polysomes.

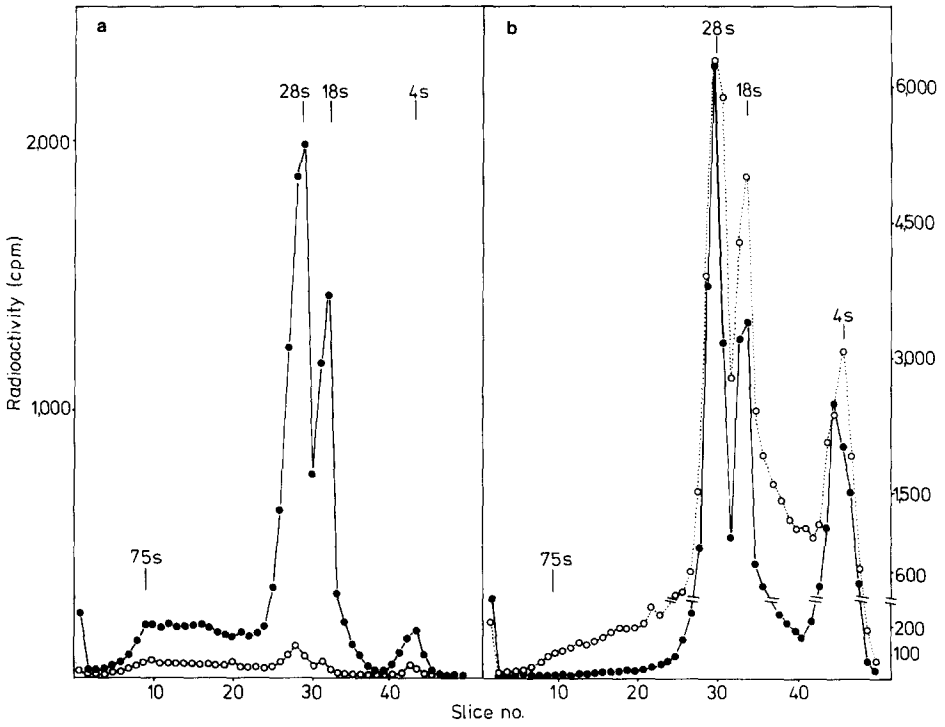

FIGURE 6 Electrophoretic analyses of RNA from the polysomal and the post-polysomal regions of sucrose gradients after sedimentation of extracts with intact polysomes or with the polysomes dissociated by EDTA. Labeled polysome extracts containing intact or dissociated polysomes were prepared and analyzed in sucrose gradients as described in the legend to Fig. 3. Fractions from the polysomal (4, 6 . . . 22) and the post-polysomal regions (24, 26 . . . 38) were pooled, and the RNA was released by Sarkosyl-pronase and precipitated by ethanol. The RNA of each sample was collected by centrifugation, redissolved, and analyzed by electrophoresis in 1% agarose gels. 6*a:* (●): RNA from the polysome region after sedimentation of the extract with intact polysomes; (○): RNA from the polysome region after sedimentation of EDTA-treated extract. 6*b:* (●): RNA from the post-polysomal region after sedimentation of the extract with intact polysomes; (○): RNA from the post-polysomal region after sedimentation of the EDTA-treated extract.

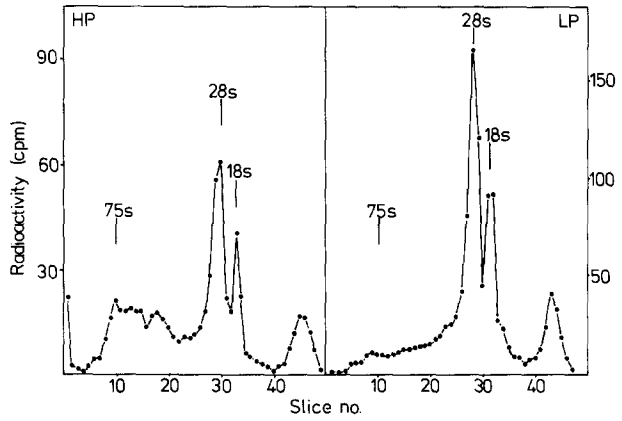

FIGURE 7 Electrophoretic analysis of long-term labeled RNA from heavy (HP) and light polysome (LP) regions of a sucrose gradient. Salivary gland polysomes were labeled, extracted, and analyzed in a 15–60% sucrose gradient as described in Materials and Methods. Fractions were pooled from the heavy (4, 6 . . . 12) and the light polysome region (14, 16 . . . 22) as shown in Fig. 3, and the RNA was released and analyzed by electrophoresis in 1% agarose gels.

11) have suggested that genetic information for salivary polypeptides is encoded in BR RNA. Grossbach (9–11) found that in salivary secretion there are five main polypeptides (up to 500,000 daltons). Furthermore, from comparisons between the galactose induced or developmental changes in the sizes of the BRs and the variations in the synthesis of the five salivary polypeptides, both Grossbach (12) and Pankow, Lezzi and Holderegger-Mähling (20) have concluded that fraction 2 (259,000 daltons, according to reference 10) is correlated with BR 2, and fraction 3 (224,000 daltons) with BR 1. It is evident that the sizes of these two polypeptides would correspond to the long coding segments in 75S RNA. However, Rydlander and Edström (24) recently modified Grossbach's extraction technique and then recorded most of the salivary polypeptides in the size range of 30,000–60,000 daltons. While their data do not rule out the possibility that there are also polypeptides of larger sizes, they point out that measures should be taken to ensure that cross-linking between the polypeptide chains not occur during the extraction. Until the various salivary polypeptide fractions have been more extensively studied, the interpretation of the polysome data as to informational content in 75S RNA must be cautious. Even if it is accepted that 75S RNA contains coding information for salivary polypeptides, it is not possible at the present time to state whether a large 75S RNA-containing polysome reflects the synthesis of one large polypeptide or the synthesis of two or more polypeptides of smaller sizes. The most urgent tasks now seem to be to further characterize the salivary polypeptides and to establish, in a suitable translational system, the nature of the coding sequences in 75S RNA from BR 1 and in 75S RNA from BR 2.

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