Localization of a Messenger RNA on the Ribosomes of Ungerminated Wheat Embryos

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Abstract

A ribosomal fraction having endogenous template activity was isolated from ungerminated wheat embryos. When the total cytoplasmic ribosomes are sedimented in a sucrose gradient and aliquots tested in a cell-free system, endogenous activity occurs in fractions sedimenting at approximately 90S and 45S whereas response to an exogenous message coincides with the A_{260} profile of the bulk of the ribosomes at 74S. The three sedimentation groups have the same buoyant density in CsCl gradients (1.65 g/cc) and analysis of RNA from the 90S, 74S, and 45S ribosome populations on polyacrylamide gels shows them to be very similar. The RNA isolated from the total ribosomal fraction yielded seven peaks on electrophoresis with molecular weights as follows: I, 1.3×10^6 daltons; II, 0.76×10^6 daltons; III, 0.4×10^6 daltons; IV, 0.25×10^6 daltons; V, 0.15×10^6 daltons; VI, 9.5×10^4 daltons; and VII, $3.6 - 5.0 \times 10^4$ daltons. Fractions I, II, and VII contain the 24S, 17S, and 5S ribosomal RNA species, respectively. Fraction VII also contains some of the transfer RNA.. Fraction V displays template activity in a cell-free system. It is suggested that messenger RNA in the dry embryo is preserved in complex form with ribosomes. These messenger RNA-ribosome complexes show endogenous activity in a cell-free system and sediment at 45S and 90S. Most of the ribosomal population consists of free ribosomes sedimenting at 74S.

1. Introduction

Many developmental systems have a latent stage in which protein synthesis is essentially inactive. In unfertilized eggs of nearly all animal species studied thus far, messenger RNA (mRNA) which survives long periods of metabolic dormancy, but which is functional after fertilization, has been demonstrated (Gross, 1968; Spirin, 1969, for reviews). It has been suggested that this mRNA is present in some systems in the form of ribonucleoprotein complexes called "informosomes" (Spirin & Nemer, 1965; Infante & Nemer, 1968) or m-RNP particles (Perry & Kelly, 1968; Henshaw, Revel & Hiatt, 1965; McConkey & Hopkins, 1965; Latham & Darnell, 1965; Perry, 1966). It has been proposed that such mRNA-protein complexes are involved in the translational control mechanism, in the transport of mRNA from the nucleus, or in the stabilization of the labile mRNA molecule (Spirin, 1969). It is not clear whether such structures in fact contain the conserved or "masked" messenger RNA present in animal oocytes and other embryonic material, nor is the mode of the association of such messenger RNA with the ribosome and its subsequent translation known.

In the dry wheat embryo, the entire apparatus necessary for protein synthesis is present and is activated by the imbibition of water in early germination (Marcus, Feeley, and Volcani, 1966; Marcus & Feeley, 1966; Marcus & Feeley, 1964). Similar results have been demonstrated in soybean seeds (Matsushita, Mori & Hata, 1968) and further studies in this system have shown the presence of RNA molecules which have template activity in the cell-free system and can be isolated in the 100,000 g supernatant of homogenized seeds (Mori, et al., 1968). Previous studies with wheat embryo have shown the presence of conserved mRNA on the basis that RNA prepared from the ungerminated embryo can enhance amino acid incorporation in a cell-free system, that this RNA will compete with labelled mRNA

formed in later stages of germination on complementary regions of wheat DNA, and that the inactive ribosomes of the ungerminated embryo can be activated *in vitro* (Marcus & Feeley, 1966; Chen, Sarid & Katchalski, 1968). Since the mRNA is present in the dry embryo in an inactive form, it is either "masked" or exists in a separate structural entity which differs from the site of translation within the cell. The present communication describes the isolation of an RNA fraction which has template activity in a cell-free system from the ribosomal fraction of ungerminated embryos. This endogenous message activity was shown to be associated with two distinct sedimentation groups; one lighter (45S) and one heavier (90S) than the 74S monoribosomes. It is suggested that the mRNA is conserved in the dry embryo in association with single ribosomes to form a complex which differs in sedimentation from usual 74S ribosomes but which can be translated *in vitro*.

2. Materials and Methods

(a) Preparation of ribosomes

Wheat embryos (Triticum vulge var. Florence) were prepared according to Johnston and Stern (1957) and stored in a dessicator at 4^0C. 10 g of dry wheat embryos were homogenized in 50 ml of PM buffer containing 0.01 M phosphate buffer, pH 7.6, 0.01 M $MgCl_2$, 0.02 M KCl, and 0.5 M sucrose. The homogenate was centrifuged twice for 20 minutes at 14,000 rpm in the Sorvall RC-2 refrigerated centrifuge and the resultant supernatant taken for centrifugation for 120 minutes at 47,000 rpm in a No. 50 rotor of a Spinco preparative ultracentrifuge. The precipitate was dispersed in 20 ml of PM buffer and recentrifuged for 60 minutes at 47,000 rpm. The resulting ribosome pellet was suspended in appropriate media for formaldehyde fixation, for sedimentation velocity in a sucrose gradient, or for RNA extraction.

(b) Isopycnic centrifugation of fixed ribosomes

Ribosomes prepared by the procedure given above were suspended in 3 ml of 0.01 M phosphate buffer, pH 7.6, containing 0.02 M KCl and 0.01 M $MgCl_2$ at a concentration of 1.5 mg/ml. Following centrifugation for 10 minutes at 12,000 rpm in the Sorvall RC-2 centrifuge, the supernatant containing the ribosomes was fixed with 6% formaldehyde (pH 7.0) for 4 days at 4°C (Spirin, Belitsina & Lerman, 1965). 0.5 ml of the fixed ribosome solution was layered onto a preformed CsCl gradient containing 6% formaldehyde and 0.2% Brig and then centrifuged at 36,000 rpm for 15 hours in the SW39 rotor of a Spinco preparative ultracentrifuge to reach equilibrium. Fractions were collected, and absorbance at 260 mμ, and refractive index determined.

(c) Sucrose density gradient centrifugation

Ribosomes were prepared as above but suspended in 3 ml. of TM buffer containing 0.01 M Tris buffer, pH 7.6, 0.01 M $MgCl_2$, and 0.02 M KCl. Aliquots of 0.2 ml. were layered onto a 5 to 20% sucrose gradient made up in TM and centrifuged for 45 minutes at 36,000 rpm. Fractions were collected and analyzed spectrophotometrically.

(d) Isolation and purification of RNA

Ribosomes prepared as above from 10 g of dry wheat embryo were suspended in 10 ml. of 0.2 M KCl prior to addition of 10 ml. of 0.1 M potassium-acetate buffer, pH 5.2. This suspension was made 1% with respect to sodium lauryl sulfate (SLS) and the solution shaken for 10 minutes at room temperature after addition of NaCl to a final concentration of 1 M. The preparation was extracted twice with redistilled phenol buffered to pH 5.2 with 0.01 M potassium-acetate buffer prior to precipitation

with two volumes of cold (-20° C) 95% ethanol overnight. The RNA was redissolved in 0.01 M Tris-HCl buffer, pH 7.6, phenol extracted a third time, and reprecipitated in cold 95% ethanol prior to use on acrylamide gels.

(e) Acrylamide gel electrophoresis

Acrylamide gel electrophoresis was done according to the technique of Loening (1967). The concentration of cross-linking agent (N, N-methylene bis-acrylamide) was 0.25% in all types of gels utilized. Electrophoresis was performed on pre-run gels in cylindrical tubes (0.6 x 9 cm) at 5 mA/tube for the time specified. Gels were made up in buffer containing 30 mM NaH_2PO_4, 36 mM Tris-HCl, and 1 mM EDTA (pH 7.8). The running buffer contained in addition, 0.2% SLS although this was not included in gels used for extraction of RNA following electrophoresis. Following electrophoresis, gels were washed in distilled water and scanned in a modified Shimadzu spectrophotometer (Gressl & Wolowelsky, 1968).

The relationship between electrophoretic mobility and log molecular weight has been shown to be linear with a range of ribosomal RNAs (Loening, 1968). Escherichia coli 23S and 16S rRNAs (1.1×10^6 daltons and 0.56×10^6 daltons) respectively, were electrophoresed with wheat ribosomal RNA samples for greater precision in determining the molecular weights of the different RNA components. The migration of marker RNAs is not included in the figures.

(f) Extraction of RNA from polyacrylamide gels

RNA components were localized in gels by scanning in a spectrophotometer as described above. The gels were frozen in solid CO_2 and the portions containing the various peaks excised. In one set of experiments, these gel segments containing RNA were placed on top of carrier gels, electrophoresed, and the RNA migrating

out of the gel segment trapped in a buffer reservoir formed by placing a dialysis membrane over the top end of the gel tube. In other experiments the segments of gel containing the various RNA components were sliced into 1 mm sections and shaken at 0 to 4°C with 0.5 ml of 0.01 M Tris-HCl buffer, pH 7.4. The dispersed gel was removed by centrifugation and the RNA precipitated overnight by the addition of two volumes of cold 95% ethanol. Recovery of RNA from gels was about 50% by either method.

(g) Charging of RNA components with labelled amino acids

RNA fractions extracted from acrylamide gels were adjusted to a concentration such that there was 20 μg of RNA in 0.5 ml of 0.01 M Tris buffer, pH 7.6. These were incubated with a mixture containing 5 μM ATP, 10 μ M KCl, 5 μM $MgCl_2$, 10 μM Tris buffer, 0.25 μC H^3-labelled amino acids, and wheat 100,000 g supernatant (0.2 mg protein) fraction containing amino-acyl enzymes. Incubation was done at 25°C for 15 minutes and the reaction stopped by the addition of 2 ml of cold 5% TCA. The precipitate was collected on GF/C filters, washed successively with 5% TCA, ethanol-ether mixture, ether, dried, and counted.

(h) Cell free system

The cell free system of wheat embryo has been described previously (Marcus & Feeley, 1964; Chen, Sarid & Katchalski, 1968). 0.4 ml of the reaction mixture contained 22 μmoles Tris buffer pH 7.8, 2.25 μmoles $MgCl_2$, 0.3 μmoles GTP, 0.4 μmoles ATP, 16 μg phosphoenol pyruvic kinase, 45 μg phosphoenol pyruvic acid, 6 μmoles mercaptoethanol, 22.5 μmoles KCl, 25 μg purified wheat tRNA, 0.02 ml supernatant from wheat (0.5 mg protein), 0.5 μC ^{14}C amino acid mixture (Amersham, 52 mC/mM) or 0.25 μC ^{14}C phenylalanine (Amersham,

380 μC/μM). No more than 100 μg ribosomes were added directly from the sucrose gradient into each tube and incorporation was allowed to proceed for 30 minutes at 30°C. The reaction was stopped by adding 3 ml of ice cold 5% TCA for 10 minutes, the tubes centrifuged for 5 minutes at 3,000 rpm, and 3 ml of cold 5% TCA added to the precipitate. The tubes were then heated for 15 minutes at 90°C, cooled, and filtered onto GF/C fiberglass filters, washed with 12 ml 5% TCA, 5 ml of ethanol-ether and 5 ml ether successively. The dried filters were counted in the Packard Tricarb scintillation counter.

(i) Base composition analysis

Base composition analysis was performed as described by Edelman, Verma & Littauer (1970). Three to five A_{260} units of RNA in 25 μl was hydrolyzed in 0.3 M KOH for 18 hours and applied to Whatman No. 1 paper. Electrophoresis was carried out at pH 3.5 in 0.86 M pyridine-acetate buffer for 90 minutes at 3000 V in a cooled electrophoresis tank. The electrophorogram was dried overnight and the nucleotide spots located with ultraviolet light and eluted with 0.3 M HCl. The samples were neutralized and the absorbancies of the nucleotide solutions at 260 mμ were determined. Concentrations were calculated using extinction coefficients of 6.8, 14.2, 11.8, and 9.9 for CMP, AMP, GMP, and UMP, respectively.

(j) Sedimentation analysis

Sedimentation velocity measurements were carried out with 0.5 to 1.0 A_{260} units of RNA obtained from acrylamide gels. A Spinco Model E analytical ultracentrifuge equipped with ultra-voilet optics was used. Readings were taken at 260 mμ at a speed of 40,000 rpm and were corrected to water at 20°C.

3. Results

(a) Sedimentation analysis of the ribosomal population

The sedimentation pattern of the ribosomal population of ungerminated wheat embryos is demonstrated in Figure 1A. The ribosomal fraction was isolated by prolonged centrifugation to allow subparticles smaller than ribosomes to pellet. The only particles revealed in the sucrose gradient sediment at 74S and no polysomes or ribosomal subparticles can be detected (Fig. 1A). When the ribosomal population was fixed with formaldehyde and spun to equilibrium in a CsCl gradient, the material bands sharply at a buoyant density of 1.565 g/cc characteristic of monosomes (Fig. 1B). On the basis of these findings it is concluded that the ribosomal population in the ungerminated embryo consists of monosomes.

(b) Composition of the RNA isolated from the ribosomal fraction

The ribosomal fraction of ungerminated wheat embryos was prepared as in Methods. The RNA was extracted by the SLS-phenol method, and 1.0 A_{260} units of purified RNA was applied to a 2.5% acrylamide gel, electrophoresed, and scanned spectrophotometrically. The absorbance profile obtained (Figure 2) closely resembled that expected of RNA isolated from ribosomes with the 24S and 17S species making up 95% of the total RNA in the preparation. For convenience, the peaks have been numbered I through VII with the 24S and 17S species being peaks I and II, respectively. The molecular weights of 1.3×10^6 daltons obtained for the 24S species in peak I and of 0.76×10^6 daltons for the 17S species in peak II agree well with values obtained for other higher plants (see Attardi and Amaldi, 1970, for review). Ribosomal RNA also contain a species of lower molecular weight which sediments at about 5S and which has been identified as peak VII in Figure 2. However, in addition to these usual ribosomal RNA species, four other minor peaks have been clearly resolved which migrate between the 17S and 5S species.

In order to identify transfer RNA molecules or aggregates containing t-RNA, each of the fractions were extracted from acrylamide gels. Extraction was done by placing gel segments containing the RNA peak on a carrier gel, and trapping the RNA which electrophoreses out of the gel segment into a buffer chamber formed by placing a dialysis membrane over the top of the gel tube. 20 μg of each of the ribosomal RNA species shown in Figure 2, and extracted from gels by the method described, were tested for their ability to charge with labelled amino acids (Table I). About half of fraction VII is capable of accepting H^3-amino acids. Therefore about one half of fraction VII consists of 5S RNA and one half of t-RNA which can be seen as a shoulder on the low molecular weight side of peak VII (Fig. 2). These two components can be resolved on gels containing higher concentrations of acrylamide. The results recorded in Table I further show that fractions III to VI do not accept amino acids and therefore do not consist of tRNA aggregates.

The RNA fractions studied were subjected to base composition analysis (Table 2). The % (G + C) is lower (46.6) for fraction V than for any of the other RNA fractions all of which have a base composition with (G + C) over 50%. Table II summarizes sedimentation data, molecular weight determination, and base composition of all of the RNA fractions.

(c) Template activity of RNA fractions isolated from acrylamide gels

Since the minor fractions (peaks III to VI) are not the usual rRNA species or t-RNA, their template activity was investigated. A test for a messenger RNA is its ability to stimulate the incorporation of labelled amino acids in a cell free system. The seven RNA fractions were again extracted from acrylamide gels and added into a cell-free protein synthesizing system prepared as described in Materials and Methods. The cpm H^3-amino acids incorporated into TCA precipitable material is plotted as a histogram and compared to stimulation by an equivalent amount of an

exogenous T4 messenger RNA preparation. Figures 3A, 3B and 3C reflect three different RNA preparations and three different experiments. In Figures 3A and 3B the RNA peaks were isolated by the method of reverse electrophoresis. In Figure 3C the RNA species were extracted by shaking sliced gel segments in the presence of 0.01 M Tris buffer pH 7.6, at 0^0C for 12 hr. In each case, fraction V stimulated the incorporation of labelled amino acids to the greatest degree. Since in Figures 3A and 3B, RNA fractions other than V also show some stimulation, it is possible that this is due to some RNA degradation by the procedure used for isolation of RNA fractions in these experiments. Reelectrophoresis of the isolated fractions according to the procedure in Figures 3A and 3B, shows a broadening of the peaks. This does not occur when the procedure of Figure 3 is utilized.

Results similar to those presented in Figure 3 were obtained when the seven RNA fractions were tested in a heterologous cell-free system using E. coli washed ribosomes. In summary, the data in Figure 3 indicate that fraction V has template activity.

(d) Template activity within the ribosomal population

When ribosomes, isolated as described previously, were layered over a 5-20% sucrose gradient and spun for 70 minutes at 36,000 rpm, in the SW30 rotor of a Spinco preparative ultracentrifuge to allow for the monosomes to band at the center of the gradient, the pattern of material absorbing at 260 mμ was similar to that of wheat embryo monosomes (Fig. 3). 0.1 ml fractions of the ribosomal material obtained from the sucrose gradient were introduced into 0.4 ml of wheat cell-free system containing wheat t-RNA, energy regenerating system, wheat enzymes, and labelled phenylalanine as described in Methods. Each fraction was challenged with 10 μg of poly U and allowed to incorporate phenylalanine for 30 minutes. The pattern of the extent of polyphenylalanine synthesis supported

by the ribosomes as assayed by measuring the radioactivity incorporated into hot TCA precipitable material follows closely that of the A_{250} profile of the major 74S band of ribosomal material (Fig. 4). This indicates the presence of free ribosomes responding to the synthetic message at the main 74S band of the gradient.

To search for endogenous template activity within the ribosomal population, the same experiment was performed except that the 0.1 ml. aliquots of ribosomal material obtained from the sucrose gradient were allowed to code for protein translation in the presence of a ^{14}C amino acid mixture in the wheat cell-free system in the absence of exogenous message. Typical results of such an experiment are presented in Figure 4. The data given show that the endogenous activity is mainly associated with fractions 4 to 6 and 11 to 13, and does not coincide with maximum absorption at 260 mμ.

The amount of radioactive amino acids incorporated per unit weight of ribosome, in an experiment in which each of the incubation mixtures was enriched with additional 50 μg of monosomes (74S) pooled from the main band of the sucrose gradient, is given in Figure 5. The data presented show that endogenous template activity can be detected in two distinct bands, one sedimenting more rapidly (approximately 90S) and one sedimenting more slowly (approximately 45S) than the main band (74S) of ribosomes. The 45S fraction displays about twice as much activity as the 90S fraction. The majority of the 74S ribosomes are devoid of endogenous template but can respond to an exogenous message (poly U).

The above experiment (Fig. 5) shows also that mRNA-protein particles, which are not active in the cell-free system because of the lack of ribosomes, are not detectable in the ribosomal fraction which has been fractionated on the sucrose gradient.

In order to study the physical nature of the complexes containing the endogenous template activity, three fractions were pooled from the sucrose gradient of the ribosomal fraction shown in Fig. 6A and their density estimated. Fraction 2 consists of 74S ribosomes, fraction 1 represents the more rapidly sedimenting complexes of about 90S, and fraction 3 represents those of approximately 45S. The pooled fractions were fixed for 4 days in 6% formaldehyde and then centrifuged to equilibrium in a CsCl gradient (Fig.6B). The results clearly show that all three fractions have the same density of 1.565 g/cc which is typical of the density of mature wheat ribosomes.

The RNA of the three fractions of Fig. 6A were also extracted with SLS and phenol and analyzed on polyacrylamide gel electrophoresis. The scan of these gels at 260 mμ shows that the RNA is typical of ribosomal RNA with most of the RNA being present in the 24S and 17S species which are present at the absorption ratio of about 2:1. On the basis of the RNA analysis and the buoyant density characteristics it might therefore be concluded that the three fractions are composed primarily of ribosomes.

4. Discussion

The existence of conserved messenger RNA in the dry seed has been shown previously (Waters & Dure, 1966; Mori, et al., 1968; Chen, Sarid & Katchalski, 1968). By isolating RNA from ribosomes of ungerminated wheat embryos and subsequent fractionation of the RNA on acrylamide gels, four minor components in addition to the usual 24S, 17S, and 5S ribosomal RNA species were detected (Fig. 2). Of these minor species, one fraction (peak V, Fig. 2) is capable of stimulating the incorporation of labelled amino acids into TCA precipitable material in a cell-free protein synthesizing system (Fig. 3). The larger ribosomal RNA molecules do not show any significant stimulation (Fig.3C). Even where some degradation has accompanied the isolation and preparative procedures (Figs.3A and 3B), fraction V

was found to stimulate the wheat as well as a heterologous (E. coli) cell-free system to a significantly higher level than the other RNA fractions utilized. The possible activation of inactive preprogrammed ribosomes by fraction V seems therefore unlikely.

Fraction V can not represent an aggregate of t-RNA molecules since it cannot be charged with labelled amino acids (Table I). The finding that the base composition of fraction V is considerably different from that of the 24S and 17S rRNA species (Table II) further suggests that the origin of fraction V is not a degradation product of larger ribosomal RNA species. In this connection it is pertinent to note that the electrophoretic profile of RNA isolated from the embryo ribosomes is highly reproducible.

The ultimate proof for fraction V being a template would be the synthesis of a defined protein. In higher cell systems, such identification has only been shown for the 9S hemoglobin message isolated from reticulocyte polysomes and subsequent synthesis of b-chains of hemoglobin (Laycock & Hunt, 1969; Lockard & Lingrel, 1969).

The role of the other minor bands (fractions III, IV, VI) is less clear. Fraction VI may be similar to the 7S rRNA which appears to be H-bonded to 28S rRNA in animal ribosomes (Pene, Knight & Darnell, 1968; Elicieri & Green, 1969; and Penman, 1968). This possibility has not been investigated in this study nor have the origins or functions of fractions III and IV been elucidated. Fraction III migrates electrophoretically similarly to rRNA of plastid origin (Loening & Ingle, 1967). We have no proof that this is the case, since plastids should be eliminated in the procedure used for the preparation of ribosomes.

When ribosomes are isolated and purified from dry wheat embryos, they consist primarily of 74S particles as observed by sedimentation velocity (Fig 1). These ribosomes will not support amino acid incorporation in vivo and have limited activity in vitro unless provided with exogenous message. The detailed analysis

of the ribosomal population presented in Fig 4 shows that the stimulation of the incorporation of H^3-labelled phenylalanine into polyphenylalanine, in the presence of poly U, coincides with the A_{260} profile of the ribosomal fraction on a sucrose gradient. An analysis for endogenous template activity, however, has shown that it is associated mainly with two distinct fractions which sediment at approximately 45S and 90S, respectively.

Messenger RNA is usually found in association with polysomes (Warner, Knopf, and Rich, 1963) but polysomes are not an obligatory prerequisite for peptide synthesis. mRNA has also been found in the form of ribonucleoprotein (RNP) complexes in loach eggs (Spirin, 1969), sea urchin eggs (Monroy, Maggio, and Rinaldi, 1965), and in a particle which can be dissociated from polysomes of mouse L cells (Perry & Kelly, 1968) and rabbit reticulocytes (Huez, et al., 1967; Burny, et al., 1969). The latter 14S RNP particle proved to contain a 9S message which could code for the b-chain of hemoglobin in vitro (Laycock & Hunt, 1969). The question thus arises whether the template activity displayed by the 45S and 90S particles in the wheat cell free system is mediated via RNP particles other than ribosomes. Titration of the various sedimentation groups with 74S ribosomes did not result in an enhancement of amino acid incorporation or a displacement of activities along the gradient (Fig. 5). Such a result could be expected only if the active complexes are saturated with respect to ribosomes, and no free messages (or RNP particles) are present. The messenger RNA could be packed in a separate entity like an RNP particle cosedimenting with ribosomes or attached to the 40S subparticle of the ribosome yielding the active 45S fraction (Figs. 4, 5). Free RNP particles can be distinguished from ribosomes by their characteristic buoyant density. All ribosomal fractions investigated here show a sharp peak at 1.565 g/cc (Fig. 6B) which is typical of mature ribosomes and not that of 1.39 - 1.45 obtained for free RNP particles (Spirin, 1969; Perry & Kelly, 1968) or the 1.49-1.51 g/cc for the 40S subunit of the ribosomes (Liau & Perry, 1969; Holder &

Lingrel, 1970). No free RNP particles could therefore be demonstrated by the experimental techniques employed on ungerminated embryos.

The RNA analysis of the three fractions shows the existence of both 24S and 17S rRNA components in the ratio of 2:1. If there were free 40S ribosome particles in the 45S fraction (Figs. 4, 5, 6A), the RNA isolated should show an excess of the 17S rRNA species. This is not the case.

The experiments of Marcus & Feeley (1966) have shown that inactive ribosomes can be activated via an energy requiring reaction, to give an active fraction sedimenting in a sucrose gradient more rapidly than the monosome fraction. It is possible that the in vitro "activated" complexes are identical with the mRNA-protein complexes described in this work.

The data presented here show, in accord with previous findings (Chen et al., 1968), that ungerminated wheat embryos contain conserved messenger RNA. The endogenous mRNA appears to be associated with well defined ribosomal fractions. The mRNA-ribosomal complexes detected might play an important role in the regulation of protein synthesis at the early stage of germination.

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Legends

FIG. 1.

A. Sucrose density gradient centrifugation of the total ribosomal fraction prepared from ungerminated wheat embryos. The ribosomal fraction was prepared as described in Materials and Methods, layered onto a 5 to 20% sucrose gradient in TM buffer and centrifuged for 45 minutes at 36,000 rpm in the SW39 rotor of the Spinco preparative ultracentrifuge. Aliquots were collected and analyzed spectrophotometrically at 260 mμ.

B. Isopycnic centrifugation in Cs Cl of the ribosomal fraction prepared from ungerminated wheat embryos. The ribosomal fraction was prepared as in A and was suspended in 0.01 M phosphate buffer containing 0.02 M KCl and 0.01 M $MgCl_2$ and fixed with 6% formaldehyde (pH 7.0) for 4 days at 4^0C. 0.5 ml of the resulting suspension was layered over a preformed CsCl gradient and centrifuged for 15 hours at 36,000 rpm in the SW39 rotor of a Spinco preparative ultracentrifuge. Fractions were collected and analyzed spectrophotometrically at 260 mμ. Buoyant density was calculated from refractive index of a few aliquots across the gradient.

FIG. 2. Polyacrylamide gel electrophoresis pattern of RNA isolated and purified from ribosomes of ungerminated wheat embryos. 1.0 A_{260} unit of RNA was applied to a 2.5% acrylamide gel and electrophoresis was carried out for 90 minutes at 5 mA/tube. The washed gel was scanned at 260 mμ. The ordinate is expressed in arbitrary units proportional to optical density. Molecular weight calculations were determined by comparison of migration with 23S (1.07×10^6 daltons) and 16S (0.56×10^6 daltons) rRNA species from *E. coli* ribosomes. For convenience, the peaks have been labelled I to VII in the order of the most slowly to the most rapidly migrating species.

FIG. 3. The in vitro template activity of several RNA fractions isolated on polyacrylamide gels from total monosome RNA of ungerminated wheat embryos. RNA was isolated and fractionated on acrylamide gels as in Fig. 2. Gel segments containing RNA peaks were placed on carrier gels and electrophoresed into a buffer chamber formed by placing a dialysis membrane over the top of the gel tube (A and B), or sliced and shaken overnight at 4^0C with .01 M Tris buffer, pH 7.6 (C). In each case 10 μg of the purified RNA fraction was added into a cell-free ribosome and supernatant system saturated with t-RNA, and the incorporation of ^{3}H-labelled amino acids into acid insoluble material followed for 15 minutes. 10 μg of T4 mRNA was added as a control. The amount of incorporation of ^{3}H-labelled amino acids into TCA precipitable material at 15 minutes is plotted as a histogram for each RNA fraction studied.

FIG. 4. Endogenous template activity within the ribosomal population. Ten grams of wheat embryos were homogenized in 50 ml. of cold SM buffer containing 0.5 M sucrose, 0.01 M Tris buffer pH 7.6, 0.02 M KCl and 0.01 M $MgCl_2$. The homogenate was centrifuged for 20 minutes at 4,000 rpm and the resultant supernatant centrifuged for 2 hr at 50,000 rpm in the Spinco No. 50 rotor. The precipitate was dispersed in 50 ml of TM buffer containing 0.01 M Tris pH 7.6, 0.02 M KCl and 0.01 M $MgCl_2$, and recentrifuged for 2 hr at 50,000 rpm. The pellet was dispersed in 3 ml. of TM buffer and centrifuged for 10 minutes at 12,000 rpm in the Servall centrifuge. 0.2 ml of the solution was layered on a 5-20% sucrose gradient and centrifuged for 75 minutes at 36,000 rpm in the SW39 rotor of the Spinco ultra-centrifuge. The gradient was fractionated in the cold and the fractions collected were kept on ice. Aliquots containing 0.1 ml. were included in an amino acid incorporating system as described in Methods. The one set of experiments included 10 μg of poly U and ^{14}C amino acid mixture without exogenous message. After 30 minutes of incorporation the reaction mixture was assayed as described in Methods. -●-●-, optical density; Δ -Δ, polyphenylalanine (poly U); o ··· o, endogenous template activity.

FIG. 5. Endogenous template activity titrated with 74S free ribosomes. Dry wheat embryo ribosomes were isolated and fractionated on sucrose gradient as in Fig. 1. Gradient fraction No. 9 containing 74S ribosomes was pooled in the cold and 10 ml of TM buffer was added. The ribosomes were centrifuged for 1 hr at 50,000 rpm in the Spinco ultracentrifuge and redispersed in TM buffer. Aliquots containing 50 μg ribosomes were added to each incubation mixture containing aliquots from the various fractions collected from the above gradient as described in Legend to Fig. 4. The endogenous template activity was measured by the amount of ^{14}C amino acids incorporated in the standard procedure as described in Methods, and expressed as cpm/mg ribosomes.

FIG. 6. Isopycnic centrifugation of complexes displaying endogenous template activity. Ribosomes derived from dry embryos were fractionated on sucrose gradient as in Fig. 1. The pooled fractions designated Nos. 1, 2 and 3 (Fig. 3A) were fixed for 4 days in 6% formaldehyde in the cold, dialyzed for 12 hr against 1 ℓ of 0.01 M phosphate buffer pH 7.6, 0.02 M KCl, 0.01 M $MgCl_2$ and 6% formaldehyde and the dialyzing buffer was changed for additional 12 hr. Half ml aliquots of ribosomes were layered over 4 ml. of preformed CsCl gradient containing 6% formaldehyde, 0.001 M phosphate buffer pH 7.6, 0.2% Brij and 1.5 g/cm^3 CsCl. The gradients were centrifuged to equilibrium for 18 hr in the SW39 Spinco rotor at 36,000 rpm and 40 fractions were collected. Density was estimated refractometrically, and the optical density was followed spectrophotometrically at 260 mμ.

TABLE I

Charging capacity for amino acids of the different ribosomal RNA fractions

RNA fraction	I	II	III	IV	V	VI	VII
cpm H^3 amino acids bound per 20 μg RNA	22	72	0	0	0	0	2240
cpm H^3 amino acids bound per 20 μg purified t-RNA		0	0	0	0	0	4892

TABLE II

Size and composition of RNA fractions isolated from ribosomes of ungerminated wheat embryos

RNA fraction	$S_{20,w}$	Molecular weight (daltons)	Base composition (mole percent) C	A	G	U	(G + C) %
I	24	1.3×10^6	25.5	23.7	29.8	22.8	54.3
II	17	7.6×10^5	25.0	22.6	30.3	22.3	55.3
III	15.2	4.0×10^5	27.9	25.0	25.0	22.1	52.9
IV	11.2-12.4	2.5×10^5	-	-	-	-	-
V	8.4	1.5×10^5	23.5	25.3	23.1	28.1	46.6
VI	7.5	9.5×10^4	28.1	23.5	25.3	23.1	53.4
VII	5.0	5.0×10^4					
	3.5	3.6×10^4	24.7	21.7	32.9	21.7	57.6
Total rRNA	heterogeneous		22.1	25.4	32.6	20.0	54.7
Pulse-label RNA	"		26.6	26.6	15.2	31.6	41.8
DNA			22.0	27.1	22.2	27.6(T)	44.2

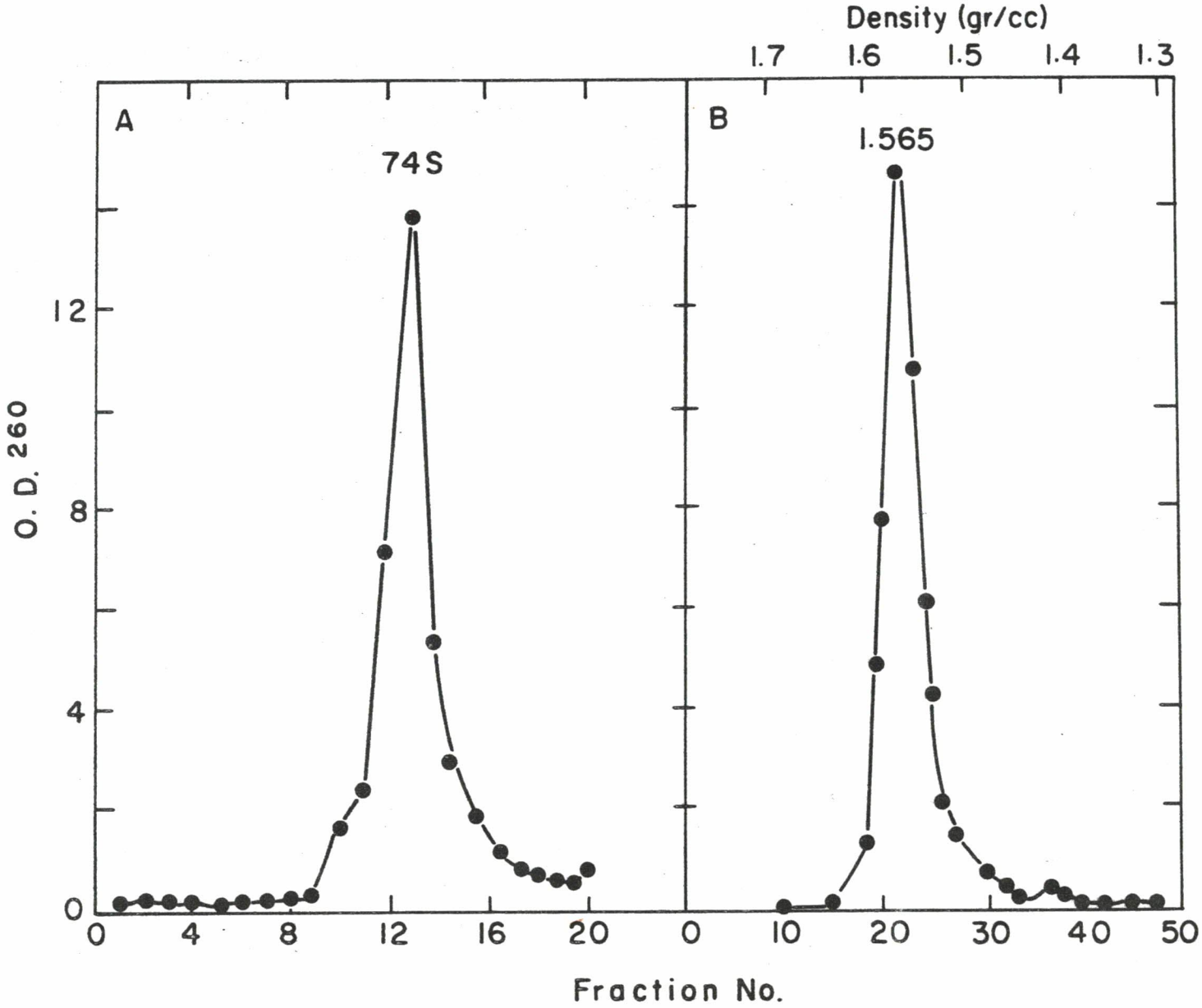
Density (gr/cc)
1.7
1.6
1.5
1.4
1.3
A
74S
B
1.565
O. D. 260
12
8
4
0
0
4
8
12
16
20
0
10
20
30
40
50
Fraction No.

Figure 1

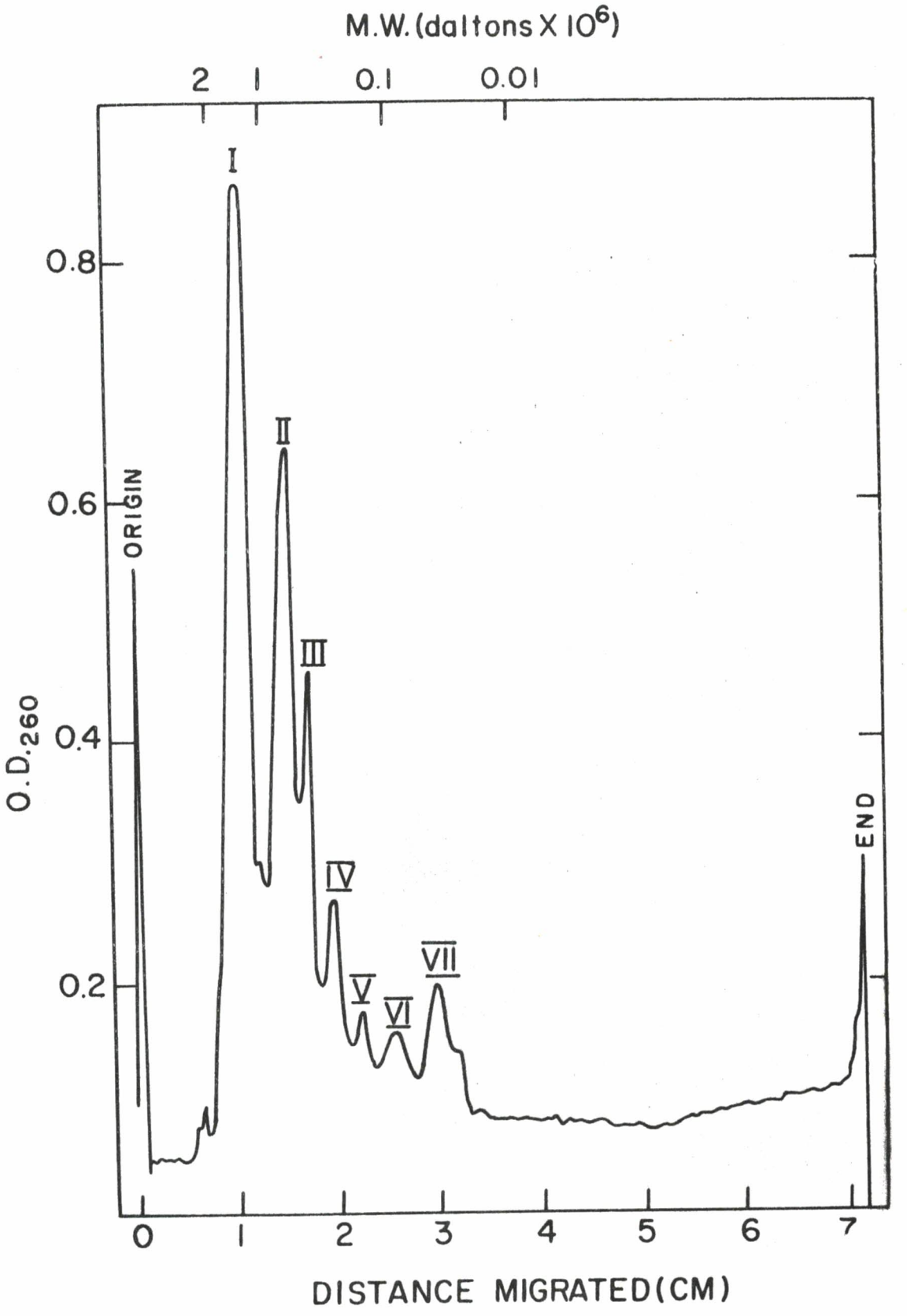
M.W. (daltons X 10^6)
2
1
0.1
0.01
I
II
III
IV
V
VI
VII
ORIGIN
END
0.8
0.6
0.4
0.2
O.D.260
0
1
2
3
4
5
6
7
DISTANCE MIGRATED (CM)

Figure 2

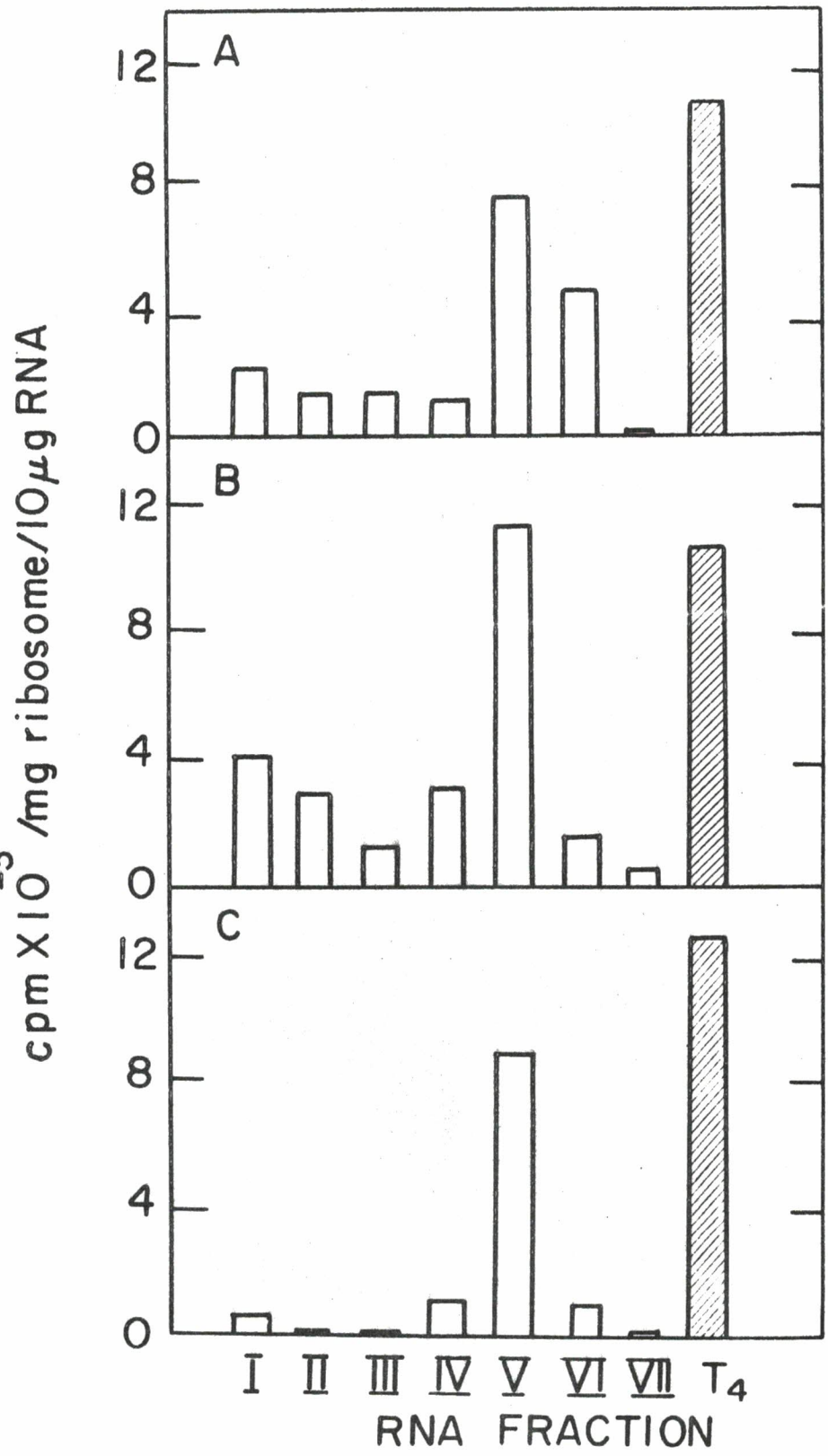
A
B
C
12
8
4
0
cpm X 10^-3 /mg ribosome/10μg RNA
I
II
III
IV
V
VI
VII
T4
RNA FRACTION

Figure 3

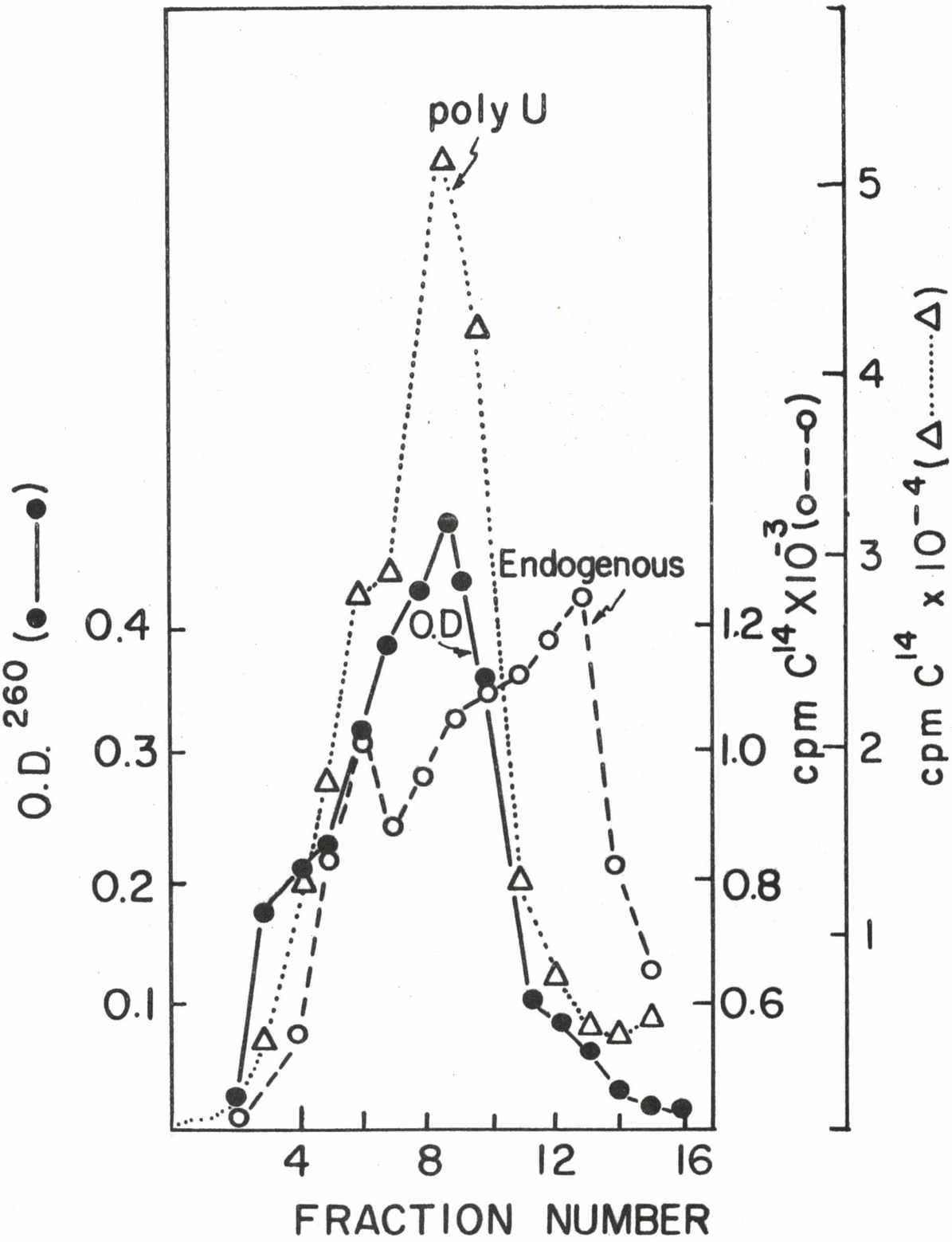
poly U
Endogenous
O.D
O.D. 260 (●——●)
cpm C14 X10-3 (o——o)
cpm C14 x 10-4 (Δ·····Δ)
0.4
0.3
0.2
0.1
1.2
1.0
0.8
0.6
5
4
3
2
1
4
8
12
16
FRACTION NUMBER

Figure 4

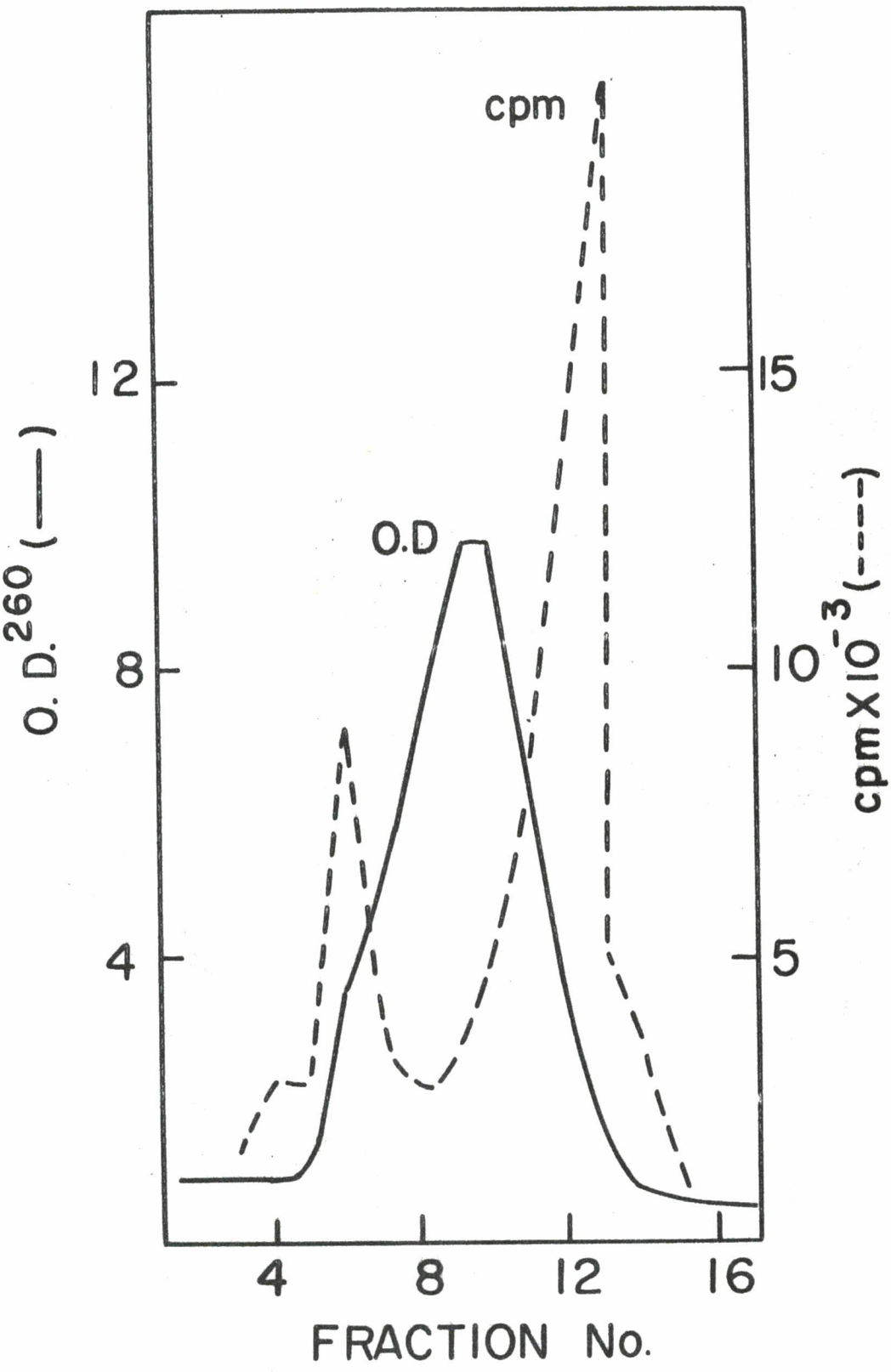

Figure 5

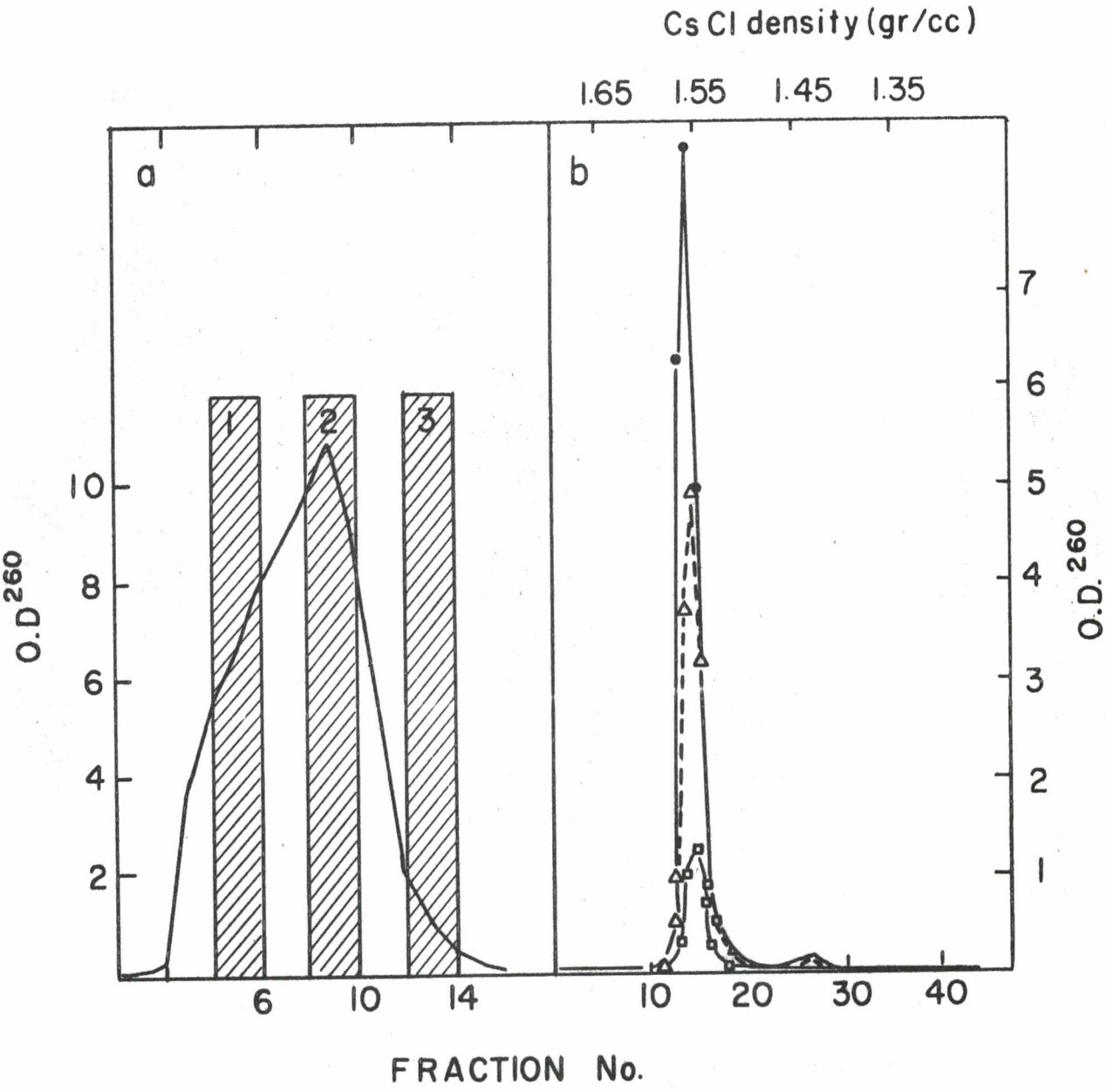
Cs Cl density (gr/cc)
1.65
1.55
1.45
1.35
a
b
1
2
3
O.D 260
O.D. 260
10
8
6
4
2
7
6
5
4
3
2
1
6
10
14
10
20
30
40
FRACTION No.

Figure 6