Subribosomal Ribonucleoprotein Particles of Developing Wheat Embryo†

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ABSTRACT: Subribosomal ribonucleoprotein particles of wheat embryos germinated for 6 hr were rapidly labeled with a sixth hour pulse of [^{3}H]uridine and had a much higher specific radioactivity, lower sedimentation rate in 5–20% linear sucrose gradients and lower buoyant density in the presence of 10–20 mM Mg^{2+} than the ribosomes with which they were isolated, in conformity with the properties of subribosomal messenger ribonucleoprotein particles of eucaryotes. The subribosomal ribonucleoprotein particles were sharply eluted from a Sepharose 6B column together with the sharp first portion of an asymmetric peak of ribosomes, and were eluted after ribosomes on DEAE-cellulose column chromatography at pH 8.0 in potassium phosphate containing 10–20 mM Mg^{2+}. On dialysis of subribosomal ribonucleoprotein particles with ribosomes against potassium phosphate (pH 8.0) lacking Mg^{2+} the particles became associated with the dissociated ribosomes, apparently with the small subunit, as determined by zonal sedimentation in sucrose gradients, equilibrium sedimentation in CsCl and DEAE-cellulose column chromatography. In the absence of Mg^{2+} ribosomes were fractionated into multiple protein fractions as well as ribonucleoprotein fractions of generally uniform specific radioactivity, apparently containing the subribosomal ribonucleoprotein particles. Experiments utilizing a sixth germination hour [^{3}H]uridine pulse followed by 6 hr of uridine chase suggested relative stability of cytoplasmic subribosomal particles and a marked influx into the cytoplasm of ribosomes bearing RNA synthesized during the sixth hour. The results suggest that the subribosomal ribonucleoprotein particles may consist of a mRNA–protein complex, which in comparison with the ribosomes have a smaller RNA:protein ratio, a more elongated structure with one dimension equal to or perhaps greater than the diameter of the ribosomes, possibly a lesser mass, and protein which is more readily eluted by salt.

Extensive work has been done on the transfer of genetic information from the DNA genome to protein synthesizing ribosomes *via* mRNA molecules transcribed from DNA (Volkin and Astrachan, 1956; *Cold Spring Harbor Symp. Quant. Biol.*, 1970) and on the synthesis of ribosomes and other ribonucleoprotein particles (Burdon, 1971; Spirin, 1969). In eucaryotes the two main ribosomal ribonucleic acids are produced by cleavage of a precursor 45S molecule in the nucleolus where 5S RNA of the large ribosomal subunit is also found (Scherrer and Darnell, 1962; Darnell, 1968). The ribosomal ribonucleic acids are associated with proteins from their earliest stages (Warner and Soeiro, 1967).

Cytoplasmic subribosomal ribonucleoprotein particles which sediment more slowly in sucrose gradients, have lower buoyant densities than ribosomes and contain messenger-like RNA have been found in embryonic and nonembryonic eucaryotic cells (Spirin, 1969). mRNA dissociated from polysomes and nuclear messenger-like RNA (heterogeneous nuclear RNA) also exist as ribonucleoprotein particles with sedimentation and buoyant density properties identical to cytoplasmic ribonucleoprotein particles (Samarina *et al.*, 1966; Kohler and Arends, 1968; Parsons and McCarthy, 1968; Henshaw, 1968; Perry and Kelly, 1968; Belitsina *et al.*, 1968; Burny *et al.*, 1969; Cartouzou *et al.*, 1969; Spohr *et al.*, 1970).

The RNA of cytoplasmic ribonucleoprotein particles and polysomal mRNA have a similar size distribution (6–35 S, mainly 10–20 S; 0.05–1.5 $\times 10^6$ daltons). Nuclear messenger-like RNA is larger (10–150 S; up to 15 $\times 10^6$ daltons) (Scherrer *et al.*, 1970) but has sequence similarity with polysomal mRNA (Teissere *et al.*, 1972). It is believed that a portion of the nuclear messenger-like RNA may be precursor to cytoplasmic mRNA and subribosomal ribonucleoprotein particles. Pulse-chase experiments suggest that a portion of subribosomal ribonucleoprotein particles may bind to ribosomes and be translated (Scherrer *et al.*, 1970).

A ribonucleoprotein fraction of low buoyant density has recently been described in germinating wheat embryo (Chen *et al.*, 1971) which was interpreted as representing newly synthesized ribosomes which contain excess protein in the immature state. The present work on the nature of this ribonucleoprotein fraction suggests the interpretation that it is a mRNA–protein complex which under certain *in vitro* conditions is capable of binding to the small ribosomal subunit.

Experimental Section

Preparation of Ribosomal Fraction of Germinating Wheat Embryo. Wheat embryos (*Triticum vulgare*) were prepared according to Johnston and Stern (1957) and stored in a desiccator at 2–4°. In a typical experiment 4–6 g of wheat embryos (0.5 g/6-cm diameter plastic petri dish containing 1% agar (Difco) and 2% sucrose) were germinated in darkness for 5 hr at room temperature (25°) by addition of 2 ml of 2% sterile sucrose/g of wheat embryo. The embryos were then washed three times with about 5 ml of 2% sucrose/petri dish, pulsed for 1 hr by addition of [^{3}H]uridine (20 Ci/mmol, Amersham-Searle; 20 μCi/g of embryo in 2% sucrose, 0.5 ml/plate), and then washed three times with 2% sucrose. In some experiments incubation was continued for up to 6 hr in the presence of 15 mg/ml of uridine. The embryos were extracted by grinding in a mortar in 0.5 M sucrose–10 mM $MgCl_2$–25 mM KCl–

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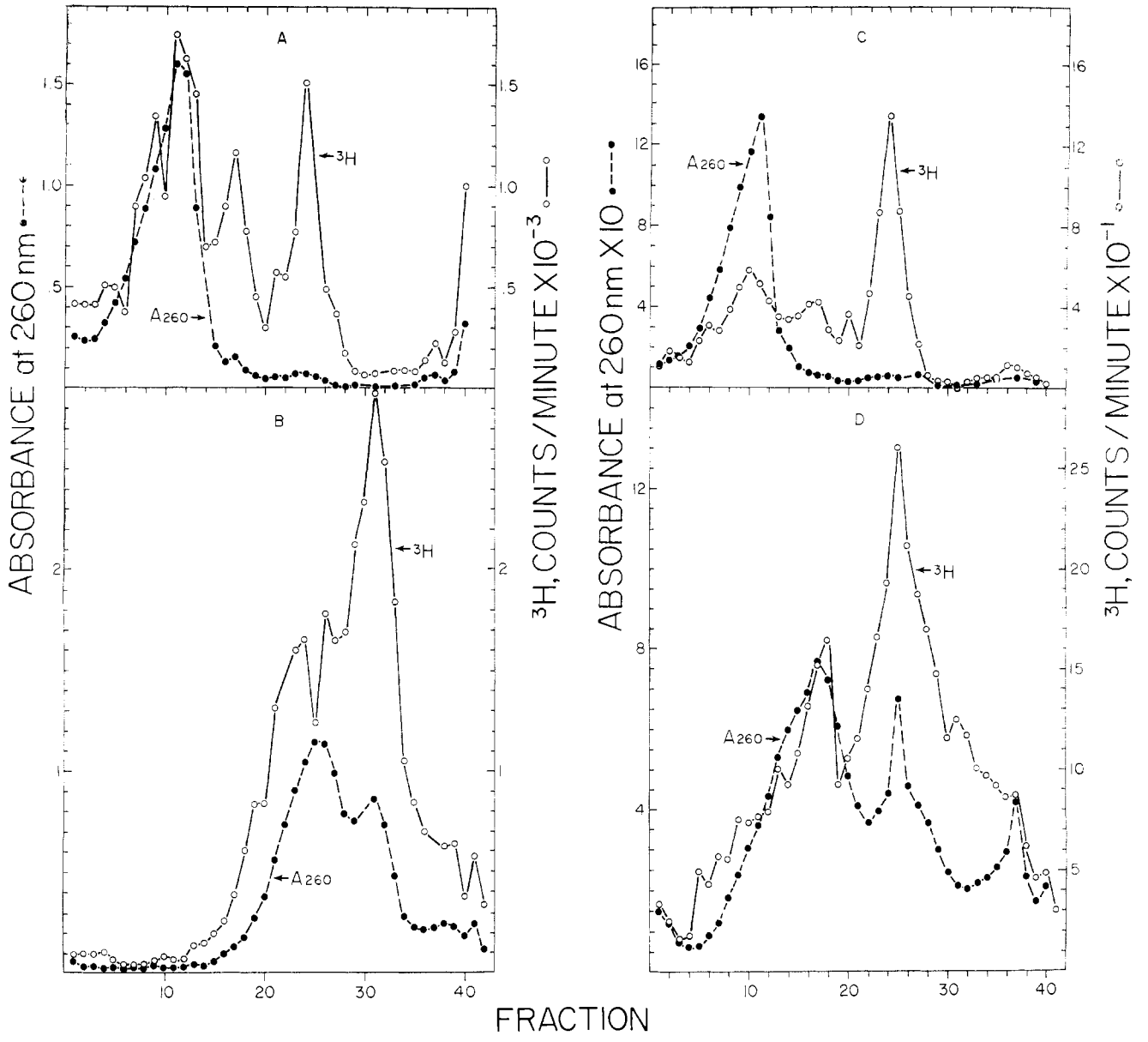

FIGURE 1: Sucrose density gradient centrifugation analysis of the ribosomal fraction of wheat embryo isolated after 6 hr of germination. Centrifugation was for 195 min (A, B, C) or 240 min (D). The $MgCl_2$ content of the gradients was 20 mM (A), 15 mM (C), and less than 1 mM in B and D. The ribosomal (A and C) and subunit (B and D) peaks are noted by their absorbance at 260 nm. The subribosomal ribonucleoprotein particle peaks to the right (direction of low density) of the ribosomal peak are characterized by their high radioactivity and low absorbance at 260 nm (A and C). (●) A_{260}; (○) ^3H radioactivity. Note the change in the radioactivity scale in C and D.

50 mM Tris-Cl (pH 7.6). The extract was centrifuged at 13,000 rpm for 20 min in a Sorvall RC2-B centrifuge. After removal of surface lipid with absorbent paper the resultant supernatant solution was centrifuged in a no. 50 rotor in a Spinco ultracentrifuge at 47,000 rpm for 2 hr. The resultant pellets were resuspended in 20 mM potassium phosphate (pH 8.0) in the presence or absence of 10–15 mM $MgCl_2$. The suspensions were further clarified by centrifugation several times at 13,000*g* and discarding the precipitates. The ribosomal fraction containing Mg^{2+} was then dialyzed overnight at 2–4° against a 1000-fold volume of 20 mM potassium phosphate (pH 8) containing 10 or 15 mM $MgCl_2$, while the ribosomal fraction lacking Mg^{2+} was similarly dialyzed in the absence of Mg^{2+}.

Sucrose Gradients. Ribosomal preparations (0.2–0.4 ml) were layered onto 12-ml sterile 5–20% sucrose gradients in 20 mM potassium phosphate (pH 8)–20 mM KCl ± 20 mM $MgCl_2$. An additional 0.5 ml of 60% sucrose in the same electrolyte was present at the bottom of the tube. Gradients were centrifuged at 36,000 rpm at 2–3° in a Spinco SW-40 rotor for 3.25–4.75 hr. Eighteen drop fractions were collected from the end of a narrow metal tube at the bottom of the centrifuge tube by means of a peristaltic pump. After addition of 1 ml of distilled water, the fractions were analyzed for absorbance at 260 nm, ^3H, and sometimes for protein.

CsCl Density Gradient Centrifugation. Aliquots of ribosomal fractions were fixed in 6% formaldehyde (distilled and neutralized) at 2° for 2–5 days and 0.5 ml was then layered over a preformed CsCl gradient and centrifuged to equilibrium in a Spinco SW-39 or SW-50 rotor at 36,000 rpm for 18–40 hr. The preformed CsCl gradient consisted of 1.5-ml volumes of 55, 44, and 33% CsCl (w/w, from bottom to top, layered in succession), and contained 2.7 mM potassium

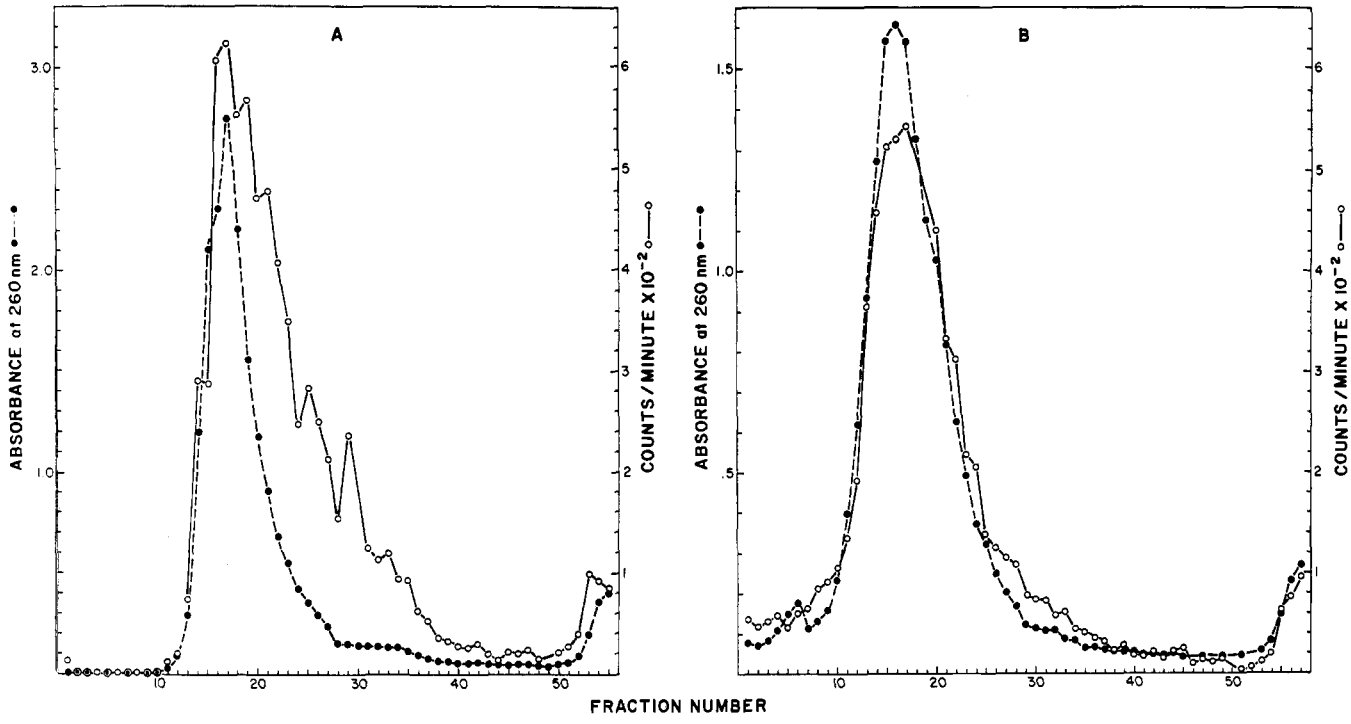

FIGURE 2: CsCl density gradient centrifugation of formaldehyde-fixed ribosomal fraction isolated from wheat embryos after 6 hr of germination. Centrifugation was at 36,000 rpm for 44 hr in a SW-50 rotor at 18°. (A) 15; (B) less than 0.1 mM $MgCl_2$ present in the ribosomal sample (see Experimental Section). (●) A_{260}; (○) ^3H cpm.

phosphate (pH 7.2), 2.7 mM NaCl, 0.4 mM $MgCl_2$, 2% formaldehyde, and 0.8% BRIJ.

DEAE-cellulose was prepared for column chromatography by removal of fines by flotation, cycling three times with 1 N NaOH, 1 N HCl, and distilled water and equilibration with 10 or 20 mM potassium phosphate (pH 8.0) with or without 10 or 15 mM $MgCl_2$.

Radioactivity of 5% trichloroacetic acid (0°) insoluble material was determined by entrapment of precipitate by filtration through Whatman GF/C 24-mm glass-fiber circles which were then thoroughly washed by filtration with 10 ml of 5% trichloroacetic acid, dried with a heating lamp, and counted in a toluene-based scintillator in a liquid scintillation counter.

Protein was determined by the method of Lowry *et al.* (1951), adapted for a 2.6-ml sample volume to maximize sensitivity.

Results

Sucrose density gradient centrifugation in the presence of 20 or 15 mM Mg^{2+} of the ribosomal fraction of what embryo exposed to [^3H]uridine during the last of 6-hr germination resulted in a single symmetrical peak of 80S ribosomes containing less than half the total radioactivity and one or several much more slowly sedimenting peaks of subribosomal ribonucleoprotein particles containing the major portion of radioactivity and little 260-nm absorbance. The subribosomal ribonucleoprotein particles thus had a much higher specific radioactivity than the ribosomes and were in much lesser amount (Figure 1A,C).

When the same zonal sedimentation was performed on the ribosomal fraction containing ribosomes which had been previously dissociated by dialysis of the fraction against 20 mM potassium phosphate (pH 8.0) lacking Mg^{2+}, the bulk of the uridine radioactivity appeared coincident with the 260-nm absorbance of the small ribosomal subunit (Figure 1B,D). Separate peaks of subribosomal ribonucleoprotein particles characterized by major peaks of radioactivity associated with little 260-nm absorbance were not observed. Under the conditions of ribosomal dissociation used, the slowly sedimenting ribonucleoprotein particles of high specific radioactivity sedimented with the small ribosomal subunit. It should be noted that the sedimentation of subribosomal ribonucleoprotein particles in the presence of 20 mM Mg^{2+} (Figure 1A,C) was not coincident with the small subunit but faster than it (Figure 1B,D; note difference in sedimentation time between C and D). A small, very slowly sedimenting third peak was sometimes seen which may represent 5S RNA containing particles or possibly a markedly unfolded ribosomal fragment or subunit (Figure 1D). Extraction of a 5S RNA–protein complex from rat liver ribosomes by formamide has recently been described (Petermann *et al.*, 1972).

CsCl density gradient centrifugation of formaldehyde-fixed ribosomes revealed a sharp ribosomal peak at a density of 1.54–1.55 g/ml which contained about half the recovered radioactivity, while half the radioactivity banded in several peaks lighter than the ribosomal peak (Figure 2A). On the other hand, ribosomes dissociated in 20 mM potassium phosphate (pH 8.0) banded in a broader peak centered at the same density. In this case the radioactivity banded symmetrically with the 260-nm absorbance peak of the ribosomes (Figure 2B). This result is consistent with the findings in sucrose density gradient experiments which suggest that [^3H]uridine containing material of high specific activity with respect to absorbance at 260 nm becomes associated with the ribosome on its dissociation.

Cosedimentation in sucrose gradients of subribosomal ribonucleoprotein particles with small ribosomal subunit after dialysis of the ribosomal fraction against phosphate

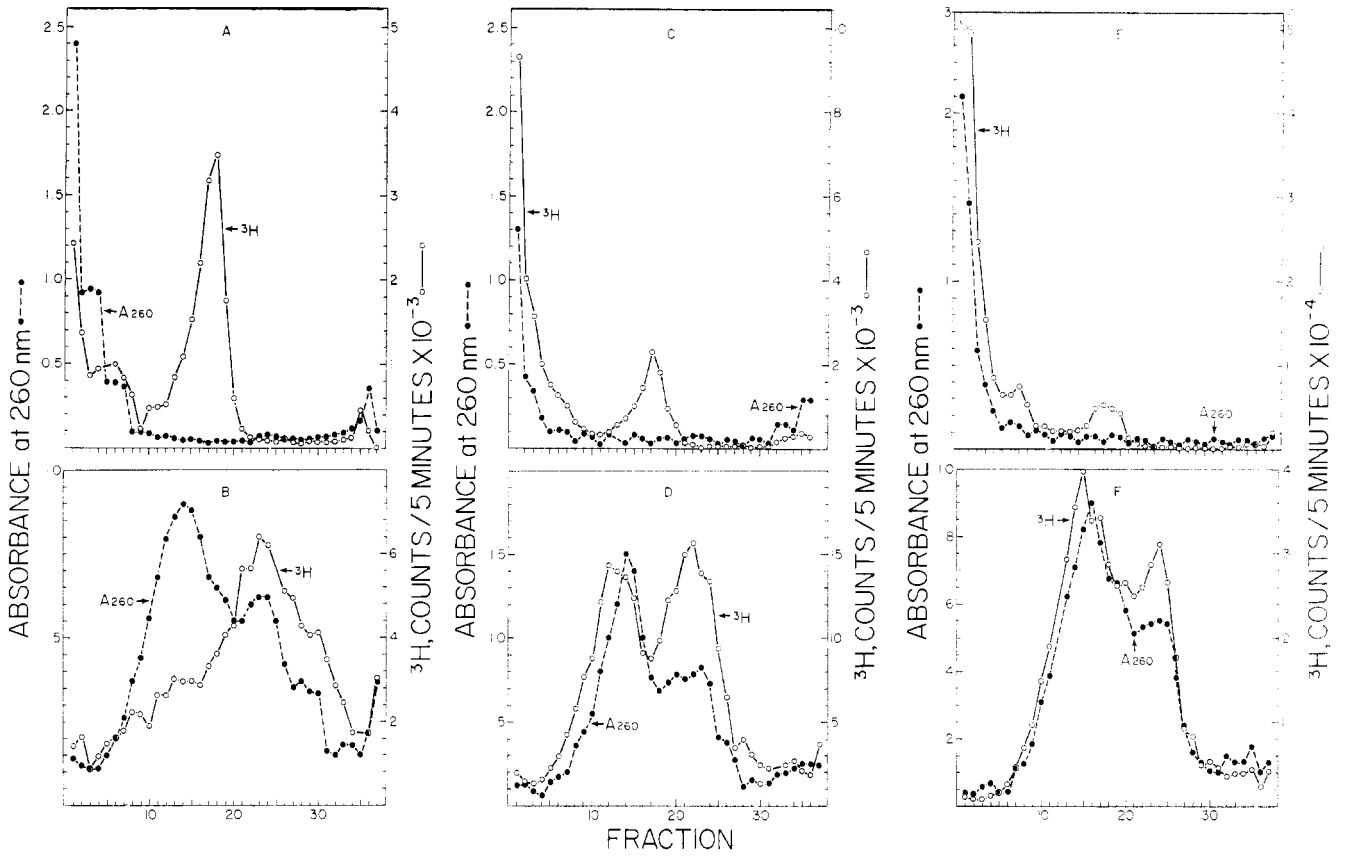

FIGURE 3: Sucrose density gradient centrifugation analysis of the ribosomal fraction isolated from developing wheat embryo. Wheat embryos were exposed for 1 hr to 60 μCi/g of [^{3}H]uridine 5 hr after onset of germination, washed twice with 0.5 M sucrose–10 mM $MgCl_2$–25 mM KCl–50 mM Tris-Cl (pH 7.6) and once with 2% sucrose, and then chased with a 60,000-fold excess of nonradioactive uridine (in 3 ml of 2% sucrose/g of embryo) for 0 hr (A, B), 2 hr (C, D), or 6 hr (E, F). The ribosomal fraction isolated in 20 mM potassium phosphate (pH 8.0) containing 10 mM $MgCl_2$ was dialyzed overnight at 2–4° against 1000 volumes of the same salt solution (A, C, E) or 20 mM potassium phosphate lacking Mg^{2+} (B, D, F) prior to gradient centrifugation (see Experimental Section) which was for 275 min at 2°. (●) A_{260}; (○) ^{3}H radioactivity. Note the 10-fold increase in the radioactivity scale in E and F.

TABLE 1: Incorporation of [^{3}H]Uridine into the Ribosomal Fraction of Developing Wheat Embryos.[a]

Hr of Uridine Chase	Radioactivity: Total[b] cpm × 10^{-3}	Radioactivity: Subribosomal RNPP[c] cpm × 10^{-3}	% of Total[b]	Increment in Small Subunit as % of Total[b,e]
0	5.1	3.3	65[d]	47
2	6.8	2.0	29	27
6	41.0	5.0	12	9

[a] Experimental details are given in the legend of Figure 3. [b] Total signifies ribosomal plus subribosomal ribonucleoprotein particle peaks. [c] RNPP, ribonucleoprotein particle. [d] In other experiments this value varied from 45 to 56%. [e] Estimation of the increment in radioactivity in small as compared to large ribosomal subunit as percentage of total radioactivity, based on the difference in specific radioactivity between small and large subunits; increment as % of total radioactivity = [(cpm of small subunit peak fraction − cpm of large subunit peak fraction)[(A_{260} for small subunit peak fraction)/(A_{260} for large subunit peak fraction)]/cpm of small + large subunit peak fractions] × 100.

buffer (Figure 1B,D) suggested the possibility that the subribosomal particles may be small subunits. This possibility was eliminated by the finding that small and large ribosomal subunits isolated from sucrose gradients and then subjected to CsCl equilibrium gradient centrifugation banded at the density of native ribosomes rather than at the lower density of subribosomal ribonucleoprotein particles (Figure 2A).

Pulse-Chase Experiments Analyzed by Sucrose Density Gradient Centrifugation. In an effort to determine the fate of ribonucleoprotein material newly synthesized during the sixth hour of germination, wheat embryos were pulsed with [^{3}H]uridine during that time period and then chased for 0, 2, and 6 hr with nonradioactive uridine (Figure 3). Although the amount of radioactivity present in subribosomal ribonucleoprotein particles varied only moderately, the fraction of total radioactivity in these particles diminished sharply from 0 to 6 hr of chase due to a marked increase in radioactivity in ribosomes (Figure 3A,C,E; Table I). This finding is compatible with the interpretation that the subribosomal ribonucleoprotein particles synthesized during the sixth hour of germination are relatively stable during the next 6 hr and that the increase in radioactivity in native ribosomes mainly reflects processing time and transport from nucleus to cytoplasm of new ribosomes containing rRNA synthesized during the sixth hour of germination, so that the ratio of cytoplasmic radioactive rRNA to mRNA steadily increases.

In accordance with the results with embryos germinated for

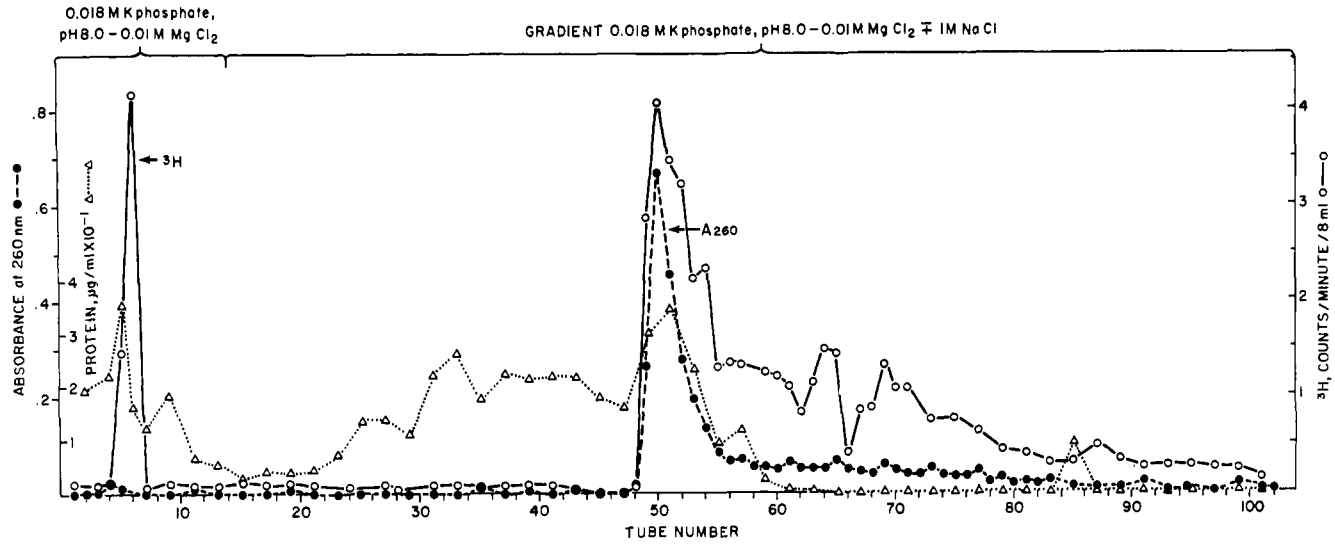

FIGURE 4: DEAE-cellulose column chromatography in the presence of Mg^{2+} of the ribosomal fraction of wheat embryos exposed to [^{3}H]-uridine (60 μCi/g of embryo) during the sixth (last) hour of germination. The ribosomal fraction (1.2 ml, A_{260} = 37) in 18 mM potassium phosphate–20 mM $MgCl_2$ (pH 8.0) was eluted from the column (1.1 × 30 cm) with 300 ml of 18 mM potassium phosphate (pH 8.0)–10 mM $MgCl_2$, followed by a linear gradient between 500 ml of the same eluent and 500 ml of eluent containing 1 M NaCl. (●) A_{260}; (○) ^{3}H radioactivity, cpm/8 ml; (△) protein, μg/ml × 10^{-1}.

6 hr (Figure 1) subjection of the ribosomal fraction to dialysis against 20 mM potassium phosphate lacking Mg^{2+} resulted in dissociation of ribosomes to subunits and disappearance of the high specific radioactivity subribosomal ribonucleoprotein particles (Figure 3B,D,F). During the 6-hr chase, the ratio of radioactivity in small as compared to large subunit decreased from about 2 to less than 1 while the ratio of specific radioactivity changed from more than 2 to only slightly greater than 1. The increment in radioactivity sedimenting with the small as compared to the large subunit expressed as percentage of total radioactivity is similar to the percentage of total radioactivity present in the subribosomal ribonucleoprotein particles (Figure 3A,C,E; Table I). This finding and the similar recovery of radioactivity as compared to 260-nm absorbance in gradients containing native as contrasted with dissociated ribosomes suggested that the higher specific activity of small subunit is largely due to attachment or less likely fortuitous cosedimentation of subribosomal ribonucleoprotein particles under the ribosomal dissociating conditions used. The fall in absolute amount of radioactivity in subribosomal ribonucleoprotein particles from 0 to 2 hr of chase suggests the possibility that some of these particles may bind to ribosomes *in vivo* during this period.

Diethylaminoethylcellulose column chromatography in the presence of magnesium of the ribosomal fraction isolated from wheat embryos germinated for 6 hr resulted in separation with a NaCl gradient of a sharp peak of ribosomes, eluted at about 0.4 M NaCl, from a small amount of 260-nm-absorbing material of much higher specific radioactivity which was eluted in a prolonged tail following the ribosomal peak (Figure 4). The high specific activity of the material following the ribosomal peak suggests that it represents the subribosomal ribonucleoprotein particles, and its prolonged elution suggests heterogeneity with respect to charge under the conditions used. The largest peak of protein was eluted with the ribosomes, but a number of peaks were also eluted throughout the gradient prior to the ribosomal peak and shortly thereafter, which are probably not structural components of the ribosomes (Salas *et al.*, 1965; Furano, 1966). Most of the high specific activity material which trailed after the ribosomal peak was not associated with detectable protein, although it is likely that some protein was associated with the RNA since RNA would not be expected to be eluted under these conditions. In some experiments a significant amount of radioactivity probably representing unpolymerized uridine or uridine derivatives was eluted in the vicinity of the void volume (Figure 4).

In contrast to the preceding results, *DEAE-cellulose chromatography in the absence of* Mg^{2+} of ribosomal fraction dialyzed against potassium phosphate lacking magnesium failed to reveal any regions of very high specific radioactivity compatible with subribosomal ribonucleoprotein particles, although the recovery of radioactivity with respect to 260-nm absorbance was equivalent to that obtained in chromatography in the presence of magnesium (Figure 5). Elution of radioactivity was symmetrical with the ribosomal ribonucleoprotein peaks. This finding is consistent with the preceding experiments which suggest that the high specific radioactivity subribosomal ribonucleoprotein particles became associated with the ribosomes under the dissociating conditions used.

Another interesting difference in the magnesium-free chromatography is the marked, new ribosomal fractionation obtained which may be a useful tool for study of ribosomal structure and function. A striking elution of protein in at least three major fractions preceded the ribonucleoprotein fractions (instead of a single native ribosomal peak) from which most of the protein was removed (Figure 5). Since somewhat higher salt concentrations were required for elution of most of the proteins than in the presence of 6 M urea (Spitnik-Elson, 1970; Spitnik-Elson and Greenman, 1971) it is likely that a combination of electrostatic and nonelectrostatic bonding forces between molecules of protein and protein and RNA are responsible for holding most of the ribosomal ribonucleoprotein complex together. Two main ribonucleoprotein peaks were eluted with about 0.5–0.7 M NaCl while a small third peak was sharply eluted with 1 M NaCl. The elution of the major peaks is less sharp and at 0.1–0.2 M higher NaCl concentration than that found for the native ribosomes (Figure 4). This difference would be expected from the large protein loss

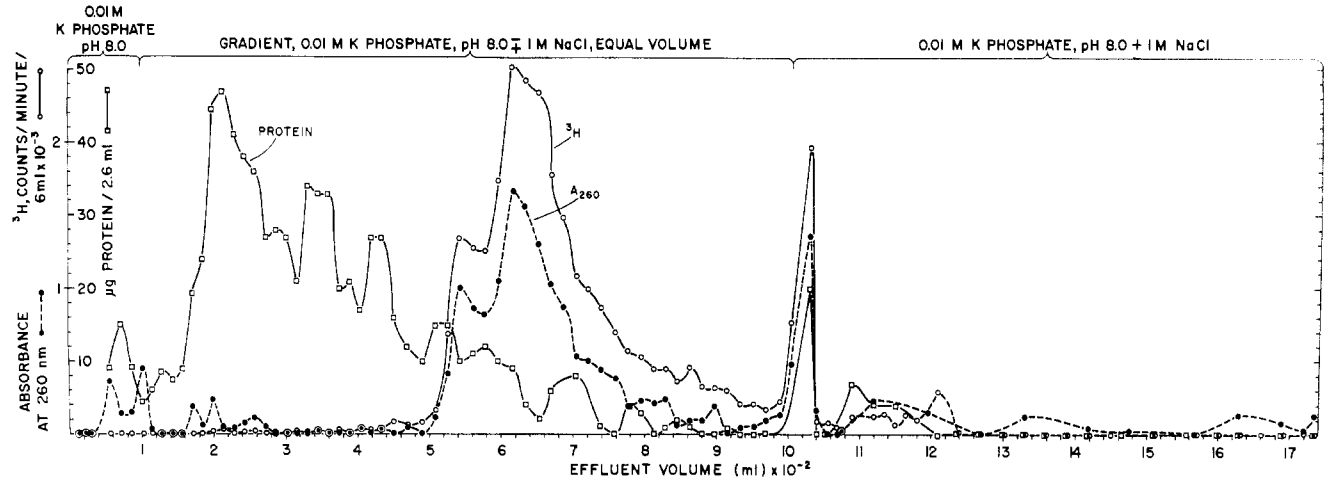

FIGURE 5: DEAE-cellulose chromatography in the absence of Mg^{2+} of the ribosomal fraction of wheat embryos germinated for 6 hr (see Experimental Section). Two milliliters of ribosomal fraction (extracted from approximately 2 g of wheat embryos and dialyzed overnight at 2° against 1000 volumes of 20 mM potassium phosphate, pH 8.0) were absorbed onto a 1.1 × 30 cm DEAE-cellulose column and eluted, in succession, by 10 mM potassium phosphate (pH 8.0); 0–1 M NaCl gradient in 10 mM potassium phosphate; and 1 M NaCl in 10 mM potassium phosphate, as indicated. (●) Absorbance at 260 nm; (○) ^3H radioactivity, cpm/6 ml × 10^{-3}; (□) μg of protein/2.6 ml.

from the ribosomal subunits. It is not clear whether these peaks may correspond to those seen in the sucrose density gradient (Figure 1D). Thus, the first ribonucleoprotein peak may correspond to small subunit (second peak in Figure 1D), the second larger peak to the large subunit (first peak at the left in Figure 1D), and the last peak to the slow sedimenting peak at the right in Figure 1D.

Sepharose 6B Gel Filtration. Filtration of the ribosomal fraction through a Sepharose 6B column resulted in elution of the ribosomes (260-nm absorbance) in a sharp peak followed by a distinct shoulder (Figure 6). The sharp break in the downward slope of the 260-nm absorbance curve divided the ribosomes into a rapidly filtering fraction, presumably of larger size particles (sharp peak), and a somewhat retarded fraction of smaller size particles (shoulder of the peak). This finding suggests the possibility that the ribosomes studied may be heterogeneous with respect to the size or perhaps physicochemical properties. The subribosomal ribonucleoprotein particle fraction which contained the major portion of radioactivity was eluted in a sharp peak coincident with the early filtering, presumably larger size ribosomes, which generally accounts for the higher specific radioactivity of the first portion of the 260-nm absorbance peak as compared to the second. The very late filtering small radioactivity peak at fraction 128 may be uridine or a small uridine-like compound on the basis of its similarity to [^3H]uridine in migration on thin-layer chromatography (silica gel, 60-min water elution).

Discussion

Ribonucleoprotein particles having lower sedimentation rate and buoyant density than ribosomes in conformity with subribosomal ribonucleoprotein particles containing rapidly labeled DNA-like RNA which have been reported in various eucaryotes and termed informosomes (Spirin, 1969) have been found in the postmitochondrial cytoplasm of germinating wheat embryo. These particles have also been reported by Chen *et al.* (1971) who have interpreted them as immature ribosomes which are rendered less dense by an extra complement of protein which is subsequently removed during a stepwise process of maturation to mature ribosomes.

The present experiments show that on dissociation of germinating wheat ribosomes in the presence of subribosomal ribonucleoprotein particles by dialysis against the same phosphate buffer lacking magnesium the subribosomal ribonucleoprotein particles became totally associated with the ribosomes, apparently with the small subunit, as determined by zonal sedimentation in sucrose gradients, isopycnic centrifugation in CsCl, and ion-exchange chromatography on DEAE-cellulose. This finding of total association of subribosomal ribonucleoprotein particles with dissociated ribosomes could be explained by assuming that this fraction contains messenger ribonucleoprotein particles which under these conditions bind to the small subunit. Alternatively, it might be argued that the subribosomal fraction consists of newly synthesized native ribosomes or small ribosomal subunit containing a large additional complement of protein which is totally removed under the dissociating conditions used (20 mM potassium phosphate, pH 8.0). Newly synthesized whole ribosomes would not account for the much higher specific activity found in the small subunit. In addition it does not seem likely that lowering magnesium would cause total dissociation of the considerable amount of postulated additional protein from the ribosomes or ribosomal subunits. Since binding of mRNA-containing subribosomal ribonucleoprotein particles to dissociated ribosomes would seem reasonable, the first possibility is favored in explanation of these findings.

In support of this interpretation is the finding on DEAE-cellulose chromatography in the presence of magnesium that ribonucleoprotein containing RNA of high specific radioactivity corresponding to the subribosomal ribonucleoprotein fraction is separated from and eluted after the ribosomes. This finding suggests that considerable protein must have been stripped from the ribonucleoprotein particle by the salt solution since it otherwise would have been expected to have been eluted prior to the ribosomes, since it clearly originally had more protein than the ribosomes as indicated by a lower buoyant density. A higher protein:RNA ratio would tend to

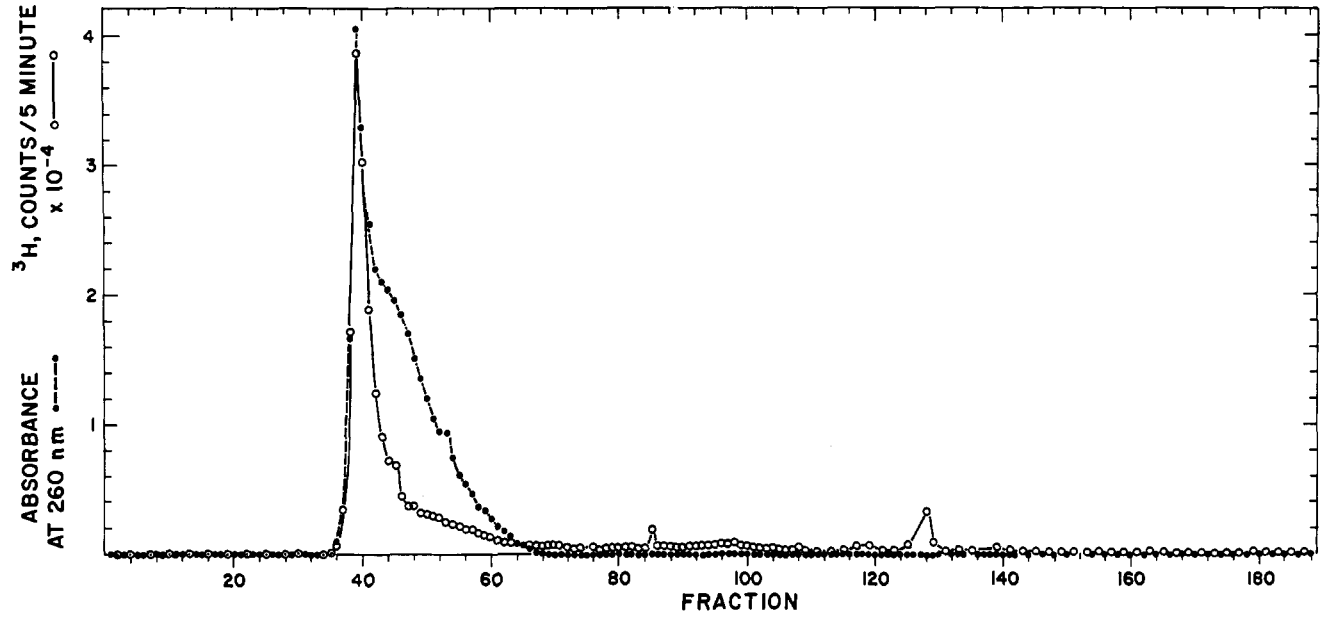

FIGURE 6: Sepharose 6B gel filtration (3.3 × 95 cm column) of wheat embryo ribosomal fraction in 20 mM potassium phosphate–10 mM $MgCl_2$ (pH 8.0). The column was previously equilibrated with 500 ml of buffer. The ribosomes can be seen to be divided into a more rapidly filtering fraction (main 260-nm absorbance peak) and a less rapidly filtering fraction (shoulder of the 260-nm absorbance peak). (●) Absorbance at 260 nm; (○) ^{3}H radioactivity.

mask more of the negative charges of the RNA, resulting in elution at a salt concentration lower rather than higher than that required for elution of ribosomes. The elution pattern observed is consistent with that expected for subribosomal ribonucleoprotein particles but not with mature ribosomes or with immature ribosomes containing additional proteins. Perry and Kelley (1968) have shown that three-quarters of the protein of subribosomal ribonucleoprotein particles is reversibly removed by exposure to 0.55 M LiCl–10 mM Mg^{2+} and that little protein was removed from ribosomal subunits under these conditions. It is conceivable that the structure of postulated immature ribosomes containing additional proteins would allow for the stripping of most of its proteins (additional as well as mature complement) on DEAE-cellulose column chromatography, but this does not seem likely.

The size distribution by gel electrophoresis of radiolabeled RNA molecules isolated from the ribosomal fraction of wheat embryos pulsed with [^{3}H]uridine during the last of 6 hr of germination (Chen *et al.*, 1971) is generally similar to the size reported for the major fraction of more heterodisperse Hela cell messenger ribonucleoprotein particle RNA determined by zonal sedimentation (Scherrer *et al.*, 1970). Perhaps the fact that almost half the radiolabeled RNA sediments in sucrose gradients with ribosomes (and generally somewhat more than half with subribosomal ribonucleoprotein particles) (Figure 1A,C) may account for the appearance of distinct 18S and 28S radiolabeled peaks corresponding to rRNA on gel electrophoresis of RNA extracted from the ribosomal fraction (ribosomes plus subribosomal ribonucleoprotein particles (Chen *et al.*, 1971). The progressive accentuation of the radiolabeled 18S and 28S RNA peaks after 2 and 6 hr of uridine chase (Chen *et al.*, 1971) may be due to the accompanying progressive increase in the proportion of radiolabeled RNA in ribosomes as compared to subribosomal ribonucleoprotein particles (Figure 3 A,C,E). Since the radioactivity in the subribosomal fraction remained relatively constant during uridine chase while ribosomal radioactivity rose substantially absolutely and relatively (Figure 3), the increasing proportion of radioactive RNA in ribosomes during uridine chase has been interpreted in this paper as due to the influx of new ribosomes into the cytoplasim from the nucleus bearing RNA synthesized during the sixth hour, rather than due to conversion of subribosomal particle RNA into rRNA.

The nature and purpose of the subribosomal ribonucleoprotein particle and its relationship to mRNA bound as messenger ribonucleoprotein to ribosomes have not been clear despite considerable investigation (Scherrer *et al.*, 1970). The apparent total binding of germinating wheat embryo subribosomal ribonucleoprotein particles to ribosomes dissociated in the presence of the particles by dialysis against phosphate buffer suggests that the subribosomal fraction is a messenger ribonucleoprotein particle which under these conditions is able to bind to the small ribosomal subunit. This finding supports the close relationship between true messenger ribonucleoprotein particles bound to polysomes and the rapidly labeled subribosomal ribonucleoprotein particles. It is not clear why a majority of subribosomal ribonucleoprotein particles do not become attached to ribosomes *in vivo* as determined by the pulse-chase experiments here reported as well as those of others (Scherrer *et al.*, 1970). Conceivably there could be a difference in the RNA, protein or conformation between the cytoplasmic ribonucleoprotein particles containing rapidly labeled mRNA which normally do or do not attach to ribosomes or a limitation of ribosomal attachment sites. Whatever the normal limitation, it is clear that under the phosphate dissociating conditions used, the subribosomal ribonucleoprotein particles appear to attach to the ribosomes.

The lower buoyant density of subribosomal ribonucleoprotein particles as compared to ribosomes indicates that they have a higher protein:RNA ratio. Cofiltration on Sepharose 6B of the subribosomal ribonucleoprotein particles with early filtering, presumably large ribosomes suggests that the two particles have similarity in size, though not necessarily

in mass. The slower sedimentation in sucrose gradients of the subribosomal ribonucleoprotein particles suggests that they have a smaller mass and/or less compact structure. A nonspherical, somewhat elongated particle, with one dimension at least as long as the diameter of the presumably larger size ribosomes, and possibly a lesser mass would be compatible with these findings. Electron microscopic evidence for such an elongated structure of at least some subribosomal particles of HeLa cells has been reported (Spohr *et al.*, 1970).

It is not presently understood why no region of high specific activity corresponding to that seen in the sucrose gradients of dissociated ribosomes (Figure 1B,D) was observed on DEAE-cellulose chromatography (Figure 5), although it may be related to the marked deproteination which preceded elution of ribonucleoprotein fractions. It appears as though under these conditions the radioactivity corresponding to the high specific radioactivity subribosomal ribonucleoprotein particles has become evenly distributed throughout the regions of ribonucleoprotein elution.

The separation of high specific activity ribonucleoprotein particles from ribosomes by DEAE-cellulose column chromatography in the presence of Mg^{2+} offers a large-scale preparative method for simultaneous purification of both. However, the subribosomal ribonucleoprotein particles so prepared have apparently been stripped of some of their protein.

Acknowledgments

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