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Stage-Specific Synthesis of Proteins Complexed to Ribonucleoprotein Particles and Ribosomes in Zoospores of *Blastocladiella emersonii*

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In *Blastocladiella emersonii* zoospores, a set of proteins was found associated with the ribosomes and free ribonucleoprotein particles distinct from the ribosomes and polyribosomes. These proteins were designated P120, P105, P64, P56, and P42 based on their molecular weights determined by gel electrophoresis. Synthesis of these proteins was detected only during late sporulation just before the time polyadenylated ribonucleic acid accumulates in the sporangia. These proteins banded in isopycnic metrizamide gradients at densities of 1.31 and 1.27 g/cm^3, which corresponded to the densities of the ribosomes and free ribonucleoprotein particles, respectively. Comparison of the distribution of the proteins in sucrose versus metrizamide gradients suggested that P105 was removed from the free ribonucleoprotein particles before complexing with the ribosomes. During germination, these proteins disappeared from the ribosomal fractions, with kinetics corresponding to the resumption of protein synthesis. Another protein (P178) was observed to bind to the ribosomes before the onset of protein synthesis during germination. Cycloheximide did not block the addition of this protein to the monoribosomes.

Zoospores of the aquatic fungus *Blastocladiella emersonii* contain dormant messenger ribonucleic acid (mRNA) which is not translated until the zoospores are induced to germinate (16, 23, 24). The zoospore mRNA is presumed to be represented at least in part by the polyadenylated [poly(A)] RNA present in the zoospores (10, 13, 21). Most of this poly(A) RNA is in particles which sediment at 80S or greater and are localized in the zoospore nuclear cap (13), a membrane-bound structure which contains essentially all of the zoospore ribosomes.

In a previous report (11) we presented evidence indicating that approximately one-third of the poly(A) RNA of the zoospore is synthesized during the final 15 to 20 min of the sporulation sequence. This suggested the possibility that factors responsible for sequestering the mRNA are not synthesized or activated until late sporulation. The latter is supported by the observation that protein synthesis occurs at reasonable rates during the earlier phases of sporulation and declines toward the end of sporulation (2, 19). We examined the proteins synthesized during late sporulation in an effort to identify proteins which could potentially regulate the translation of the zoospore mRNA. Our data indicate that there is a set of proteins which appear during late sporulation just before the time at which the poly(A) RNA accumulates, and these proteins disappear during germination with kinetics which correlate with the resumption of protein synthesis. There is also a protein which is synthesized during late sporulation and binds to the ribosomes just before the onset of protein synthesis during early germination.

MATERIALS AND METHODS

Growth of cultures. *B. emersonii* was grown in 1-liter cultures in PYG medium (peptone, 1.25 g/liter; yeast extract, 1.25 g/liter; glucose, 3 g/liter) as described previously (10). For germination experiments, zoospores were inoculated into Lovett's defined medium (16) modified to contain 50 mM KCl as suggested by Soll and Sonneborn (25).

Preparation of post-mitochondrial supernatants and ribosomal pellets. Post-mitochondrial supernatants and ribosomal pellets were prepared essentially as described by Gong and Lovett (5). Briefly, cells were collected by centrifugation and quick frozen at −70°C. Frozen cells were ground in a cold mortar and resuspended in 5 ml of homogenization buffer, which contains 50 mM Tris(hydroxymethyl)-aminomethane (Tris) (pH 8.6), 250 mM NaCl, 50 mM $MgCl_2$, 250 mM sucrose, 25 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 2% Triton X-100. Cell suspensions were then homogenized in a Duall no. 23 glass tissue homogenizer (Kontes Glass Co.) and centrifuged at 12,000 × *g* for 10 min to yield a pellet

fraction and a post-mitochondrial supernatant. Ribosomes were pelleted by layering the post-mitochondrial supernatant over 2 ml of TMK buffer (10 mM Tris [pH 8.6], 50 mM $MgCl_2$, 10 mM KCl, 25 mM ethylene glycol-bis(β-aminoethyl ether)*N,N'*-tetraacetic acid, 1 M sucrose) and centrifuging the sample at 48,000 rpm in a Beckman 50 Ti rotor at 4°C for 95 min except where indicated otherwise. In cases where the post-mitochondrial supernatant was to be assayed directly on sucrose gradients, sucrose was omitted from the homogenization buffer and cells were resuspended in 2 ml.

Sucrose gradients. Linear 10 to 35% sucrose gradients were prepared in gradient buffer (10 mM Tris [pH 7.6], 100 mM KCl, 10 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride). Post-mitochondrial supernatants containing 4 optical density units were layered on the gradients and centrifuged at 35,000 rpm in a Beckmen SW41 rotor at 5°C. Gradients were fractionated with an ISCO model 640 gradient fractionator.

Metrizamide gradients. Metrizamide [2-(3-acetamido-5-*N*-methylacetamido-2,4,6 triiodobenzamido)-2-deoxy-D-glucose] was purchased from Accurate Chemical, Hicksville, N.Y., as the analytical grade. Gradients were prepared by dissolving 2 g of metrizamide in 2.5 ml of buffer (100 mM Tris, [pH 8.6], 200 mM KCl, 20 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride). This solution was cooled to 4°C, after which the volume was adjusted to 5 ml by the addition of 4°C water and the ribosome suspension. Mineral oil was layered over the metrizamide-ribosome mixture, and the gradient was formed by centrifugation at 36,000 rpm for 42 to 44 h at 5°C in a Beckman 50 Ti rotor. Gradients were fractionated by piercing the bottom of the gradient tube with an 18-gauge needle and collecting fractions dropwise. The density of the fractions was determined by measuring the refractive index of an aliquot from selected samples and extrapolating from a standard curve. Radioactivity in metrizamide fractions was measured directly by using a Triton X-100-based scintillation cocktail. Metrizamide fractions were diluted with an equal volume of aqueous buffer before addition to the Triton cocktail.

Analysis of labeled poly(A) RNA from sucrose gradients. Fractions from sucrose gradients were immediately adjusted to 10 mM Tris (pH 7.6)-200 mM NaCl-0.1% sodium dodecyl sulfate (SDS) in a final volume of 1 ml. Samples were heated to 60°C for 2 min, cooled to room temperature, and passed over polyuridylic acid filters as described by Sheldon et al. (22). Nonbound RNA was estimated by measuring the trichloroacetic acid-precipitable radioactivity in a 0.2-ml portion of the sample which passed through the polyuridylic acid filter.

Analysis of proteins from sucrose and metrizamide gradients. Fractions were immediately adjusted to 10% trichloroacetic acid and incubated at 4°C for at least 16 h. Precipitates were collected by centrifugation and washed twice with a 6:4 mixture of 95% ethanol-electrophoresis sample buffer (62.5 mM Tris [pH 6.8], 2% SDS, 10% glycerol, 5% 2-mercaptoethanol). Precipitates were then dried under a stream of air, dissolved in 25 μl of electrophoresis sample buffer, heated to 90°C for 2 min, and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography.

SDS-PAGE and autoradiography. SDS-PAGE was carried out as described by Laemmli (14) on slab gels (140 by 120 by 0.75 mm), using 10% acrylamide in the resolving gel. Electrophoresis was carried out at 21°C at a constant current of 5 mA per slab during stacking and 15 mA per slab in the resolving gel. After electrophoresis, gels were stained overnight with 0.05% Coomassie brilliant blue in methanol-acetic acid-water (5:1:5) and destained with 10% acetic acid. After destaining, gels were soaked for 1 h in methanol-glycerol-water (4:0.25:5.75) and then dried on a Bio-Rad model 224 gel drier. Autoradiography was carried out with Kodak X-Omat R film XR-1.

RESULTS

Accumulation of proteins during late sporulation. Previous work with *B. emersonii* (2) had shown that the rate of protein synthesis decreases continuously during the later stages of sporulation. To determine whether any proteins were synthesized during late sporulation at or near the time at which the large accumulation of poly(A) RNA occurs (11), plants were given a continuous pulse with [^{3}H]methionine throughout the sporulation sequence. There was a dramatic increase in the incorporation of label at 3 h into sporulation, which is just before the time at which poly(A) RNA accumulates in the sporangia (Fig. 1). At 3 h the uptake of methionine into the cells also increased, and thus, at least a portion of the increase in incorporation of label was due to an increased uptake of labeled methionine. However, increased uptake alone does not entirely explain the increased incorporation at this time since the labeled methionine pool (uptake minus incorporation) increased by only 5×10^4 cpm whereas the incorporation data increased by 1.1×10^5 cpm.

Zoospore release occurred in these cells at about 4 h. Since zoospores do not synthesize detectable amounts of protein (17, 24) and the total counts incorporated does not decline for at least 2.5 h after the burst of accumulation (Fig. 1), it appears that these late sporulation proteins are stable in the zoospore.

Analysis of ribosomal pellet proteins synthesized at various stages of sporulation. Since the increase in the accumulation of labeled protein occurred just before the accumulation of poly(A) RNA during late sporulation, it was of interest to determine whether any proteins were synthesized stage specifically during late sporulation. Johnson et al. (13) reported that most of the poly(A) RNA in the zoospore sedimented in sucrose gradients in the region of 80S or greater. Thus, it was of interest to investigate the proteins found in ribosomal pellets in an attempt to detect proteins which may poten-

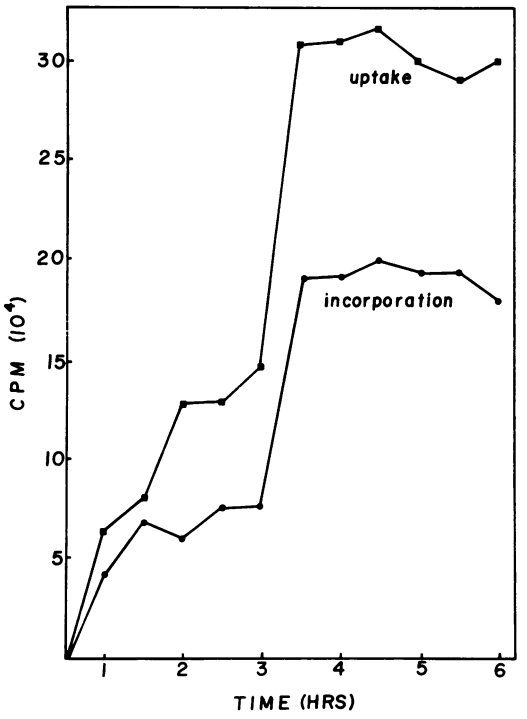

FIG. 1. *Accumulation of [^{3}H]methionine-labeled protein in cells continuously labeled during sporulation. A total of 10^7 cells were grown for 15 h in PYG medium and induced to sporulate by transfer to 200 ml of buffered $CaCl_2$. After transfer, cells were labeled with 2 μCi of [^{3}H]methionine per ml (specific activity, 4.6 Ci/mmol). At the indicated times, replicate 1-ml samples were taken and trichloroacetic acid-precipitable radioactivity was measured. Uptake data were obtained by washing replicate 1-ml samples with sporulation solution containing 10^{-4} M unlabeled methionine.*

tially regulate the translation of the poly(A) RNA. From zoospore ribosomal pellets we consistently detected prominently labeled proteins with molecular weights of 120,000, 105,000, and 64,000 which were designated P120, P105, and P64, respectively (Fig. 2, lanes 2, 4, 6, and 8). These proteins were associated with a background of minor proteins, the most prominent of which had molecular weights of 56,000, 42,000, and 30,000 (designated P56, P42, and P30). Throughout the course of the experiments reported here, P56, P42, and P30 were not as consistently prominent as the P120, P105, and P64 proteins. These six proteins are not labeled in detectable amounts in cells labeled for 30 min at 60, 120, and 150 min into sporulation (Fig. 2, lanes 1, 3, and 7). However, cells labeled from 180 to 210 min (Fig. 2, lane 5) had some label in P105, indicating that this is the first protein to become associated with the ribosomal pellet material. Labeling of these proteins is reduced after 210 min into sporulation (Fig. 2, lane 8). Since cells labeled continuously from 180 min until zoospore release (Fig. 2, lane 6) showed prominent labeling of P120, P105, and P64, these data

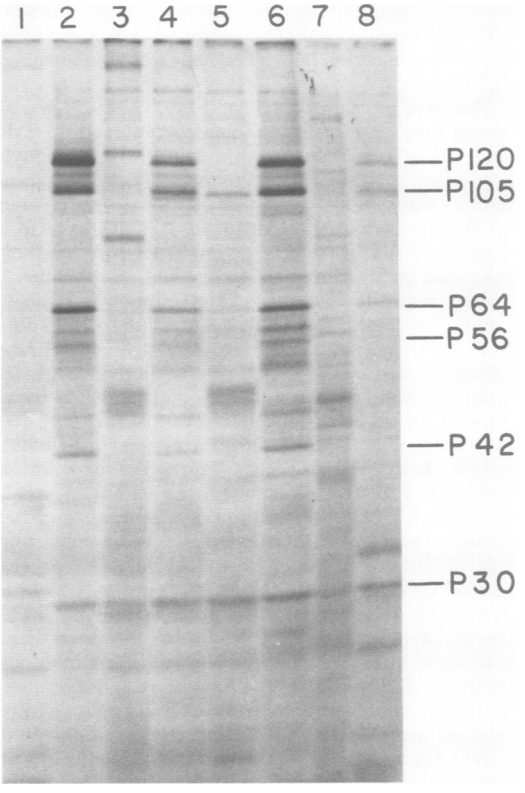

FIG. 2. *Autoradiogram of ribosomal pellet proteins synthesized at various stages of sporulation. Cells were grown and induced to sporulate as in the legend to Fig. 1. At various times, 25 ml of the sporulating culture was transferred to a 125-ml flask and labeled with 2 μCi of [^{35}S]methionine per ml (specific activity, 1.5 Ci/mmol). After labeling, ribosomal pellets were isolated and analyzed by SDS-PAGE and autoradiography as described in the text. Each sample contained 1 optical density unit (260 nm) of ribosomes. Phosphorylase B (molecular weight, 94,000), bovine serum albumin (68,000), ovalbumin (45,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (21,000) were used as standards for molecular weight estimations. Labeling times for the various samples were as follows: lane 1, 60 to 90 min; lane 2, 60 min until zoospore release; lane 3, 120 to 150 min; lane 4, 120 min until zoospore release; lane 5, 180 to 210 min; lane 6, 180 min until zoospore release; lane 7, 150 to 180 min; lane 8, 210 min until zoospore release. Time zero refers to the time at which transfer of the cells from PYG growth medium to the buffered $CaCl_2$ sporulation solution was complete. Zoospores released at about 4 h.*

suggest that the proteins are synthesized between 180 and 210 min into sporulation but do not become associated with the ribosomal pellet fraction until after 210 min. This point is consistent with the data in Fig. 1, which show that the accumulation of labeled protein occurs between 180 and 210 min and ceases after this time. The data do not preclude the possibility that these proteins are also synthesized before 180 min into sporulation.

Changes in ribosome pellet proteins during germination. To determine whether ribosome pellet proteins are stable or disappear during germination, sporangia were labeled during late sporulation and the labeled zoospores were germinated in a medium containing sufficient unlabeled methionine to prevent labeling of protein during germination. After 15 min of germination, P120, P105, and P64 were present in the ribosomal pellets at levels similar to those of the zoospore (Fig. 3). A noticeable decrease was observed after 30 min of germination, with a marked decline after 45 and 60 min. The decrease in the labeled proteins correlated with the kinetics of resumption of protein synthesis during germination (data not shown). This is the result one would expect for proteins potentially involved in blocking protein synthesis in zoospores. At 15 min into germination, the most obvious change in the ribosomal pellet proteins was the addition of a protein with a molecular weight of 178,000 (P178) which remained associated with the ribosomes at later stages of germination (Fig. 3). Very little label was detected in this band at the zoospore stage, and only a slight decline was observed after 60 min of germination. It appeared that P178 became associated with the ribosomes before the onset of protein synthesis during germination. Additional evidence for this was obtained by germinating prelabeled zoospores in cycloheximide. Under these conditions P178 associated with the ribosomes, indicating that this protein is made during sporulation and becomes associated with the ribosomes in the absence of protein synthesis. Cycloheximide blocked the disappearance of P120, P105, and P64 at least until 60 min into germination, suggesting that the disappearance of these proteins is somehow coupled to protein synthesis (data not shown).

Distribution of protein on sucrose gradients. A preliminary analysis of the ribosomal pellet proteins on sucrose gradients indicated that P120, P105, P64, P56, and P42 sedimented in both the subribosomal (20S to 80S) and the ribosome-polysome region (80S and above) of the gradient. P30 sedimented primarily in the 80S region. Post-mitochondrial supernatant

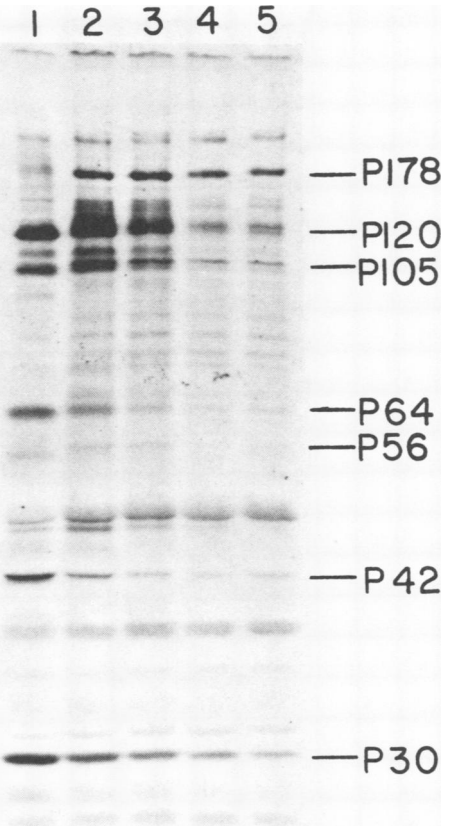

FIG. 3. *Loss of ribosomal pellet proteins during germination. A 200-ml culture of sporangia was labeled with 2 μCi of [^{35}S]methionine per ml (specific activity, 1.5 Ci/mmol) from 170 min into sporulation until zoospore release. Zoospores were collected and resuspended in cold defined medium with 1 mM methionine added. A portion was removed for ribosomal pellet isolation, and the remainder was diluted with defined medium plus 1 mM methionine to a final concentration of 2 × 10^6 zoospores per ml. At 15-min intervals, samples were removed, and the ribosomal pellets were isolated and analyzed as described in the legend to Fig. 2. Samples in each lane contained 1 optical density unit (260 nm) of ribosomes. Lane 1, Zoospores; lane 2, 15 min; lane 3, 30 min; lane 4, 45 min; lane 5, 60 min. The molecular weight of P178 was determined on a separate gel in which myosin (molecular weight, 200,000), β-galactosidase (116,500), phosphorylase B, bovine serum albumin, and ovalbumin were used as markers.*

fractions were analyzed on sucrose gradients to get a better estimate of the extent to which these proteins are found in subribosomal particles (Fig. 4). In these preparations the majority of the labeled P120, P105, P64, P56, and P42 proteins sedimented in the 20S to 80S region of the gradient (Fig. 4, lanes 3, 4, and 5) but these

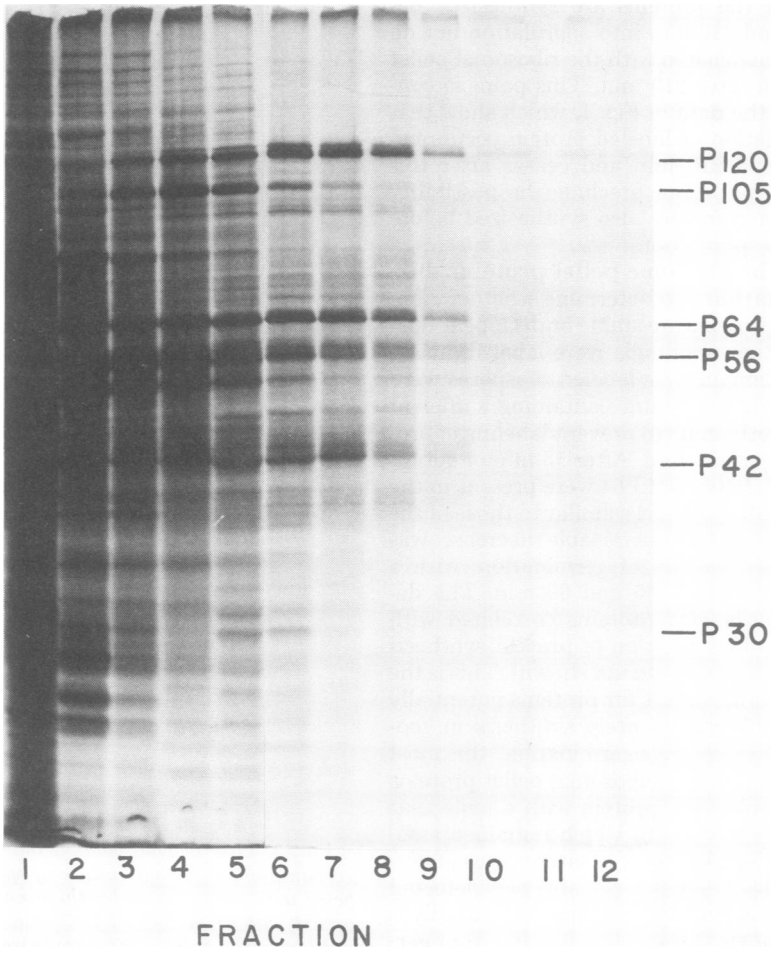

FIG. 4. *Autoradiogram of sucrose density gradient analysis of zoospore post-mitochondrial supernatant proteins labeled during late sporulation. Sporangia were labeled from 170 min into sporulation until zoospore release. A post-mitochondrial supernatant was prepared and centrifuged for 2 h on linear 10 to 35% sucrose gradients. Fractions of 0.6 ml were collected and analyzed by SDS-PAGE and autoradiography. Only the top 12 fractions are shown. Fractions 5 and 6 contained the 80S ribosomes.*

proteins were also easily detected in the polysome region of the gradient (Fig. 4, lanes 7, 8, and 9). In the polysome region P120 was enriched relative to P105, whereas in the subribosomal region these proteins contained approximately equal amounts of radioactivity.

Sucrose gradient analysis of a post-mitochondrial supernatant prepared from 30-minute germlings yielded results similar to those obtained from zoospores. The most obvious change in the pattern of labeled protein at this stage was the appearance of P178 in the region of the 80S ribosomes (Fig. 5, lane 6). In repeated attempts we were unable to detect P178 in the polysome region of the profile and only a slight P178 band was detectable in the subribosomal region, suggesting that this protein is limited to the monoribosomes. P178 was also found in the 80S ribosome region when cells were germinated in cycloheximide (data not shown). Data in Fig. 4 and 5 were obtained from cells labeled during late sporulation at a time corresponding to the large accumulation of labeled protein noted in Fig. 1. There is a large amount of label which remains at the top of the gradient when post-mitochondrial supernatants are analyzed, indicating that the proteins which are synthesized during late sporulation represent a spectrum of proteins and are not limited to those found in ribosomal pellets.

Distribution of poly(A) RNA on sucrose gradients. Since the proteins synthesized during late sporulation sedimented in both the subribosomal and the ribosome-polysome region of

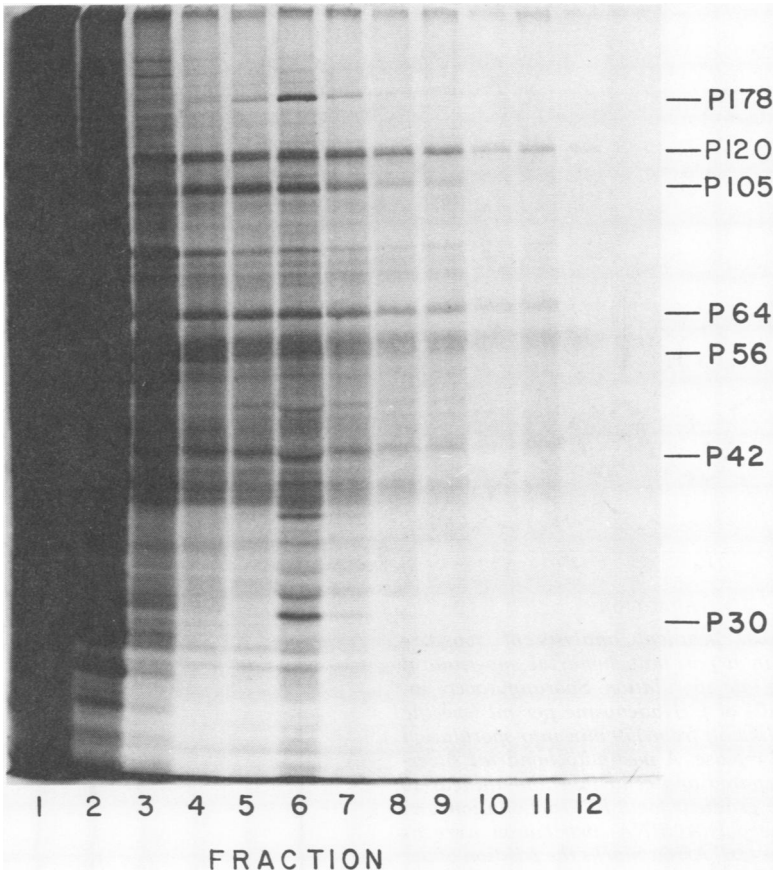

FIG. 5. *Autoradiogram of sucrose density gradient analysis of post-mitochondrial supernatant proteins from 30-min germlings. Zoospores, labeled as described in the legend to Fig. 4, were grown in defined medium with added methionine. After 30 min, germlings were collected and analyzed as described in the legend to Fig. 4. The 80S monoribosome peak was found primarily in fraction 6, with trace amounts of ribosomes in fractions 5 and 7.*

the sucrose gradients, it was of interest to determine the distribution of the zoospore poly(A) RNA on similar gradients. Analysis of the sedimentation of [^{3}H]adenosine-labeled RNA from zoospore post-mitochondrial supernatants is shown in Fig. 6 and 7. Poly(A) RNA labeled during late sporulation sedimented in both the subribosomal and the ribosomal region of the gradient (Fig. 6). Labeled poly(A) RNA was found near the top of the gradient in the 10S to 20S region, where one would expect to find free RNA. The remainder was spread throughout the gradient, with a slight peak at 80S. Nonadenylated RNA on the gradient sedimented primarily in the subribosomal region, with a peak at about 60S. In a similar experiment in which the sedimentation of labeled RNA in ribosomal pellets was analyzed, poly(A) RNA was found only in the ribosome-polysome region, with very little poly(A) RNA radioactivity at the top of the gradient in the region of 0 to 50S. In contrast to the RNA labeled during late sporulation, poly(A) RNA labeled during growth (Fig. 7) sedimented as a discrete peak with the 80S ribosomes. Non-adenylated RNA (primarily ribosomal RNA) was also associated with the 80S ribosomal peak. With this labeling protocol only a minor fraction of the adenylated or nonadenylated RNA was detected in the subribosomal or polysomal region of the gradient.

Analysis of RNA and proteins on metrizamide gradients. From the sucrose gradient analysis of the labeled RNA and proteins it was not possible to distinguish whether the RNA and proteins in the 80S and above region were cosedimenting with the ribosomes and polysomes as a free complex or were actually bound to the ribosomes. Since free ribonucleo-proteins (RNPs) usually have a buoyant density distinct from the ribosomes (6, 7, 20), separations based

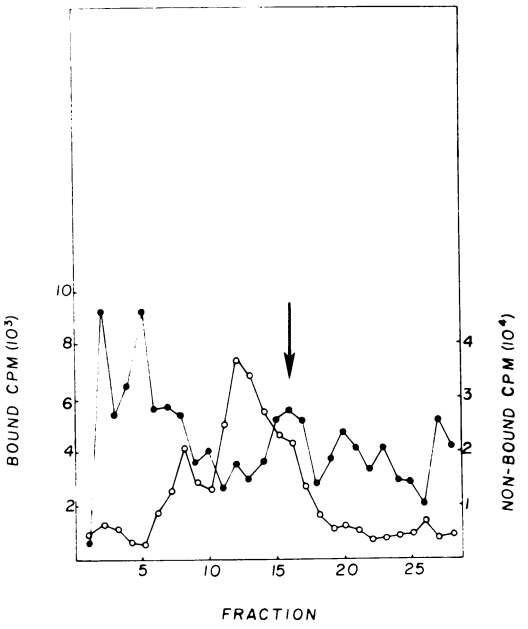

FIG. 6. *Sucrose gradient analysis of zoospore poly(A) RNA in a post-mitochondrial supernatant labeled during late sporulation. Sporangia were labeled with 2 μCi of [³H]adenosine per ml (specific activity, 20 Ci/mmol) from 170 min into sporulation until zoospore release. A post-mitochondrial supernatant was prepared and centrifuged as a linear 10 to 35% sucrose gradient for 3 h. Fractionation and analysis of the poly(A) RNA distribution were as described in the text. Arrow marks the position of the 80S ribosomes. Symbols:* (○) *nonbound radioactivity;* (●) *bound [poly(A)] radioactivity.*

on density are usually used to distinguish between cosedimenting particles and RNA or protein bound to the ribosomes. In our experiments, metrizamide was selected as the density medium since its use does not require prior fixation of the RNPs with formaldehyde or glutaraldehyde, which would preclude identification of the labeled proteins by SDS-PAGE. Radioactivity in proteins from ribosomal pellets prepared from cells labeled during late sporulation banded in metrizamide at a density slightly less than that of the ribosomes (Fig. 8). RNA labeled during late sporulation banded in metrizamide with a peak closer to that of the ribosomes, with some RNA radioactivity in the region coinciding with the density of the labeled protein peak (Fig. 8). SDS-PAGE analysis of the labeled proteins banded in metrizamide is shown in Fig. 9. The majority of the ribosomal proteins banded in fractions 5 and 6 (ρ = 1.31 g/cm^3), with slight amounts found in fractions 7, 8, and 9 (data not shown). The autoradiogram from this gel (Fig. 9) revealed that P120, P105, P64, and P56 banded in fractions 5 to 9. In fractions 5 and 6, P120 was more heavily labeled than was P105, as was observed in the polysome region of the sucrose gradients in Fig. 4. In the region of the metrizamide gradient corresponding to an average density of 1.27 g/cm^3 (fractions 7, 8, and 9), the labels in P120 and P105 were about equal, as was the case in the subribosomal region of the sucrose gradient (Fig. 4).

Ribosomal pellets used for the metrizamide analysis were obtained from post-mitochondrial supernatants centrifuged for 3 h rather than the normal 95 min since direct sucrose gradient analysis (Fig. 4) of post-mitochondrial supernatants revealed that a majority of the labeled proteins under investigation sedimented in the subribosomal region. Attempts to analyze the post-mitochondrial supernatant directly on metrizamide were unsuccessful because the proteins which remained at the top of the sucrose gradient (Fig. 4) banded with a broad density that overlapped

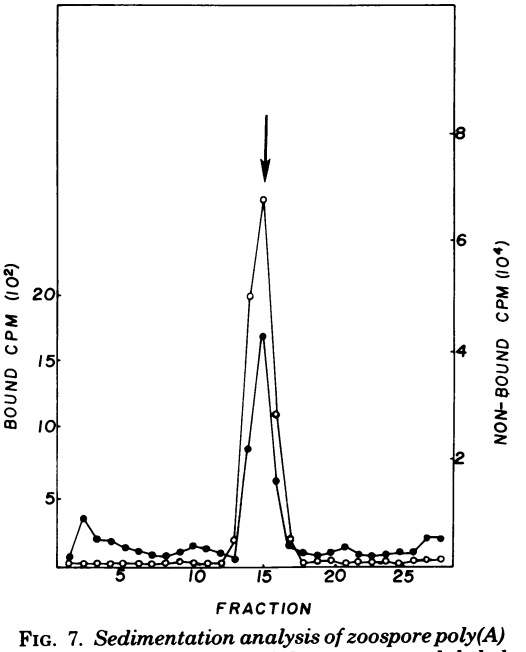

FIG. 7. *Sedimentation analysis of zoospore poly(A) RNA in a post-mitochondrial supernatant labeled during growth. Growth-phase plants were labeled with 2 μCi of [³H]adenosine per ml (specific activity, 20 Ci/mmol) from 14 to 15 h into growth. Plants were than induced to sporulate by transfer to buffered $CaCl_2$ containing 1 mM adenosine, which blocks further labeling of the RNA in these cells. After release, zoospores were collected and analyzed on sucrose gradients as described in the legend to Fig. 6. Arrow marks the position of the 80S ribosomes. Symbols:* (○) *nonbound radioactivity;* (●) *bound [poly(A)] radioactivity.*

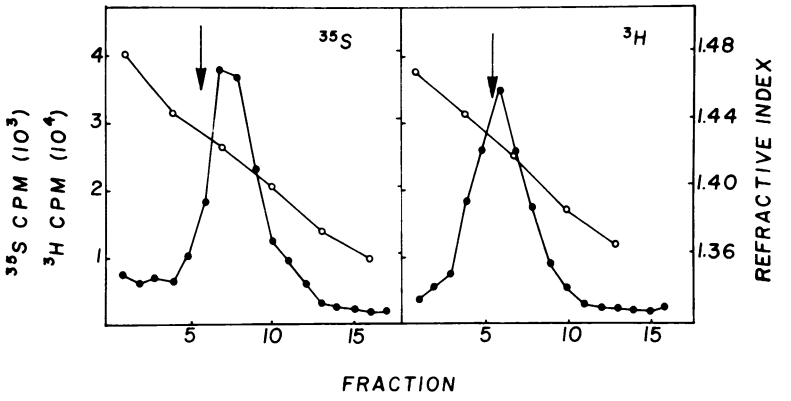

FIG. 8. *Distribution of labeled RNA and protein on metrizamide gradients. Sporangia were labeled with 2 μCi of either [^{35}S]methionine or [^{3}H]adenosine per ml from 170 min into sporulation until zoospore release. Zoospores were collected and ribosomal pellets were isolated as described in the text, except the post-mitochondrial supernatant was centrifuged for 3 h at 48,000 rpm to pellet the RNPs. Metrizamide gradients were prepared and analyzed as described in the text. Arrows mark the position of the ribosomes. (●) Counts per minute (cpm); (○) refractive index.*

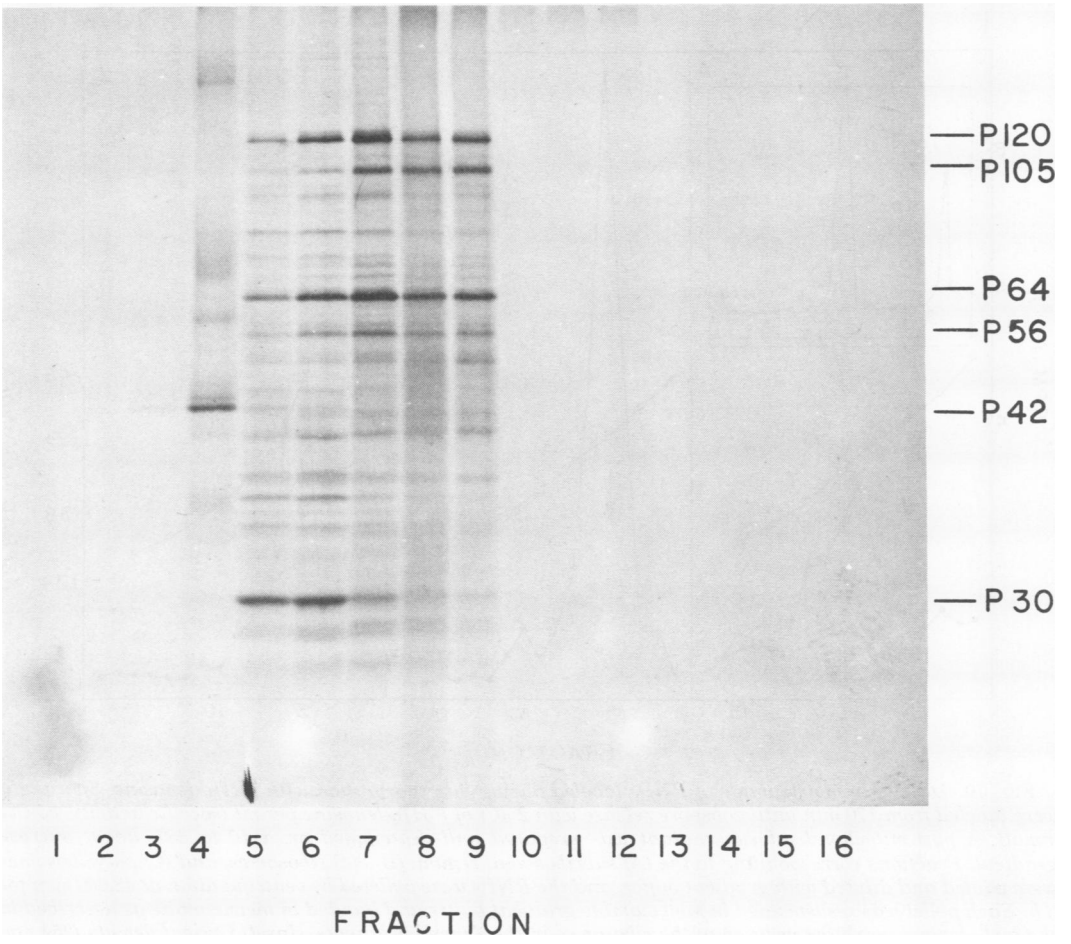

FIG. 9. *SDS-PAGE analysis of proteins in ribosomal pellets banded in metrizamide. Fractions containing [^{35}S]methionine-labeled proteins in Fig. 8 were analyzed by gel electrophoresis and autoradiography. The numbers above each lane refer to the fraction numbers in Fig. 8.*

1.27 g/cm^3 and interfered with identification of P120, P105, P64, P56, P42, and P30.

In the subribosomal region, the sucrose gradient data from Fig. 4 and 6 indicated that P120, P105, P64, P56, and P42 were bound to RNA to form an RNP complex with an average sedimentation coefficient of <80S. To obtain further evidence that these proteins are bound to RNA, cells were labeled with [^{3}H]adenosine during late sporulation. After zoospore release, a post-mitochondrial supernatant fraction was prepared and centrifuged on a sucrose gradient. Fractions corresponding to the subribosomal, ribosomal, and polysomal regions were pooled, centrifuged to pellet RNPs, and then analyzed on metrizamide gradients. Labeled RNA which sedimented in the subribosomal region banded in metrizamide with a density of 1.27 g/cm^3 (Fig. 10A). This coincides with the density at which the label in P120 and P105 is approximately equal (Fig. 9). In contrast, labeled RNA from both the ribosome (Fig. 10B) and the polysome (Fig. 10C) regions of the sucrose gradient banded in metrizamide at 1.31 g/cm^3, which corresponds to the density of the ribosomes (Fig. 9). Very little of the labeled RNA which sediments in the ribosome-polysome region of the sucrose gradients bands at a density of 1.27 g/cm^3.

DISCUSSION

During late sporulation in *B. emersonii*, proteins are synthesized and stored in the zoospore. Data in Fig. 1 reflect the net accumulation of labeled proteins, which is a balance between protein synthesis and degradation. Since the rate of protein synthesis decreases to low levels during the later stages of sporulation (2) it would appear that the increased accumulation observed at 3 h into sporulation is due in part to a substantial decrease in proteolysis. This conclusion is supported by the work of Lodi and Sonneborn (15) in which they observed a high rate of proteolysis (about 12%/h) during sporulation.

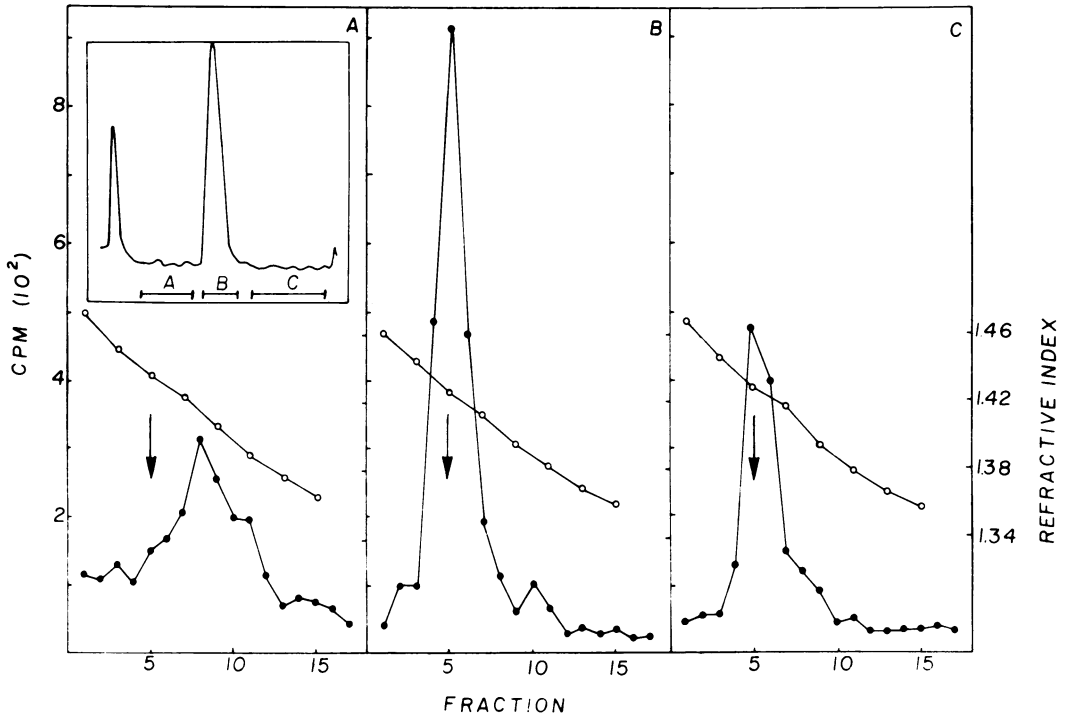

FIG. 10. *Density in metrizamide of RNPs labeled during late sporulation with [^{3}H]adenosine. Sporangia were labeled from 170 min until zoospore release with 2 μCi of [^{3}H]adenosine per ml (specific activity, 33 Ci/mmol). A post-mitochondrial supernatant was prepared and centrifuged on a 10 to 35% linear sucrose gradient. Fractions corresponding to the (A) subribosomal region, (B) 80S ribosomes, and (C) polyribosomes were pooled and diluted with gradient buffer, and the RNPs were pelleted by centrifugation at 48,000 rpm for 3 h. Each pellet was resuspended in metrizamide gradient buffer and banded in metrizamide as described in the text. Arrows mark the position of the ribosomes in the density gradients. (Inset) Optical density (254 nm) profile of the sucrose gradient showing the regions pooled for metrizamide analysis.*

The enzyme activity presumably responsible for this proteolysis is released from the cells to the medium at approximately the same stage at which we observe the large accumulation of labeled protein.

Of the proteins synthesized during late sporulation P178 represents a potential activator of protein synthesis since it binds to the ribosomes before the onset of protein synthesis and remains bound after protein synthesis begins (Fig. 3). We have no evidence as to the function of this protein, but the observation that it binds almost exclusively to monoribosomes (Fig. 5) suggests that it may function in 80S initiation complex formation or activation. Within the zoospore, ribosomes are apparently inactivated by an inhibitor which binds to the ribosomes and is removed after the cells are induced to germinate (2). Removal of this inhibitor is a possible function for P178 since the zoospore ribosomes are over 90% monoribosomes (5) (Fig. 10). If P178 did function to remove the inhibitor, then our inability to detect P178 in the germling polysomes indicates that the protein remains bound to the ribosome only until the inhibitor is removed. Gong and Lovett (5) have reported that elongation factor activity is localized in the extra cap cytoplasm separate from the nuclear cap-bound ribosomes. Although P178 may play a role in elongation, we consider this less likely since elongation factors should also be found in the polysome region of the profile.

Evidence reported in this paper indicates that P120, P105, P64, P56, and P42 exist in the zoospore as an RNP complex. Particles which sediment in sucrose between 20S and 80S characteristically have approximately equal amounts of label in P120 and P105 (Fig. 4 and 5), whereas those which sediment at >80S characteristically have more label in P120 than in P105 (Fig. 4 and 5). The 20S to 80S particles band in metrizamide at 1.27 g/cm^3, whereas ribosomes band at 1.31 g/cm^3. Late-sporulation RNA which sediments between 20S and 80S also bands at 1.27 g/cm^3 (Fig. 10A), and at least a portion of this RNA is poly(A) RNA (Fig. 6). In other systems messenger RNP (mRNP) usually bands in metrizamide at a lower density than the ribosomes (3, 8), whereas purified RNA bands at 1.17 g/cm^3. The non-adenylated RNA which sediments between 20S and 80S (Fig. 6) has not been characterized, but it may represent non-adenylated mRNA since ribosomal RNA synthesis is greatly reduced at this time (11) and the non-adenylated RNA labeled at this stage sediments heterogeneously, as does the poly(A) RNA. The possibility that this non-adenylated RNA is mRNA is supported by our earlier evidence (10, 12) that zoospores contain RNA which is not polyadenylated until cells are induced to germinate.

The data in Fig. 4, 6, 9 and 10 are consistent with the idea that P120, P105, P64, and P56 are part of a free mRNP complex when these particles sediment between 20S and 80S, but the evidence for this is not conclusive. There is little evidence currently available on mRNP in fungi but RNP particles (presumptive mRNP) have been reported for *Dictyostelium* (4), *Neurospora* (18), and *Physarum* (1). Nonpolysomal particles (free RNP) from *Physarum* post-mitochondrial supernatants and *Dictyostelium* nuclei sediment in sucrose gradients with characteristics similar to the *B. emersonii* subribosomal particles in the present report. The molecular weight of the proteins from the *Physarum* RNPs were not reported but the RNPs from *Dictyostelium* had proteins with a broad molecular weight range extending above the 120,000 and below the 30,000 proteins reported here.

P120, P105, P64, and P56 are apparently bound to the ribosomes and polysomes when they are found in this region of the sucrose gradients. Late-sporulation RNA which sediments at ≥80S bands in metrizamide with the ribosomes at 1.31 g/cm^3. Also, at 1.31 g/cm^3 the pattern of label found in P120 versus P105 is similar to the relative amounts of label in these two proteins from the polysome region of sucrose gradients. This implies that P105 is removed from the subribosomal particles before they are bound to the ribosomes. In this context it is interesting that a model for translational control of mRNP has been proposed in which it is postulated that removal of mRNP-associated proteins allows translation of free mRNP (9).

From a number of reports, the evidence currently available suggests that at least a partial explanation for the block in protein synthesis at the zoospore stage in *B. emersonii* is related to elongation. Gong and Lovett (5) reported that, although elongation factor activity is present in the zoospore, it is primarily localized outside the nuclear cap away from the ribosomes. Concomitant with the onset of protein synthesis, the nuclear cap breaks down, releasing the ribosomes (23), and presumably allowing them to interact with the elongation factors. Data in Fig. 6, 7, and 10 indicate that poly(A) RNA is associated with the ribosomes in an initiation complex, which is in agreement with the idea that zoospores are somehow blocked at the elongation step. Whether all of the ribosomes in the zoospores are complexed to poly(A) RNA or mRNA cannot be determined from the available evidence. However, it seems clear from the data in Fig. 6 and 10 that at least some of the zoospore

poly(A) RNA is not complexed with the ribosomes.

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