CHEMICAL AND PHYSICAL CHARACTERIZATION OF "NUCLEAR CAPS" ISOLATED FROM *BLASTOCLADIELLA* ZOOSPORES

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ABSTRACT

LOVETT, JAMES S. (Purdue University, West Lafayette, Ind.). Chemical and physical characterization of "nuclear caps" isolated from *Blastocladiella* zoospores. J. Bacteriol. **85**:1235–1246. 1963.—Electron micrographs of *Blastocladiella* zoospores have shown the nuclear cap to contain essentially all of the small (250 to 300 A) electron-dense particles of the cell. Preparations of clean, whole nuclear caps were isolated to study the composition of the intact organelles and their particulate contents. The cap is strongly basophilic, and is composed of 60% protein and 40% ribonucleic acid (RNA). It represents 18% of the dry weight, and contains 69% of the total RNA, of the spore. The amino acid composition of cap proteins is similar to the ribosomal protein of other organisms. The nuclear cap contents have been extracted and isolated by high-speed centrifugation. More than 95% of the material has a sedimentation coefficient of 83S in 0.005 M Mg. The 83S particles form aggregates at higher Mg concentrations and dissociate to yield 63S and 41S peaks at low Mg concentrations. Purified cap particles contain 37% protein and 63% RNA. The RNA has a nucleotide composition (in moles per cent) of 18.5% cytidylic, 26.2% adenylic, 31.8% guanylic, and 23.5% uridylic acid. The particles contain a latent ribonuclease, which can be activated by urea, and are susceptible to degradation by added pancreatic ribonuclease. The available evidence supports a concept of the zoospore nuclear cap as an unusual intracellular "packet" of ribosomes.

The massive, crescent-shaped nuclear cap is a characteristic feature of the motile spores produced by fungi in the *Blastocladiales*. During the past 50 years, the function of this organelle, found only during the motile phase, has been the subject of considerable speculation. It has been variously suggested to be a "food body" (Barrett, 1912), to function in growth (Hatch, 1938), to be associated with motility (Wilson, 1952), and to be involved in protein synthesis during spore germination (Turian, 1956*a*; Rorem and Machlis, 1957). Hatch (1935) found the nuclear cap to be Feulgen-negative, and concluded from its apparent staining with Janus green that it was an aggregate of chondriosomes. Using a combination of cytological and enzymatic techniques, Turian (1955, 1956*b*) clearly established the ribonucleoprotein (RNP) nature of the nuclear caps in *Allomyces* gametes. Turian and Kellenberger (1956) further showed that the cytochemically demonstrable basophilia of the nuclear cap was exactly correlated with the presence of closely packed, electron-dense particles in the same region of ultrathin sections. In fact, the nuclear cap was found to contain most, if not all, of the cellular particles identical in size and appearance to the ribosomes of other organisms.

The functional significance of the nuclear cap was emphasized by the observation (Hatch, 1935; Emerson, 1941) that it disintegrated during zoospore germination. Blondel and Turian (1960*a*, *b*) have recently documented the formation of the nuclear cap during gamete differentiation in *Allomyces*, and the dispersion of its particles to the cytoplasm during germination of zoospores and zygotes. This sequential, subcellular compartmentalization and release of the ribosomes appears to be a phenomenon unique to the lower fungi. The restriction of the nuclear cap to the motile, nongrowing, spore phase strongly suggests that it may play a significant role in the cellular control of protein synthesis and, in turn, of development. The isolation and characterization of nuclear caps, reported here, have been undertaken to provide the ground work for an analysis of nuclear cap function in *Blastocladiella* zoospores.

MATERIALS AND METHODS

Zoospore harvest. Heavy suspensions of actively motile zoospores were obtained by repeatedly flooding about 20 large cultures of mature first-generation zoosporangial plants (grown on 15-cm petri plates with Difco Cantino PYG Agar at 20 C) with distilled water until the majority had discharged spores (McCurdy and Cantino, 1960). For very large harvests, the procedure was modified by the substitution of ten large aluminum cake pans (20 × 30 cm) covered with aluminum foil. The zoospore suspensions were filtered through cotton to remove any germlings or plants loosened by the washing process, and stored on ice until the harvesting was complete. Zoospore suspensions were concentrated by 2 min of centrifugation at 755 × *g* in a Servall refrigerated centrifuge at 2 C. The loosely packed spores were resuspended in ice-cold 0.001 M $MgCl_2$ or a balanced salt solution (0.0067 M $MgCl_2$, 0.0003 M $CaCl_2$, 0.0034 M potassium phosphate buffer, at pH 7.0) with a Pasteur pipette, pooled, and recentrifuged. The washing procedure was repeated a second time; the final washed pellet was resuspended in a small volume of $MgCl_2$ or salts solution, and stored on ice until used.

Electron microscopy. Washed zoospores were fixed for 2 hr at 2 C in 1% osmium tetroxide buffered at pH 7.1 with HCl-Veronal acetate containing 0.001 M $MgCl_2$. After fixation, the spores were quickly rinsed with buffer and the lightly centrifuged pellet was mixed with an equal volume of 2% agar (Kellenberger, Ryter, and Sechaud, 1958). After chilling, the solidified material was cut into tiny cubes (less than 1 mm^3) and dehydrated through a graded series of alcohols. The fixed and dehydrated blocks of spores were finally infiltrated and embedded in Araldite epoxy resin (Glauert and Glauert, 1958). After hardening for 48 hr at 48 C, the material was sectioned by means of a glass knife in a Servall Porter Blum microtome. Sections were mounted on Formvar-coated grids and examined in a Philips model 100A modified electron microscope. Photographs were taken with Kodak P426 fine-grain positive 35-mm film.

Isolation of zoospore nuclear caps. An International model C clinical centrifuge with a four-place, 15-ml, swinging head was used for all centrifugations in the nuclear cap isolations, and all steps were carried out at 0 to 5 C. Unstained and toluidine blue-stained (after 30 sec of fixation in 1% osmic acid fumes) material was routinely examined microscopically to verify the recovery and degree of purification attained at each stage of the procedure. Washed zoospores in 0.001 M $MgCl_2$ (see harvest procedure) were homogenized by rapidly forcing thick suspensions six times through a hypodermic needle (no. 27). The resulting 4 to 5 ml of yellowish homogenate were centrifuged in a 5-ml round-bottom tube for 15 min at 55 × *g* to sediment the unbroken nuclear caps. The supernatant, containing the soluble materials and most of the cellular debris, was discarded. The nuclear cap pellet was thoroughly resuspended with a Pasteur pipette in 2 ml of medium A/4 (0.075 M sucrose, 10% glycerol, 0.003 M $MgCl_2$, at pH 6.9) and centrifuged for 20 min at 55 × *g*. The supernatant was discarded, the pellet thoroughly dispersed in 3 to 4 ml of medium A/4, and the centrifugation repeated. The supernatant was again discarded and the pellet resuspended in ca. 1 ml of medium A/4. Samples (0.3 ml) of the suspension were then carefully layered on the surface of 20 to 80% sucrose gradients in 15-ml round-bottom plastic tubes. (Sucrose percentages are expressed as g/100 ml; all sucrose solutions contained 0.003 M $MgCl_2$.) The gradient tubes were centrifuged for 5 min at 365 × *g*. Under such conditions, the nuclear caps sedimented in a discrete band ca. 8 mm wide to a region nearly three-fifths of the way down the gradient (i.e., to the zone of 50 to 60% sucrose). The nuclear cap-rich band was removed from below by means of a long hypodermic needle (no. 18) bent to a hook on the end and attached to a gentle suction device. To remove the sucrose and concentrate the clean nuclear caps, the suspensions removed from the gradients were diluted 2.5-fold with 0.003 M $MgCl_2$ and centrifuged for 10 min at 770 × *g*. The pure white caps were finally suspended in 0.003 M $MgCl_2$, pooled, and washed twice in the same solution. If it was necessary to store the purified caps, they were pelleted and covered with 1 ml of medium A (0.319 M sucrose, 40% glycerol, 0.003 M $MgCl_2$, at pH 6.9). Under these conditions, nuclear cap preparations held on ice were stable for up to 1 week.

Isolation of nuclear cap contents. The contents of nuclear caps were released by grinding with acid-washed, powdered glass in a very small Pyrex mortar. For grinding, the caps were suspended in 0.5 to 1.0 ml of TMK solution [0.01

M tris(hydroxymethyl)aminomethane (tris)-HCl buffer, pH 7.4; 0.01 M $MgCl_2$; 0.01 M KCl]. The mixture was ground and extracted at least four times with 2- to 3-ml portions of TMK buffer. After each extraction, the suspension was centrifuged for 5 min at 4,300 × *g*, the supernatant saved, and the sediment returned to the mortar. The combined supernatants were then centrifuged for 15 min at 25,000 × *g* in a Servall refrigerated centrifuge to remove any heavy or aggregated material. The resulting supernatant was in some cases directly examined in a Spinco model E analytical centrifuge. Routinely, however, such supernatants were centrifuged for 120 min at 105,000 × *g* in a Spinco model L preparative centrifuge. The pellet obtained was allowed to resuspend in 2 ml of TMK buffer. The suspension was diluted and subjected to a second cycle of low- (25,000 × *g*) and high- (105,000 × *g*) speed centrifugation. The resulting glassy-clear, colorless pellet was finally allowed to resuspend in 1 to 2 ml of TMK buffer. This nuclear cap particulate fraction was used to obtain the data described under Results.

Analytical procedures. Ribonucleic acid (RNA) was determined by the orcinol method of Dische (1955) on hot trichloroacetic acid digests (5% trichloroacetic acid, 100 C, 20 min). The individual RNA nucleotides were measured spectrophotometrically after KOH hydrolysis and separation by paper electrophoresis (Davidson and Smellie, 1952). The nucleotides in desalted hydrolysates were separated on Whatman no. 1 filter paper with ammonium formate buffer at pH 3.5 and a potential of 1,000 v (Smith, 1955). Individual nucleotides and similarly treated controls were eluted with 0.1 N HCl (12 hr), and the quantities were estimated spectrophotometrically using the wavelengths of maximal extinction. The diphenylamine procedure of Burton (1956) was used to measure deoxyribonucleic acid (DNA). Phosphorus was analyzed by the Fiske and SubbaRow (1925) method and total nitrogen by sulfuric acid digestion and nesslerization (Umbreit, Burris, and Stauffer, 1957). Protein was determined by the biuret technique (Gornall, Bardawill, and David, 1949). The amino acid composition of cap proteins was determined on material hydrolyzed for 8 hr with 6 N HCl at 121 C in a sealed ampule. After removing the excess acid, the amino acids were redissolved in water and assayed by the procedure of Piez and Morris (1960) with a Technicon Amino Acid Autoanalyzer. Ribonuclease activity was measured by the release of acid-soluble ribonucleotides. After incubation, 0.5 ml of the RNA and protein of assay mixtures was precipitated at 0 C in 0.15 ml of a mixture of 0.75% uranyl acetate-25% perchloric acid. After centrifugation, the clear supernatant was appropriately diluted and the optical density determined at 260 mμ.

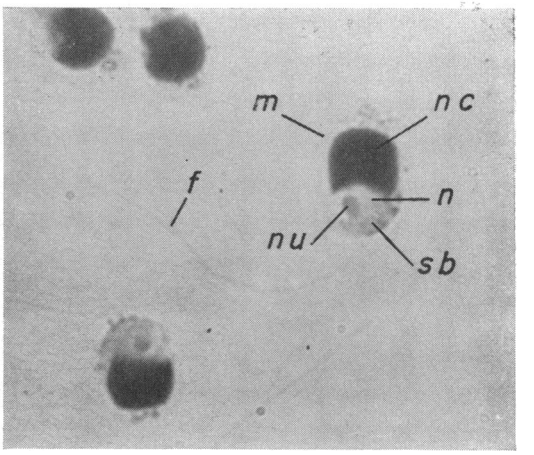

FIG. 1. *Photomicrograph of intact Blastocladiella zoospores suspended in a balanced salt solution. Fixed for 30 sec with 1% osmic acid vapor and stained with 0.01% toluidine blue. The nuclear cap (nc), nucleus (n), nucleolus (nu), "side body" (sb), flagellum (f), and spore membrane (m) are indicated.* × *2,000.*

RESULTS

The general appearance of *Blastocladiella* zoospores is shown in the photomicrograph of Fig. 1. The massive nuclear cap is very obvious and its intense, basophilic staining with toluidine blue is in striking contrast to the essential absence of stain in the cytoplasm. The important details of spore morphology are illustrated more clearly in the electron micrograph of Fig. 2. A complete discussion of zoospore fine structure is to be published elsewhere (Cantino et al., J. Gen. Microbiol., *in press*). But it is important to note here the position of the cap symmetrically enveloping the upper two-thirds of the spore nucleus. The particulate contents of the cap are surrounded by a typical cytoplasmic, double-layered membrane and are separated

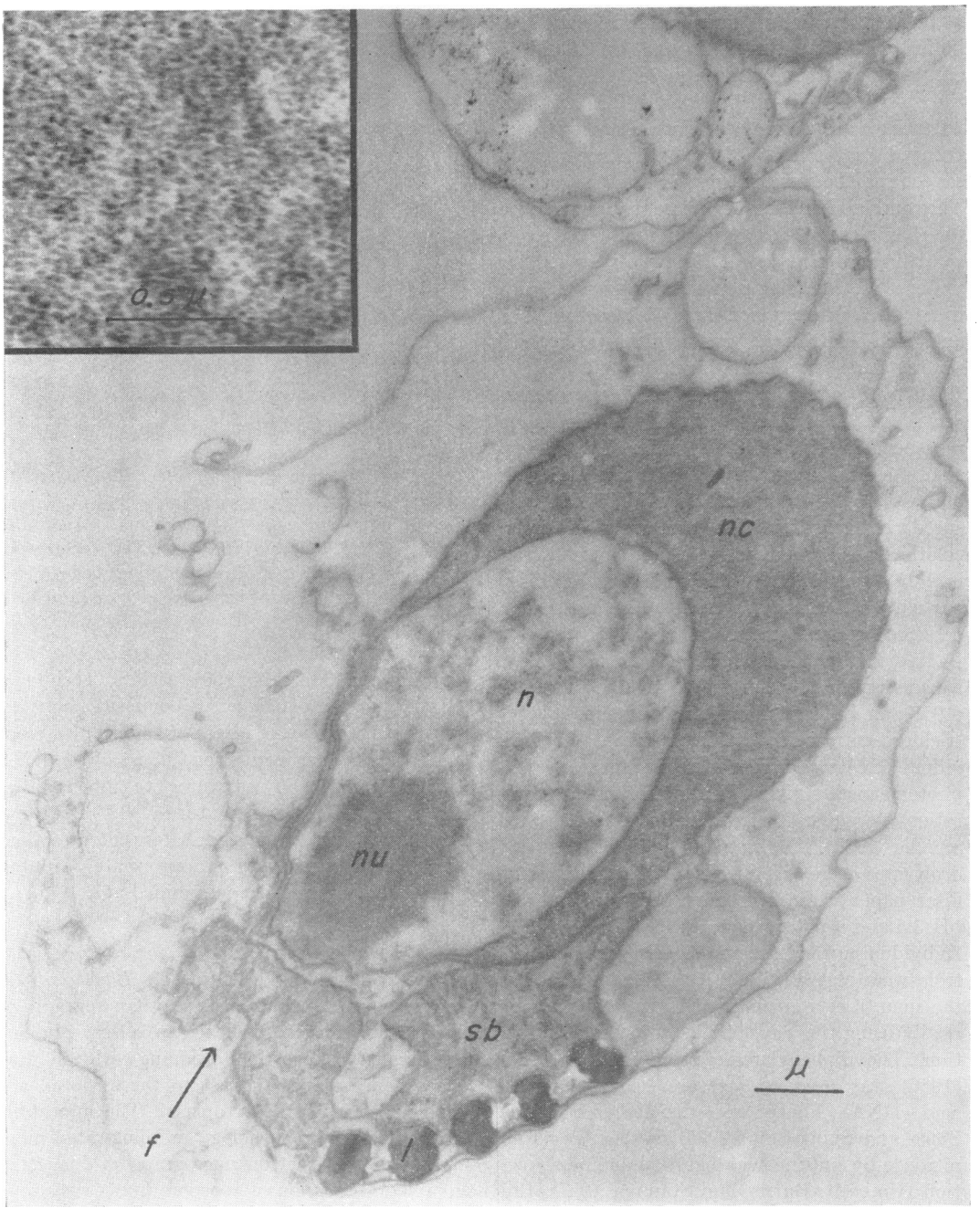

FIG. 2. *Electron micrograph of a longitudinal section through a spore. The nuclear cap (nc) is filled with small electron-dense particles and surrounded by a typical cytoplasmic membrane. The nucleus (n), nucleolus (nu), lipid granules (l), and "side body" or mitochondrion (sb) are also shown. The approximate position of flagellum insertion (f) is indicated.* × *12,800. The insert illustrates the particulate contents of the cap at higher* (× *37,000*) *magnification.*

from the nucleus by only the membrane of the nuclear envelope proper. The extranuclear cytoplasm is essentially devoid of small electron-dense particles of equivalent size. The extent to which the nuclear cap membrane encloses the nucleus has not been established, but it can be seen (Fig. 2) to extend to very near the base of the nucleus, i.e., to the region of the nucleolus. The insert in Fig. 2 pictures the granular contents of the cap at higher magnification. The discrete, closely packed particles predominantly fall in the range of 250 to 300 A.

In preliminary attempts to isolate the nuclei and nuclear caps of *Blastocladiella* zoospores, I used conventional nuclear isolation procedures. These were unsuccessful because the concentrations of sucrose normally used for such procedures were extremely hypertonic to the osmotically sensitive spores. As a result, they caused the flexible outer membrane to collapse tightly around the nucleus and other intracellular structures. When spores were suspended in these media, conditions which permitted cell breakage in a sonic oscillator, Nossel shaker, or French pressure cell also led to extensive fragmentation of the nuclear caps. In contrast, with hypotonic (0.001 M) $MgCl_2$ solutions the cells were easily broken by very mild treatment. The latter medium also led to the osmotic rupture of the nucleus and the large mitochondrion or "side body" (see Fig. 2), both of which would interfere with the isolation of the nuclear caps. The result was to leave the osmotically insensitive caps free and apparently undamaged in the homogenate. Figure 1 illustrates the appearance of zoospores in a normal osmotic environment, and Fig. 3 the condition of spores after two washes in 0.001 M $MgCl_2$. Dense suspensions of these swollen cells were easily and completely broken, by being forced through a fine needle, and gave excellent yields of intact nuclear caps.

Once released from the nucleus, the nuclear caps were readily purified by differential centrifugation. Because of their large size and high density, the caps rapidly sedimented through concentrated sucrose solutions even at low centrifugal forces. A photomicrograph of a purified nuclear cap preparation obtained by our isolation procedure is shown in Fig. 4. Attempts to carry the purification further were not successful, primarily because of the sensitivity of these large structures (ca. 4 × 5 μ) to mechanical damage during repeated pelleting and resuspension. In

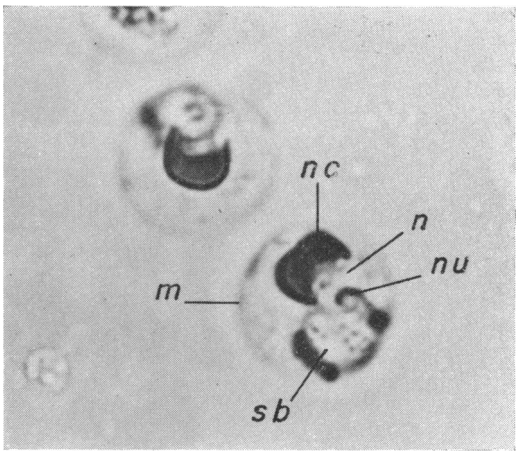

FIG. 3. *Photomicrograph of zoospores washed twice in 0.001 M $MgCl_2$. The swollen condition of the previously inconspicuous "side body" (sb) is particularly obvious. Fixed, stained, and labeled as for Fig. 1.* × *2,000.*

any case, additional purification did not seem to be required. The final preparations looked clean microscopically, retained their normal appearance (compare Fig. 1 and 4), and carried only traces of contaminating DNA (see Table 1). Typical yields of pure white nuclear caps ranged from 30 to 50 mg (dry weight), depending upon the number of spores (4×10^9 to 7×10^9) used.

With a reproducible procedure available for isolating nuclear caps in adequate yield, attention was turned to their structure and chemical composition. The close similarity in staining characteristics, appearance, and ultrastructure between *Blastocladiella* zoospores and *Allomyces* gametes (Turian, 1955, 1958; Turian and Kellenberger, 1956) implied a similar RNP composition of their nuclear caps. Purified cap preparations were therefore analyzed for their content of protein, RNA, DNA, nitrogen, and phosphorus. The results (Table 1) substantiated these expectations, previously based only upon cytological evidence from *Allomyces* gametes. A value of ca. 40% RNA was obtained both by analysis of the total nucleotides released by alkaline hydrolysis and by the direct orcinol method. A content of 60% protein was determined by the biuret reaction, and also from the sum of the component amino acids released by HCl hydrolysis. Thus, the sum of protein plus RNA, estimated by two independent methods, accounts for nearly 100% of the total dry weight of the

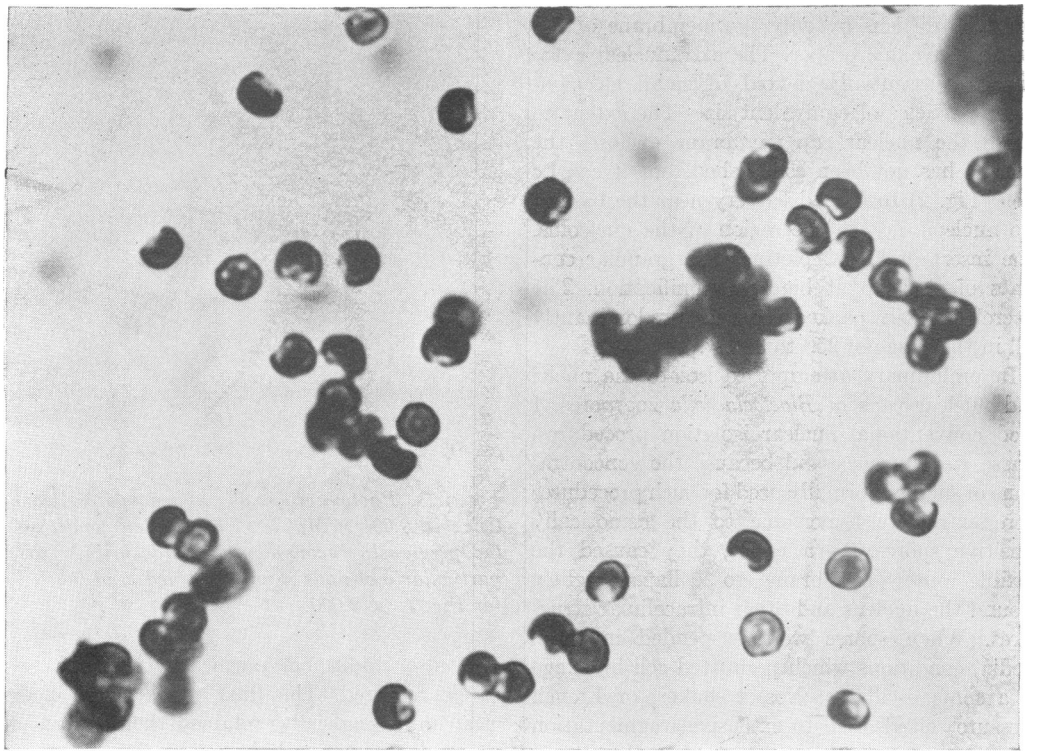

FIG. 4. *Photomicrograph of a purified preparation of zoospore nuclear caps. Material fixed and stained as indicated for Fig. 1.* × *1,200.*

cap. The data for the total phosphorus and nitrogen content are also in agreement with a composition of 40% RNA and 60% protein.

Careful determination of the dry weight per spore (7.6×10^{-5} μg), RNA per spore (8.1×10^{-6} μg), and dry weight per nuclear cap (1.39×10^{-5} μg) has permitted an estimate of the contribution of the cap to the whole spore. The cap was found to contribute 18.5% of the dry weight and 68.6% of the RNA per spore. These values lent further credence to the concept of the nuclear cap as an organelle "packaging" a preponderance of the spore ribosomes. To further verify the ribosomal nature of the cap, the nucleotide composition of the RNA was determined on alkaline hydrolysates (Table 2). Similarly, the amino acid composition was determined as a check on the type of protein present (Table 3).

All of the preceding data were consistent with the "packaged ribosome" hypothesis. But to provide incontrovertible evidence, it was necessary to isolate and examine the contents of the caps. The Schleiren pattern of the material recovered after grinding and two cycles of low- and high-speed centrifugation in 0.01 M Mg is illustrated in Fig. 5*a*. The final 105,000 × *g* pellet was completely colorless and glass-clear before resuspension. In buffer with 0.01 M $MgCl_2$, a majority of the material had a sedimentation coefficient of 83S. A lesser, rapidly migrating component had a coefficient of 125S. The effect of magnesium concentration on the particles is also shown in Fig. 5*a*, *b*, and *c*. The Schleiren pattern of Fig. 5*b* was obtained with cap extracts prepared in 0.005 M Mg^{++} and examined immediately after 15 min of centrifugation at 25,000 × *g*. At this Mg^{++} concentration, the fast peak observed in 0.01 M Mg^{++} (Fig. 5*a*) is practically nonexistent, and the 83S component represents most of the material present. After 6 hr of dialysis vs. 0.0001 M Mg^{++}, the same preparation (Fig. 5*c*) displays, in addition to the reduced 83S peak, two new peaks of 41S and 63S. These must have resulted from dissociation of the 83S fraction.

The cap particle preparations used for chemi-

cal analyses were identical to those used for Fig. 5*a*, because material isolated in buffer containing 0.01 M $MgCl_2$ and 0.01 M KCl was much more stable than that prepared in lower magnesium and potassium concentrations. The data obtained for total protein, total RNA, and RNA nucleotides of the isolated cap contents are given in Table 4. It is not yet known whether the small difference in base ratios between caps and cap particles represents a loss of specific materials during isolation. The question of loss during preparation is again raised by the marked difference in the RNA-protein ratio of cap particles (63:37) and whole nuclear caps (40:60). How much of this is due to the expected elimination of a membranous fraction vs. actual loss of cap contents is at present a moot point. To check this, one must examine the residual and soluble fractions normally discarded during isolation of the cap contents.

The cap particles were also examined for their stability and for the presence of the latent ribonuclease normally associated with ribosomes (Elson, 1959*b*). The results obtained with material prepared in 0.005 M and 0.01 M magnesium are illustrated in Fig. 6 and 7, respectively. The preparations isolated and assayed in low magnesium concentrations were very unstable at 36 C, with or without the addition of urea. Under the conditions used for Fig. 6, 4 M urea caused a rapid initial release of nucleotides (by the particle ribonuclease) which quickly leveled off. Turbidity was observed within 5 min of the start of the incubation, and the proteins had precipitated out of solution at the end of 30 min. Without urea, the RNA was degraded at an essentially exponential rate from the start of the incubation. In this case, little or no precipitation occurred but the material was obviously unstable in the virtual absence of magnesium ions. The control curve of Fig. 7 demonstrates that material prepared and assayed in 0.01 M magnesium was quite stable for 30 min at 30 C. Nevertheless, urea (2 M) again led to an initially rapid release of nucleotides followed by an equally fast inactivation. In fact, concentrations of urea effective in releasing the activity of the particle ribonuclease consistently led to the rapid denaturation of the proteins and consequent secession of the reaction.

Added crystalline pancreatic ribonuclease (10 μg/ml; Sigma Chemical Co., St. Louis, Mo.) also caused a rapid degradation of the otherwise stable particle RNA (Fig. 7). This activity resulted in the formation of turbidity and eventual precipitation of the proteins in a manner similar to that obtained with urea. Despite their sensitivity to external ribonuclease, the nuclear cap contents isolated in 0.01 M magnesium underwent only slight autodegradation during 48 hr of storage at 0 to 2 C.

TABLE 1. *Chemical composition of isolated nuclear caps*

Constituent	Dry weight	Per cent of total dry weight
	μg/mg	
Experiment 1		
RNA nucleotides	402.6	38.1*
Phosphorus (total)	40.2	4.0
RNA phosphorus (calculated)	36.2	3.6
Nitrogen (total)	156.6	15.7
Protein amino acids	696.3	60.1†
DNA		<1.4
RNA + protein		98.2
Experiment 2		
RNA (orcinol)	428.9	42.9
Protein (biuret)	599.4	59.9
RNA + protein		102.8

* As RNA.
† As protein.

TABLE 2. *Nucleotide composition of nuclear cap ribonucleic acid**

Nucleotide	Moles per cent	Molar ratio
Cytidylic acid	19.4	1.00
Adenylic acid	27.3	1.41
Guanylic acid	28.1	1.45
Uridylic acid	25.2	1.30

* Ratio of purines–pyrimidines = 1.24. Ratio of adenylic acid + uridylic acid–guanylic acid + cytidylic acid = 1.11.

DISCUSSION

The material isolated from purified cap preparations is basically similar in its characteristics to the RNP particles, or ribosomes, of other organisms. The sedimentation coefficient of 83S is higher than the 74S of pea seedlings (T'so, Bonner, and Vinograd, 1956), or the 70S of

TABLE 3. *Amino acid composition of nuclear cap protein*

Amino acid	Amount (g/100 g of protein)	Per cent
Alanine	10.6	8.8
Arginine	12.5	10.4
Aspartic acid	8.9	8.0
Cystine	0.5	0.4
Glutamic acid	16.8	13.9
Glycine	6.8	5.7
Histidine	2.9	2.4
Isoleucine	4.6	3.8
Leucine	9.7	8.0
Lysine	13.7	11.4
Methionine	1.4	1.1
Phenylalanine	4.4	3.7
Proline	4.7	3.9
Serine	4.4	3.6
Threonine	5.5	4.6
Tyrosine	3.0	2.5
Valine	7.0	5.8
Total	121.6	

Tetrahymena (Plesner, 1961) and *Escherichia coli* (Tissieres and Watson, 1959). It is very similar to the 83S material of rat liver (Hamilton and Petermann, 1959) and the 80S of yeast (Chao and Schachman, 1956; Morgan, 1962). Nuclear cap particles respond to varied magnesium concentration in a manner similar to that described for ribosomes of other cells. High magnesium concentrations lead to partial aggregation, presumably of the principal 83S component, to form heavier 125S aggregates. This corresponds to the association of *E. coli* 70S particles to form 100S dimers (Tissieres et al., 1959), and the 83S of rat liver to form 110S and 140S aggregates (Hamilton and Petermann, 1959). Low magnesium results in dissociation of the 83S material to form two new smaller units of 63S and 41S (ratio ca. 2:1), nearly identical to the 60S and 40S similarly obtained from the 80S particles of yeast (Chao, 1957; Morgan, 1962).

The 63% RNA of cap particles is higher than the 45% of yeast (Chao and Schachman, 1956) and the 35% of pea seedling (T'so et al., 1956), but the same as the 60 to 65% reported for *E. coli* (Tissieres et al., 1959; Elson, 1959). The nucleotide composition of the cap particle RNA also resembles that of *E. coli* (Spahr and Tissieres, 1959) and a variety of other bacteria (Miura, 1962). It differs considerably from the low adenylic and high cytidylic acid content found in calf thymus RNP (Wang, 1962).

The susceptibility of otherwise-stable RNP particles to degradation by external pancreatic ribonuclease has been shown for material from pea seedlings (T'so et al., 1956) and *E. coli* (Elson, 1959*a*). Ribonuclease action in both cases led to turbidity and, with *E. coli* ribosomes, to precipitation of the proteins. The nuclear cap RNP behaves in an identical fashion and reacts similarly to the presence of urea, again mimicking the behavior of *E. coli* ribosomes (Elson, 1959*a*). The cap particles contain a characteristic "latent" ribonuclease (Elson, 1959*b*), and the release of this activity by urea further indicates their ribosomal nature. The cause of the subsequent inactivation in urea is uncertain, but urea-induced denaturation of pancreatic ribonuclease is known to occur (Nelson and Hummel, 1962). Vigorous and repeated pipetting or a single freezing-and-thawing cycle also cause a significant denaturation of the cap material. Whether this instability is due to the suspending medium or to an inherent susceptibility of the material itself remains to be established.

An additional characteristic in which the zoospore RNP resembles typical ribosomes is the amino acid composition of its protein. Although whole caps, rather than their contents, were analyzed, the amino acid content is remarkably similar to that of ribosomes from a variety of other plants and animals (T'so, Bonner, and Dintzis, 1958; Wang, 1962). This is particularly true with regard to their low sulfur amino acid content and high basic amino acid content. Since the caps are essentially filled with the RNP particles (see Fig. 2), the amino acid composition of a whole cap is probably reasonably close to that of its particulate contents. The observed similarity to ribosomal proteins of other cell types supports this assumption.

Finally, the magnitude of the nuclear cap contribution to the total RNA of the spore, i.e., 69%, and the lack of such particulates in the cytoplasm clearly imply the equivalence of its contents to the ribosomal component of more conventional cells. The fraction of the total cellular RNA represented by the particulate RNP has been estimated as 60 to 80% for pea seedling (T'so et al., 1956), 78 to 90% for yeast (Kihara, Hu, and Halvorson, 1961) and nearly 90% in *E. coli* (Tissieres and Watson, 1958).

In conclusion, little doubt remains that the nuclear caps of *Blastocladiella* zoospores are aggregates of particles which resemble typical ribosomes in their chemical and physical properties. Because of the similar fine structure and staining characteristics of the caps in *Blastocladiella* zoospores and *Allomyces* gametes, it is more than probable that the caps of the latter have a similar structure and composition. These results therefore confirm, and significantly extend, those of Turian (1955, 1956*a*, *b*) and Turian and Kellenberger (1956) obtained by cytological and electron microscopic techniques.

In addition to the zoospores and gametes of the *Blastocladiales*, nuclear caps have been described in zoospores of the two remaining orders of the posteriorly uniflagellate phycomycetes, the *Monoblepharidales* (Sparrow, 1960), and *Chytridiales* (Koch, 1961). These observations have been limited to nonspecific staining and light microscopy. Nevertheless the near-universal presence of the nuclear cap in the motile cells of a large assemblage of organisms, cells with such diverse functions as sexual and asexual reproduction, clearly implies a common role of some kind for this intriguing and unique organelle.

Turian (1956*a*, 1962) has suggested that during germination the dissolution of the cap is involved in the activation of the RNA- and protein-synthesizing systems of *Allomyces* zygotes by removing the cytoplasmic barrier between the cap RNP and the mitochondria. Current concepts concerning the function of ribosomes in protein synthesis certainly make it tempting to assign the nuclear cap a morphogenetic role in the control of this biosynthetic process. The cap exists only during the motile phase when no detectable growth occurs, and it breaks down at a very very early stage during spore germination when growth, and presumably protein synthesis, begin. In this system, the nuclear cap could serve as a storage depot of the RNA and protein subunits utilized for synthesis during the early stages of growth. This would necessitate extensive degradation and resynthesis if all new ribosomes were to be formed. Turian and Cantino (1959) have shown that the concentration of DNA per cell doubles prior to the first nuclear division, whereas very little, if any, change occurs in the total RNA. However, turnover of RNA into new ribosomes would not have been detected by the methods used. An alternative, and attrac-

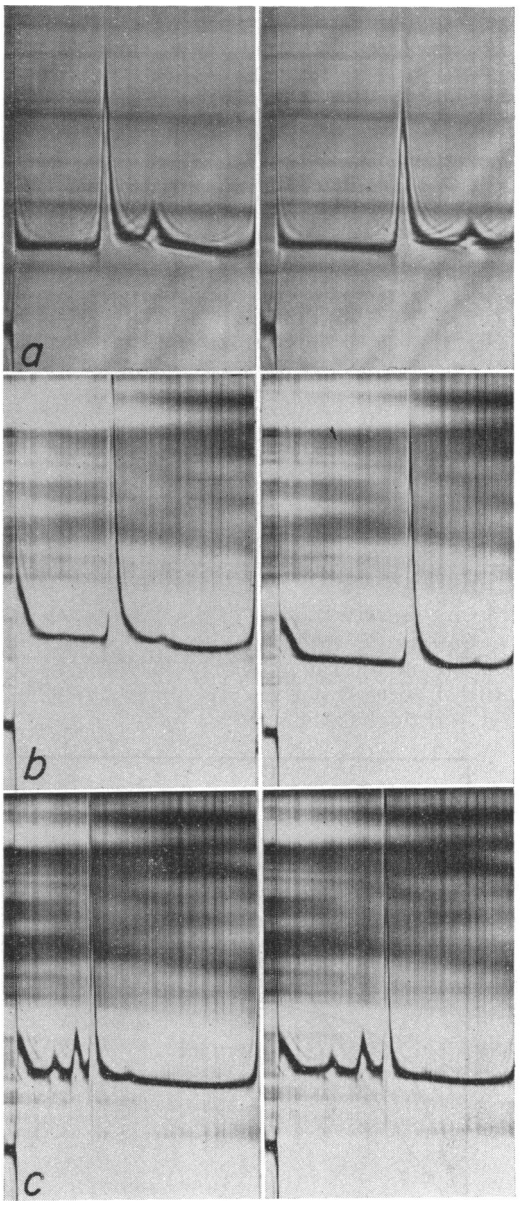

FIG. 5. *Schleiren photographs of nuclear cap ribonucleoprotein particles. Sedimentation was from left to right. The first pictures were taken 3 to 4 min after reaching 50,740 rev/min with 2-min intervals between exposures. (a) Particles isolated and washed in TMK buffer containing 0.01 M* $MgCl_2$ *. Bar angle, 42 degrees. (b) Particles extracted in TMK buffer with 0.005 M* $MgCl_2$ *. Bar angle, 45 degrees. (c) Material used for (b) after 6 hr of dialysis vs. TMK containing 0.0001 M* $MgCl_2$ *. Bar angle, 46 degrees.*

tive, hypothesis would be that the nuclear cap is a "package" of functionally complete, but spatially isolated, and therefore inactive, ribosomes. Such ribosomes, if preprogrammed for the specific proteins to be formed, could initiate rapid synthesis immediately after their release from the cap and association with the enzymes, cofactors, transfer RNA, etc., necessary for the functional biosynthetic system. At present there is insufficient evidence to warrant a choice between these or other plausible alternatives. The method of cap formation during zoospore differentiation, its activity, if any, in the spore, and the function of its contents during the early stages of germination are being examined in an attempt to understand the developmental role of this unusual intracellular structure.

TABLE 4. *Chemical composition of isolated nuclear cap particles**

Nucleotide†	Moles per cent	Molar ratio
Cytidylic acid	18.5	1.00
Adenylic acid	26.2	1.42
Guanylic acid	31.8	1.72
Uridylic acid	23.5	1.27

* RNA (orcinol) = 62.7% of total dry weight. Protein (biuret) = 37.3% of total dry weight.

† Data in the table show the RNA nucleotide composition. Ratio of purines–pyrimidines = 1.38. Ratio of adenylic acid + uridylic acid–guanylic acid + cytidylic acid = 0.99.

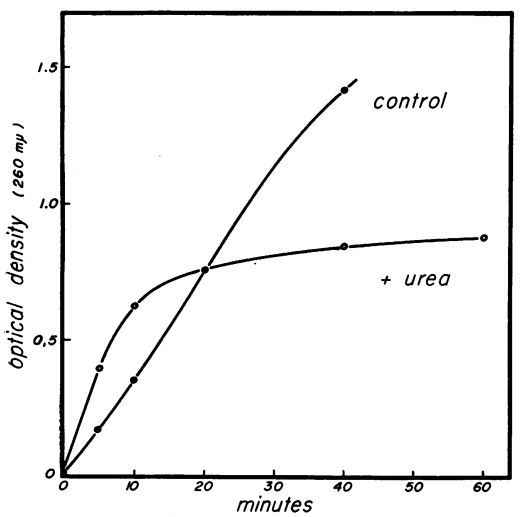

FIG. 6. *Stability of nuclear cap ribonucleoprotein particles incubated in low concentrations of magnesium. The cap particles were isolated in 0.01 M tris-HCl (pH 7.4), 0.005 M $MgCl_2$, and 0.01 M KCl. ●, control assay containing 0.05 M tris-HCl (pH 7.4), 0.0016 M $MgCl_2$, and 1.22 mg of ribonucleoprotein particles per ml; ○, control mixture plus 4 M urea and 0.01 M NaCl; 36 C. Release of acid-soluble RNA-nucleotides measured by ribonuclease assay procedure.*

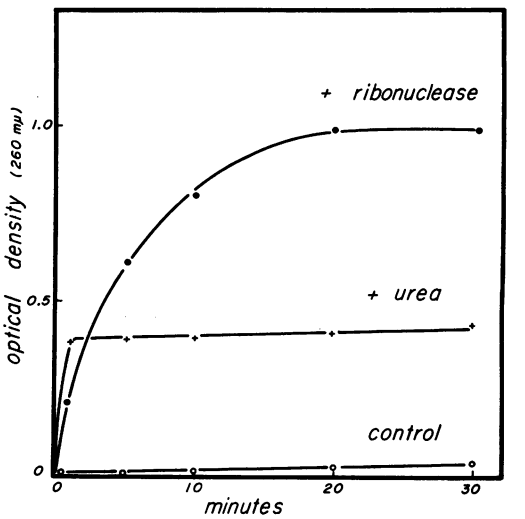

FIG. 7. *Stability of nuclear cap ribonucleoprotein particles incubated in high concentrations of magnesium. The cap particles were isolated in 0.01 M tris-HCl (pH 7.4), 0.01 M $MgCl_2$, and 0.01 M KCl. ○, control assay containing 0.01 M tris-HCl (pH 7.4), 0.01 M $MgCl_2$, 0.01 M KCl, and 0.87 mg of ribonucleoprotein particles per ml; +, control mixture plus 2 M urea; ●, control mixture plus 10 μg/ml of pancreatic ribonuclease; 30 C. See Fig. 6 for procedure used.*

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