

## Differential Protein Synthesis During Sporulation in the Slime Mold *Physarum polycephalum*

BRIGITTE M. JOCKUSCH,<sup>1</sup> HELMUT W. SAUER,<sup>2</sup> DONNA F. BROWN,  
KARLEE L. BABCOCK, AND HAROLD P. RUSCH

McArdle Laboratory for Cancer Research, Medical Center, University of Wisconsin, Madison, Wisconsin 53706

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The size distribution and synthesis of polypeptide chains and the polysome patterns were studied during sporulation of the slime mold *Physarum polycephalum*, and were compared with nonsporulating controls. The proteins were divided into a 27,000 × *g* supernatant (buffer-soluble proteins) and a pellet (buffer-insoluble proteins) while still native. The sodium dodecyl sulfate complexes of the denatured proteins were separated on polyacrylamide gels containing urea. The following differences were found between sporulating and nonsporulating cultures. (i) The distribution of the soluble proteins into bands from sporulating and control cultures was the same in stained patterns; however, there was a slight shift toward increased synthesis of larger polypeptide chains in the radioactivity patterns of the soluble proteins in sporulating cultures. (ii) The amount of histones in the sporulating cultures was less than 30% of the values in the controls. Also, histone synthesis was reduced to less than 10% of that in the nonsporulating controls. In addition, proteins in three defined regions, corresponding to molecular weights of 70,000 to 75,000 (I), 55,000 (II), and 41,000 (III), were synthesized in sporulating cultures at a rate at least twice that in controls. Polypeptides corresponding to peaks I and II could be extracted from purified walls of mature spores. (iii) The polysome pattern as revealed by sucrose density centrifugation showed a breakdown of heavy polysomes at 3 hr after illumination, with their reappearance 4 hr later. The latter pattern, however, differed from that of the nonsporulating control in that the amount of light polysomes was reduced. This might account for the reduction in histone synthesis.

The plasmodium of *Physarum polycephalum* exists as a syncytium during growth and differentiates to a cellular state only toward the end of sporulation, when cell walls appear and separate the nuclei into spores. The plasmodium, kept in the dark, may be induced to sporulate by starving it on a non-nutrient salts medium (sporulation medium) for at least 4 days and then exposing it to light for 4 hr (2). Three hours after the illumination period, the changes leading to spore formation become unalterably fixed, and the mold will sporulate even if it is returned to the growth medium. The first morphological change indicating the onset of sporulation is the appearance of beading of the plasmodial strands, noted about 8 hr after the exposure to light.

Since proteins are known to play an important role during morphogenesis in many different organisms, we were interested in determining whether changes in protein can be detected prior to and concurrently with the earliest morphological alterations in *Physarum*. On the basis of work done previously, one might expect that three classes of proteins would be involved in the changes accompanying sporulation: enzymes, as shown in sporulating cellular slime molds (15, 20, 21); structural proteins, as observed in the formation of bacterial spores (8, 18, 19); and nuclear proteins, since deoxyribonucleic acid (DNA) synthesis and mitosis at the end of the starvation period are an essential prerequisite before sporulation can be induced by illumination (16). The aim of the present paper was to determine whether changes in the patterns of these three types of proteins could be detected at various times during sporulation.

<sup>1</sup> Present address: Max-Planck-Institut für Biologie, Abteilung Melchers, 74 Tübingen, West Germany.

<sup>2</sup> Present address: Zoologisches Institut der Universität Heidelberg, 69 Heidelberg, West Germany.

## MATERIALS AND METHODS

**Culture of the organism.** *P. polycephalum* was grown in axenic culture in shake flasks (12). Surface plasmodia were formed by coalescence of microplasmodia from 3-day-old shake-flask cultures on petri dishes (11), and sporulation was induced as described previously (2).

**Labeling procedures.** To compare the protein extracts from nonsporulating controls and sporulating cultures, the following experimental procedure was used. After 4 days of starvation, some cultures were illuminated for 4 hr; the control cultures were kept in the dark. Two hours after the illumination period, two cultures (one illuminated and one nonilluminated) were put on petri dishes with fresh sporulation medium (2), each containing 100  $\mu$ c of 4,5-<sup>3</sup>H-L-leucine (Schwarz BioResearch, Inc., 1.0 mc/ml, 2 c/mmole) in 1.8 ml of sporulation medium. Five hours later the cultures were cut in half, and one-half of each culture was harvested. The other halves were left on the plates for 2 hr longer. Thus samples of starved and illuminated cultures were obtained 7 and 9 hr after the end of illumination, and they had been labeled for 5 and 7 hr, respectively. In this and all the following experiments samples were harvested by being rinsed in ice-cold tap water and frozen in liquid nitrogen.

To study amino acid incorporation, six cultures were put on petri dishes containing fresh sporulation medium with 4,5-<sup>3</sup>H-L-leucine (6 c/mmole, Schwarz BioResearch Inc.), at a concentration of 1  $\mu$ c per ml of medium, after a starvation period of 4 days in the dark. Four hours later, three of them were illuminated for 4 hr, and samples were taken at various times before and after the illumination period. Each sample consisted of two halves from two different cultures. For pulse-label experiments, sporulating and control cultures were labeled for 2 hr with 1  $\mu$ c of <sup>3</sup>H-leucine. Two halves of two different cultures were harvested at various times.

**Protein extraction.** Amino acid incorporation into acid-insoluble material was determined, the extraction procedure being modified after Daniel and Baldwin (3). Frozen samples were thawed in 5% trichloroacetic acid in 50% acetone and extracted twice. The pellets were then extracted three times with cold 0.25 M perchloric acid and twice with hot 0.5 M perchloric acid (30 min at 70 C). The pellets were solubilized in 0.4 N NaOH, and the radioactivity was determined in a Packard scintillation counter.

Protein extracts from all samples were prepared in the following way. The frozen samples were thawed in 1.0 ml of 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 7.5, plus 0.5% Cleland's Reagent and disrupted by sonic treatment for 1 min (Branson Sonifier, model LS 75, setting 6). The extract was kept on ice for 30 min and then separated into a supernatant and a buffer-insoluble pellet by centrifugation at 27,000  $\times$  g for 15 min (Sorvall Superspeed RC 2-B). The supernatant was made up to 67% acetic acid plus 0.5% 2-aminoethanethiol (2-AET) to precipitate polysaccharides (6). The extract was kept on ice for 30 min and centrifuged again (27,000  $\times$  g, 15 min, Sorvall). This residual

pellet was discarded; the supernatant contained the buffer-soluble, acetic acid-soluble proteins. The buffer-insoluble pellet was reextracted by sonic treatment (1 min, setting 6) in 0.7 ml of 67% acetic acid plus 0.5% 2-AET. The extract was kept on ice for 30 min, and the acid-insoluble residue was separated (27,000  $\times$  g, 15 min, Sorvall) and discarded. The supernatant, containing the buffer-insoluble, acetic acid-soluble proteins was dialyzed, as were the buffer-soluble proteins, against distilled water plus 0.5% 2-AET for 2 hr, and against 0.1 M sodium acetate plus 0.5% 2-AET overnight. The extraction of the insoluble proteins with acetic acid yielded more than 90% of the proteins in solution, with less than 10% of the polysaccharides which would interfere with the subsequent gel electrophoresis (6).

To solubilize the protein components of the spore walls, 1 mg of clean, dry spore walls was extracted by sonic treatment for 30 min in 0.7 ml of 67% acetic acid plus 0.5% 2-AET; the extract was then treated as described for the buffer-insoluble extract above. Isolated histones, extracted from isolated nuclei with CaCl<sub>2</sub> (13), were dialyzed overnight against the same electrophoresis buffer.

All protein samples were precipitated with acetone (final concentration, 75%) plus 2-AET, washed once with acetone, and redissolved in the electrophoresis buffer containing 0.1 M Tris-hydrochloride (pH 7.5), 8 M urea, 1% (w/v) SDS (sodium dodecyl sulfate), and 0.5% Cleland's Reagent at 60 C.

Protein contents of the buffer-soluble and -insoluble proteins thus obtained were determined by a modified Folin method (9), with bovine serum albumin used as a standard. Carbohydrate contents were determined with glucose used as a standard (4). Radioactivities were determined in a Packard scintillation counter by mixing a portion of each sample with 10 ml of scintillation fluid ("ANPO," containing 2,5-diphenyloxazole,  $\alpha$ -naphthylphenyloxazole, naphthalene, dioxane, xylene, and ethanol). The radioactivity measurements of the acetone supernatants were not higher than that of the background.

**Gel electrophoresis.** Electrophoresis was carried out on 7.5% polyacrylamide gels, modified after Shapiro et al. (17), with a Canalco Electrophoresis Apparatus model 1400. Gels (100 by 6 mm) and buffer system used were as described previously (6). Duplicate gels were run for each sample. Approximately 250  $\mu$ g of protein was layered on each gel to be utilized for staining. In every experiment, 50  $\mu$ g of lysozyme, also treated with SDS and urea, was run on an extra gel as a standard protein. All samples contained bromophenol blue as marker dye, and electrophoresis was carried out until the dye had reached the bottom of the tube (50 v, 6.5 mamp/gel, 4 hr). One of the duplicates was fixed and stained with Amido Black 10B; densitometer tracings were obtained with a Canalco microdensitometer, model E, connected to a Sargent SRL recorder (6). The duplicates utilized for counting were run with various amounts of protein, but with equal amounts of radioactivity. They were fractionated in a gel crusher and eluted in counting vials as described previously (6). Radioactivities were determined with the same scin-

tillation fluid and counter mentioned above. Counting efficiency was approximately 15%.

Isolated histones and spore wall extracts were subjected to the same gel electrophoresis and compared with the buffer-soluble and -insoluble patterns. For molecular weight determination (17), the following standard proteins were used: Transferrin (molecular weight 72,000), ovalbumin (molecular weight 45,000), Bromegrass Mosaic Virus (subunit: molecular weight 20,000), and lysozyme (molecular weight 14,400).

**Polysome preparation.** Polysomes were prepared by a modification of the method of Mittermayer et al. (10). Frozen plasmodia were homogenized in a medium containing 0.2 M sucrose, 0.05 M KCl, 0.0015 M MgCl<sub>2</sub>, and 0.1 M Tris-hydrochloride (pH 7.3) in a Potter-Elvehjem homogenizer, and centrifuged at 30,000 × g for 7 min at -2 C. The supernatant was adjusted to 0.5% sodium deoxycholate, layered over sucrose gradients (10 to 50% sucrose in 0.05 M Tris-hydrochloride, 0.005 M MgCl<sub>2</sub>, 0.025 M KCl, pH 7.3), and centrifuged at 25,000 rev/min for 2.5 hr at -12 C (Spinco L 2, SW25 rotor). The tubes were punctured at the bottom, and the ultraviolet-absorbing material was monitored in a flow cell by a spectrophotometer (Beckman DB) and recorded as optical density at 260 nm (Sargent recorder, model SRL). The flow rate was controlled by a peristaltic pump (approximately 1 ml/min).

## RESULTS

In continuous as well as in pulse-label experiments, the illuminated cultures incorporated much less radioactive leucine than did the non-illuminated ones, even during the period of exposure to light (Fig. 1). This difference increased with time at least up to 9 hr after the illumination period and resulted in lower specific activities for proteins extracted from illuminated as compared with control cultures (Table 1). This might reflect differences in turnover, uptake, or pool size (or combinations of these factors) of leucine between the control and the sporulating cultures. A comparison of the amounts of the buffer-soluble and the buffer-insoluble proteins revealed that both control and sporulating cultures contained about 1.5-fold as much buffer-insoluble as buffer-soluble protein (Table 1).

Separation of proteins by gel electrophoresis is shown in Fig. 2, 3, and 4. Seven hours after illumination, the synthesis pattern of proteins from the illuminated cultures was already distinct from that of the nonilluminated controls. Whereas the differences in the buffer-soluble proteins were only minor after 7 hr (Fig. 2), two major differences were observed in the patterns derived from insoluble proteins. First, there was distinctly increased incorporation in several of the more slowly moving bands (Fig. 3; I, II, III). Second, less than 10% as much incorporation

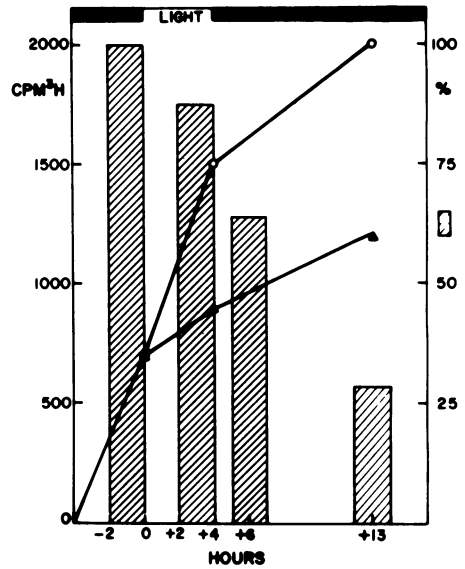


FIG. 1. Leucine incorporation. After a starvation period of 4 days and 4 hr, some cultures were illuminated for 4 hr (top of the graph) while the controls were kept in the dark. Line: continuous labeling (from 4 hr before to 7 hr after the illumination period) of nonilluminated (○) and illuminated (▲) cultures with 4,5-<sup>3</sup>H-leucine (1 μc/ml). Bars: plasmodia labeled for 2-hr periods with 4,5-<sup>3</sup>H-leucine (1 μc/ml) and illuminated as indicated on top of the graph. Incorporation at the end of each period is expressed as the percentage of the value for the culture prior to illumination, which is given as 100%. In both experiments, incorporation was measured as specific activity in hot perchloric acid-insoluble material.

was found in the histone fraction of illuminated cultures as compared with that of the controls. Under our conditions, the histones were extracted by acetic acid from the 27,000 × g pellet of homogenized plasmodia and identified by comparison with the electrophoretic mobility of isolated histones. Nine hours after illumination, the differences in protein synthesis were essentially the same as after 7 hr, although more pronounced [Fig. 2 (c, d) and Fig. 3 (c, d)]. In the buffer-soluble proteins (Fig. 2c), there was a general increase in incorporation of those polypeptides that migrated to fractions 10 to 35 of the gel, although the radioactivity could not be localized in specific bands.

The densitometer tracings obtained with gels identical to those used for radioactivity measurements were similar for sporulating and for control cultures. The patterns of soluble proteins (not shown) consisted mainly of a broad, densely stained region, with no bands discernible. All material which could be stained with Amido Black

TABLE 1. Amounts and specific activities of protein fractions of sporulating and control cultures<sup>a</sup>

| Hr <sup>b</sup> | Protein fraction | Controls                           |                    |                                   | Sporulating cultures               |                    |                                   |
|-----------------|------------------|------------------------------------|--------------------|-----------------------------------|------------------------------------|--------------------|-----------------------------------|
|                 |                  | Protein <sup>c</sup><br>( $\mu$ g) | Ratio <sup>d</sup> | Specific<br>activity <sup>d</sup> | Protein <sup>c</sup><br>( $\mu$ g) | Ratio <sup>d</sup> | Specific<br>activity <sup>d</sup> |
| 7               | Buffer-soluble   | 1,680 $\pm$ 103                    | 1.69 $\pm$ 0.18    | 1,740 $\pm$ 289                   | 1,020 $\pm$ 138                    | 2.06 $\pm$ 0.28    | 1,215 $\pm$ 231                   |
| 7               | Buffer-insoluble | 2,800 $\pm$ 184                    |                    | 5,438 $\pm$ 413                   | 2,100 $\pm$ 177                    |                    | 3,601 $\pm$ 411                   |
| 9               | Buffer-soluble   | 1,050 $\pm$ 238                    | 1.42 $\pm$ 0.11    | 2,619 $\pm$ 247                   | 1,480 $\pm$ 162                    | 1.62 $\pm$ 0.14    | 1,846 $\pm$ 299                   |
| 9               | Buffer-insoluble | 1,500 $\pm$ 411                    |                    | 8,017 $\pm$ 754                   | 2,400 $\pm$ 193                    |                    | 4,300 $\pm$ 762                   |

<sup>a</sup> Two halves of two different cultures were assayed in one experiment. All values are averages of four different experiments, and are given with standard deviations. The standard deviations of the protein contents reflect mainly the variability in size of the plasmodia.

<sup>b</sup> Hours after the end of the illumination period of the sporulating cultures. The nonsporulating controls were kept continuously in the dark and harvested at the same time.

<sup>c</sup> Protein contents were determined from two halves of plasmodia and averaged over four different experiments.

<sup>d</sup> The ratios of insoluble/soluble proteins and the specific activities were determined for each experiment and then averaged. The specific activities are expressed as counts per minute per microgram.

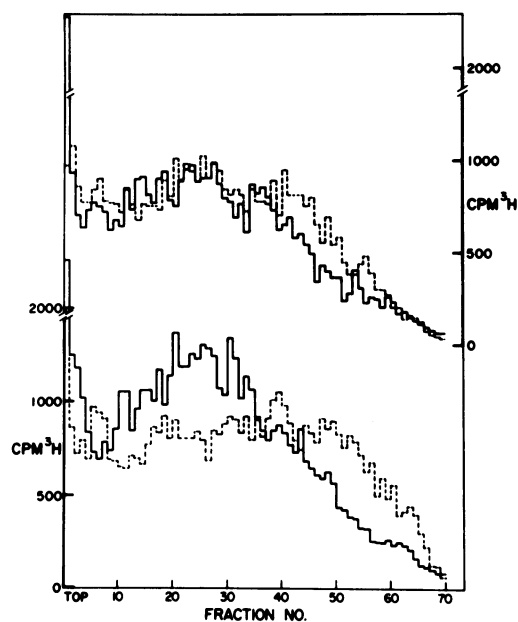


FIG. 2. Radioactivity patterns of buffer-soluble proteins in SDS-urea-polyacrylamide gels. Upper half: — (a), cultures 7 hr after illumination, --- (b), nonilluminated controls at the same time. Lower half: — (c), cultures 9 hr after illumination, --- (d), controls at the same time. Proteins were extracted with Tris-buffer, precipitated with acetone, and dissolved in the SDS-urea-containing electrophoresis buffer before being subjected to electrophoresis. The patterns were obtained by fractionating the gels after the run, eluting, and measuring the radioactivity for each fraction.

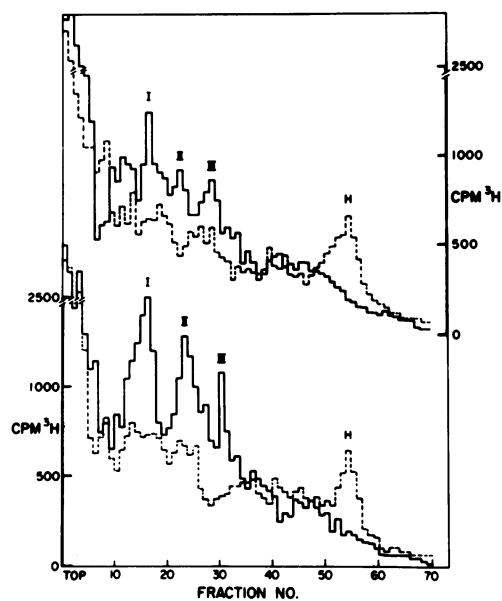


FIG. 3. Radioactivity patterns of buffer-insoluble proteins extracted with acetic acid. Further treatment of the proteins and gel electrophoresis were as described in Fig. 2. Upper half: — (a), cultures 7 hr after illumination, --- (b), nonilluminated controls at the same time. Lower half: — (c), cultures 9 hr after illumination, --- (d), controls at the same time. H: histone-containing region, identified by comparison with isolated histones in the same gel system. I, II, III: Peaks with approximately twofold increase in synthesis.

migrated into the gel. The patterns of buffer-insoluble proteins showed a number of distinct bands (Fig. 4); much of the material stayed on

top of the gel. The tracings of control and illuminated cultures, 7 and 9 hr after illumination, revealed no differences in the upper half of the gels. By comparing duplicate gels with each

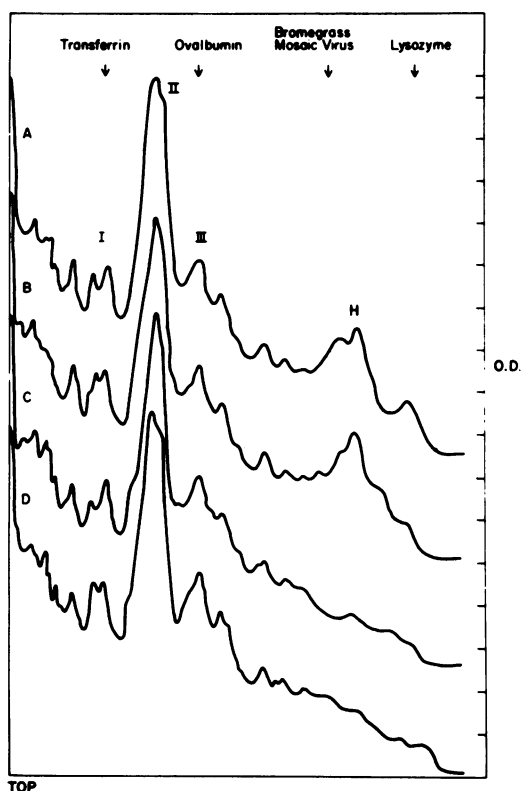


FIG. 4. Densitometer tracings of buffer-insoluble proteins, run as duplicates to the gels in Fig. 3 and stained with Amido Black 10B. Patterns derived from nonilluminated controls harvested 7 hr (A) and 9 hr (B) after the illuminated plasmodia were exposed to light, and from illuminated cultures 7 hr (C) and 9 hr (D) after illumination. H: histone-containing region. I, II, III: Bands which contained the peaks showing increased synthesis in Fig. 3, identified by comparison of  $R_F$  values. The arrows at the top indicate the positions of the standard proteins with known molecular weights run on extra gel in the same experiment: transferrin, molecular weight 72,000; ovalbumin, molecular weight 45,000; Bromegrass Mosaic Virus, subunit, molecular weight 20,000; and lysozyme, molecular weight 14,400.

other and with the migration of lysozyme used as a marker protein, the peaks I, II, and III in the radioactive patterns could be identified in the densitometer tracings. The radioactive peak I (Fig. 3) was included in the double peak marked I in the densitometer tracings (Fig. 4). Peak II appeared as the most prominent peak in the stained patterns (Fig. 4, II); peak III was also present (Fig. 4, III). The molecular weights of all three peaks were determined by comparing their  $R_F$  values with those of standards run in SDS-urea (17). The migration distances of the standard proteins used are indicated in Fig. 4.

The approximate molecular weights are: peak I, 70,000 to 75,000; peak II, 55,000; peak III, 41,000.

To determine whether protein bands with increased synthesis during morphogenesis were part of the structure of the spore wall, we extracted total proteins from isolated spore walls with acetic acid. The results are given in Fig. 5. Three bands were produced in an SDS-urea gel, two of which had exactly the same migration distances and therefore the same molecular weights as peaks I and II in the buffer-insoluble proteins. The third band with increased synthesis in the plasmodia during sporulation did not appear in the spore wall proteins; instead, a more slowly moving band was present, i.e., a larger polypeptide which might coincide with another band in the buffer-insoluble patterns of the plasmodia (Fig. 5, IV). Since the amount of spore wall material available was very limited (1 mg), we could not perform any other experiment at this point.

A comparison of the histone-containing region of the stained gels showed that the illuminated cultures not only synthesized much less histone but also contained less (Fig. 4, H). To prove that this result was reliable, we investigated two major possibilities for artifacts. (i) The histone region in the gel patterns could be masked by ribosomal polypeptides which have  $R_F$  values similar to those of the main histone bands when bound to SDS (Jockusch, unpublished data). However, ribosomes were separated from the buffer-insoluble pellet in the first centrifugation step. Thus, ribosomal proteins would be included in the patterns of buffer-soluble and not in the patterns of buffer-insoluble proteins. (ii) The observed differences in the amount of histones between sporulating and control cultures could result from differences in the extractability of histones before and after illumination. This was excluded by using a nonselective method (67% acetic acid) for extraction and by determining the amounts of proteins left in the acetic acid-insoluble pellets; the same amounts (5 to 10%) of nonextractable proteins were found in sporulating and control cultures.

Since small polysomes have been reported to synthesize histones, we looked at the patterns of polysomes derived from cultures in different stages of sporulation, in order to correlate polysome patterns with cessation of histone synthesis after illumination (Fig. 6). A typical profile from a culture after 4 days of starvation is shown in Fig. 6A. The first two peaks, probably comprised of the ribosomal subunits and the monosomes, constitute a large proportion of the profile, but four more peaks are distinguishable in addition

to a broader peak of heavier material. During illumination the pattern was very similar to Fig. 6A. However, 3 hr after the end of illumination most of the ultraviolet-absorbing material was located in the first two peaks on top of the gradient (Fig. 6B); these results indicate a pronounced breakdown of the heavy polysomes at that time. Seven hours after illumination we observed an increase of the heavy polysome material and a decrease in the lighter fractions (Fig. 6C, arrow), compared with the patterns of the earlier stages.

### DISCUSSION

The induction of sporulation in the plasmodia of *P. polycephalum* depends on starvation and illumination (2). Since *Physarum* cannot sporulate without exposure to light, the effects of starvation alone can be separated from the changes associated with the differentiation of sporangia. In the present study, we therefore compared proteins of starved, illuminated cultures with those of starved, nonilluminated ones. The total proteins were divided into a buffer-soluble and a buffer-insoluble fraction, both of which were soluble in acetic acid. From the separation procedure used in this study, it can be concluded that enzymes and proteins derived from particles of ribosomal and subribosomal size are included in the buffer-soluble fraction, and membrane proteins are in the buffer-insoluble fraction. Starvation alone results in a number of biochemical changes, e.g., proteins decrease in amount (2). Whereas in growing plasmodia there is approximately twice as much buffer-soluble as buffer-

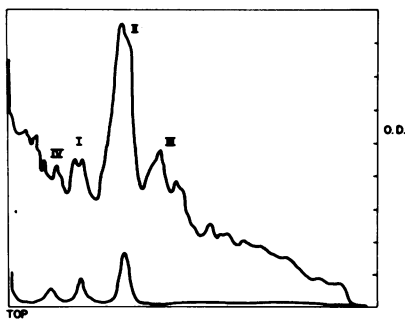


FIG. 5. Comparison between buffer-insoluble, acetic acid-soluble proteins extracted 9 hr after illumination from sporulating cultures (upper curve), and an acetic acid extract of proteins of isolated spore walls (lower curve). The extracts were subjected to the same type of gel electrophoresis as in Fig. 2 to 4. Proteins were stained with Amido Black 10B, and tracings were taken with a densitometer. I, II, III: See legend of Fig. 4; IV: see text.

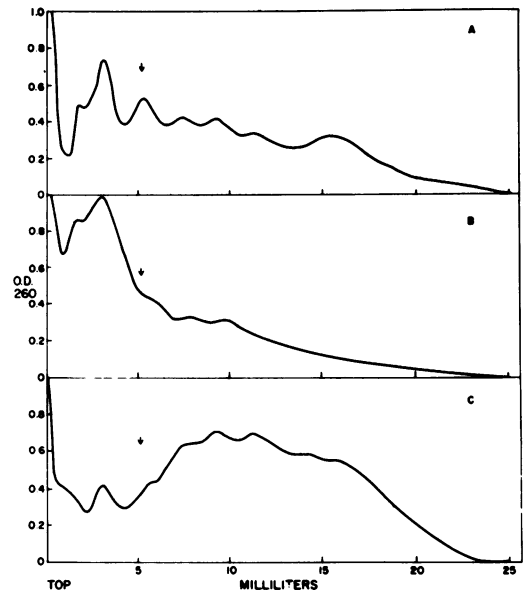


FIG. 6. Typical polysome patterns derived from starving and sporulating cultures. (A) Culture after 4 days of starvation; (B) 3 hr after illumination, at commitment to sporulation; (C) sporulating culture, 7 hr after illumination. The patterns show sucrose gradient profiles recorded as optical density at 260 nm. The arrows indicate the position of the light polysomes.

insoluble protein (Jockusch, unpublished data), there is considerably more buffer-insoluble than -soluble protein present after more than 4 days of starvation (Table 1). This shift is probably caused, at least in part, by a more rapid synthesis of new insoluble proteins at this time, as indicated by the higher specific activity of the insoluble proteins.

The densitometer tracings of the slower-moving polypeptide bands of control and illuminated cultures indicate that there is no major difference in the relative amounts of the different size classes of proteins between nonsporulating and sporulating cultures. By comparing the radioactivity patterns of proteins of sporulating and control cultures in polyacrylamide gels, we observed an increased synthesis in certain size classes of both the soluble and insoluble proteins in sporulating cultures (Fig. 2, 3), although the synthesis of total proteins in sporulating cultures was decreased (Table 1, Fig. 1). In the buffer-insoluble proteins, this increase was very pronounced as soon as 7 hr after illumination and was confined to three peaks in the more slowly moving bands. By comparison of radioactivity patterns with stained band patterns, we found that these peaks of increased synthesis corresponded to polypeptide chains with molecular weights of 70,000 to 75,000, 55,000, and 41,000.

Extraction of purified spore walls revealed only three polypeptide bands in stained patterns when complexed with SDS. It is a reasonable assumption that each peak of this simple pattern represents a small number of polypeptide chains, most likely one, since the spore walls themselves contain a highly purified subfraction of the cellular protein. The same cannot be said for any peak in the pattern of total or soluble proteins, but one would expect the number of polypeptide species to be considerably reduced in the insoluble fraction. Peaks of increased synthesis in the insoluble fraction are even less likely to represent a large number of different polypeptides that happen to be of similar size. On these grounds, it is suggested that the two peaks of increased synthesis observed might reflect to a large extent the synthesis of spore wall protein.

Changes in the buffer-soluble proteins occurred somewhat later than was the case for the insoluble proteins, and the changes were not confined to any particular bands, probably because too many protein species were present in this extract. It is interesting that Zeldin and Ward (22) reported differences in a certain fraction of the soluble proteins in sporulating and nonsporulating *Physarum* cultures, especially in  $\alpha$ -amylase activity. It is unlikely, however, that the "plasmoidal stage" in their report is comparable to our starved control, especially since they grew the plasmodia on oatmeal.

The cessation of histone synthesis after the illumination period may be explained by assuming that the illuminated cultures were synchronous and in the G<sub>2</sub> phase, since synchronous mitosis is known to occur 13 hr after illumination (16). The synthesis of histone in the nonilluminated cultures at the same time, on the other hand, may result from the fact that there is less mitotic synchrony under starvation conditions (5). Since histone messenger ribonucleic acid was found in the light polysome fraction of HeLa cells (1, 14) and sea urchin embryos (7), the changes in polysome patterns derived from cultures before and after illumination suggest a relationship between the disappearance of light polysomes and the cessation of histone synthesis.

The difference in the relative amount of histones present in total protein extracts from starved and illuminated cultures requires further clarification. If the 1:1 relationship of histone:DNA, which was observed for growing cultures (13), holds true also for sporulating ones (Mohberg and Rusch, *in press*), one would have to assume a breakdown of DNA, or possibly of whole nuclei, in the process of sporulation. Disintegration of nuclear contents, "ghost" formation, and breakdown of

whole nuclei prior to sporangia formation have been reported previously (5). Further investigations of this problem are in progress.

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