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Variation in membrane proteins of developmentally aberrant mutants in Dictyostelium mucoroides.

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Variation in Membrane Proteins of Developmentally Aberrant Mutants in *Dictyostelium mucoroides*

A Thesis

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

University of Nebraska at Omaha

by

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THESIS ACCEPTANCE

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Introduction

Cellular slime molds of the genus Dictyostelium have attracted the interest of developmental biologists for many years. They are used extensively to investigate the factors controlling development in multicellular organisms. This is largely due to the unique growth and developmental characteristics which the dictyostelids exhibit. Unlike higher eukaryotes, in which cell growth and morphogenesis coincide and are often interdependent, the cellular slime molds possess distinct and separate growth and developmental phases. This greatly simplifies the study of developmental processes making the cellular slime molds valuable research organisms. Other factors which make the dictyostelids desirable research organisms are: 1)They follow a simple developmental pattern when compared to higher eukaryotic organisms. Cell differentiation occurs from a single cell type, a free-living uninucleate myxamoeba, into two new cell types, stalk cells and spore cells. 2)Growth and development occurs rapidly when compared with other eukaryotes. Depending upon

environmental conditions a mature cell population may be obtained in a few days. 3)Suitable environmental conditions for growth and development are relatively inexpensive and easy to provide. 4)Mutagenesis and cloning of individual strains is readily accomplished.

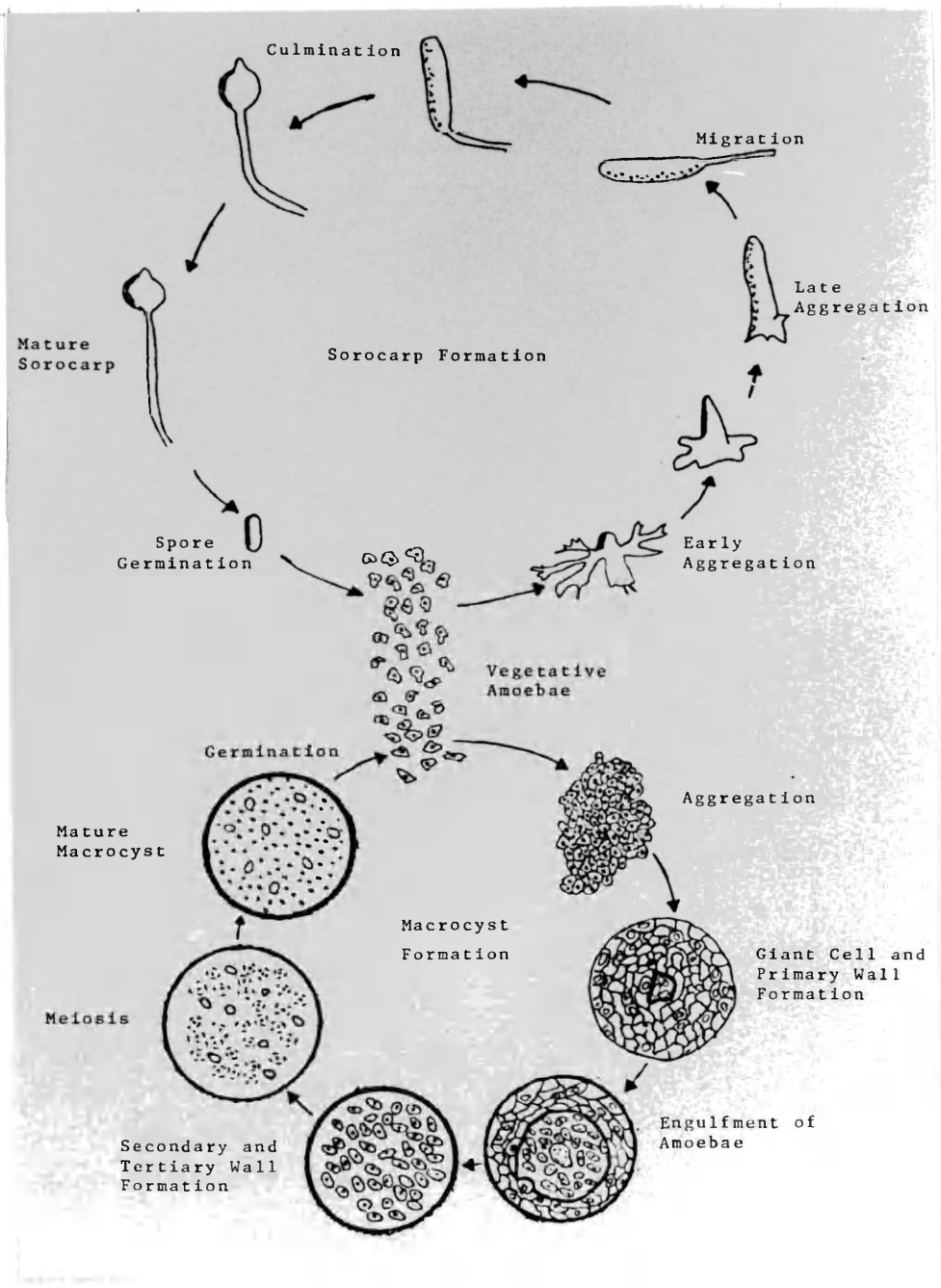
Although much of the previous developmental work has been performed with Dictyostelium discoideum; the first cellular slime mold discovered, Dictyostelium mucoroides (Brefeld, 1869), was the organism chosen for this investigation. One reason for this is the homothallic nature of the sexual cycle of D. mucoroides. Any molecular differences observed during the various stages of the life cycle can be attributed to developmental events and not to factors introduced by different mating types. A second reason is that relatively little research has been performed at the molecular level with D. mucoroides.

Development in D. mucoroides begins with unicellular free-living myxamoebae. Upon exhaustion of their nutrient supply, the myxamoebae develop along either one of two separate pathways. The asexual pathway involves the conversion of vegetative myxamoebae into the spore cells and stalk cells that form a fruiting structure known as a sorocarp. The sexual pathway involves the

conversion of vegetative myxamoebae into a multicellular structure known as a macrocyst. The developmental pathway chosen is determined by environmental conditions such as light, moisture, and certain chemicals.

The sorocarp pathway or asexual cycle (Fig. 1) begins with spore germination. Each individual spore gives rise to a single, uninucleate myxamoeba. The vegetative myxamoebae feed on bacteria and increase their numbers through binary fission. Upon exhaustion of the nutrient source certain myxamoebae will establish themselves as aggregation centers and begin to send out cyclic-AMP pulses. These cAMP pulses attract other myxamoebae toward these centralized locations to form a multicellular structure known as a pseudoplasmodium (Chisolm et al., 1984). At this stage the pseudoplasmodium enters a short migration period during which cell differentiation begins. Myxamoebae in the anterior end of the pseudoplasmodium form stalk cells. As the pseudoplasmodium moves forward on the stalk it leaves behind the basal stalk structure. Migration ceases when the pseudoplasmodium begins its final transformation into the mature fruiting body; this begins culmination. In this stage, all forward movement stops and the pseudoplasmodial tip begins a vertical orientation. Stalk

Figure 1. The life cycle of Dictyostelium mucoroides.


Culmination
Migration
Late
Aggregation
Mature
Sorocarp
Sorocarp Formation
Spore
Germination
Early
Aggregation
Vegetative
Amoebae
Germination
Aggregation
Mature
Macrocyst
Macrocyst
Formation
Giant Cell and
Primary Wall
Formation
Meiosis
Engulfment of
Amoebae
Secondary and
Tertiary Wall
Formation

cells continue to form with the progressive vacuolization of myxamoebae, cellulose wall deposition, and cell death. Culmination ends with the movement of the differentiated spore cells to the apex of the stalk to complete the final fruiting structure known as the sorocarp. Environmental factors which favor sorocarp development are high phosphate, low calcium substrates, the presence of light, and relatively dry conditions (Raper, 1940; Gezelius and Wright, 1965).

The macrocyst pathway or sexual cycle (Fig. 1) is favored when the "starved" vegetative myxamoebae are on a very moist, high calcium, low phosphate substrate, in the absence of light (Nickerson and Raper, 1973). Under these conditions the vegetative myxamoebae form loose cAMP mediated aggregates and surround themselves with a membranous covering. This covering or primary wall resembles a slime sheath and was first described by Blaskovics and Raper (1957). As development proceeds, a large, uninucleate, cytophagic cell or zygote is formed by the fusion of two vegetative myxamoebae (Filosa and Dengler, 1972). According to Dengler et al., (1970) this "giant" cell continues to grow by engulfing and digesting the remaining myxamoebae (forming endocytes) until it comes into contact with the primary wall. At

this time a secondary wall, composed largely of cellulosic material (Blaskovics and Raper, 1957), is secreted along the inside of the primary wall. This is followed by the secretion of a final tertiary wall inside the cellulosic wall. The nucleus of the original zygote then undergoes a meiotic division and a series of mitotic divisions to form a mature macrocyst. The life cycle becomes complete when the mature macrocyst germinates and releases the free-living, vegetative myxamoebae.

The dissection of an organism's developmental pathway can be greatly facilitated by the isolation of mutants. The cellular slime molds are especially suited for this type of analysis because the vegetative myxamoebae are haploid, thus the isolation and identification of mutational aberrations is easier than in diploid developmental systems. For example, the functions of N-acetylglucosaminidase, which allows for normal pseudoplasmodial formation and migration (Dimond et al., 1973), and UDPG-pyrophosphorylase, which is necessary for cellulose production and other culmination events (Dimond et al., 1976), have been elucidated in D. discoideum by analyzing mutants that are deficient for these gene products. Siu et al. (1977) used aggregate-

less mutants of D. discoideum to determine that the temporal sequence of the accumulation of plasma membrane proteins in wild type could be blocked at eight separate stages. With further study of developmentally aberrant mutants a better understanding of the factors controlling developmental processes may be obtained.

Previous studies with D. discoideum have shown that cell interactions are important during development (for review, see Loomis, 1982). Since the plasma membrane is the active site of intercellular communication, as well as contact with its external environment, investigators have had an interest in the plasma membrane's role in differentiation. Research in D. discoideum has shown: 1)The plasma membrane contains the receptor sites which bind cAMP, signalling the start of aggregation (Mullens and Newell, 1978). 2)Without cell adhesion during aggregation, development will discontinue (Newell, 1971). 3)The ability of the dictyostelids to develop along a particular pathway is dependent upon the ability of the plasma membrane to collect and process data about the surrounding environmental conditions (Loomis, 1982). Other researchers have looked at the structural components of the plasma membrane. For example, Siu et al. (1977) looked at the composition of the plasma membrane

proteins in developing D. discoideum and their variations over time. Siu et al. observed that proteins with molecular weights of 38,000, 36,500, and 10,000 to 12,000 rapidly accumulate during the first six hours of development and then disappear after twelve hours. They also found higher molecular weight proteins being synthesized in the later hours of development.

The purpose of this investigation was to look for variations in the membrane proteins between wild type and developmentally aberrant strains of D. mucoroides. By obtaining induced mutants which only develop to certain stages in the sorocarp and/or macrocyst pathway and by comparing their proteins with those of the appropriate stage of development in wild type cells, a profile of those membrane proteins which are important for a particular stage of development might be obtained. In this study, the developmentally aberrant mutant strains which were isolated fell into one of two categories; those which were phenotypically stopped at various stages within the life cycles of both the sorocarp and macrocyst pathways and those which showed some developmentally aberrant characteristic in the mature fruiting structures. Membrane proteins were extracted from the developing myxamoebae of both the wild type and

developmentally aberrant strains at various times and then separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The composition of the resulting protein profiles showed numerous similarities and some striking dissimilarities.

Materials and Methods

Organism:

Dictyostelium mucoroides strain Dm-7 and developmentally aberrant strains derived from it were utilized in this investigation. All strains were grown in conjunction with Escherichia coli B/r as a nutrient source for the myxamoebae. Stock cultures of all organisms were maintained on 0.6% lactose-peptone agar plates (lactose 6.0 g/l, peptone 6.0 g/l, agar 15.0 g/l).

Mutagenesis:

Approximately 1.5 x 10^5 spores from the sorocarps of D. mucoroides and a single loopful of E. coli B/r were inoculated into each of four 250 ml Erlenmeyer flasks containing glucose-yeast-peptone medium (Weber and Raper, 1971). These flasks were placed in a 23 C shaking water bath and incubated for 45 hours, at which time the myxamoebae concentration was approximately 1.0 x 10^6 myxamoebae/ml, corresponding to late exponential phase. At this time 150 ug/ml of the mutagen N-methyl-N'-nitro-N-nitrosoguanidine was added to each flask and incubation continued. At the end of 30 minutes, with about 10% of the myxamoebae surviving mutagenesis, the

myxamoebae were harvested from the liquid culture by centrifugation in a Sorvall centrifuge, using an HL-4 swinging bucket rotor for 15 minutes at 1500 rpm (455 x g). The myxamoebae were then washed three times with cold (4 C) phosphate buffer (0.025 M, pH 6.5) centrifuging as above between each wash. The final pellet was resuspended in phosphate buffer, diluted, and spread at a concentration of 200-300 myxamoebae/plate, along with E. coli, onto 0.6% lactose-peptone agar. Plates were then incubated in the light at 23 C and monitored every 24 hours for plaque development. Myxamoebal clones which exhibited unusual development were transferred to 0.6% lactose-peptone agar plates three consecutive times. Those strains which continued to be developmentally aberrant for sorocarp formation were then examined under conditions favoring macrocyst development.

Growth Conditions:

In order to obtain a large number of myxamoebae for development, the following procedure was utilized for wild type as well as each developmentally aberrant strain of D. mucoroides. Thirty-six 250 ml Erlenmeyer flasks containing 40 ml of GYP medium were inoculated with approximately 1.5×10^5 spores and/or myxamoebae

and were incubated in 23 C shaking waterbaths until a cell density of 2.5-3.5 x 10^6 myxamoebae/ml was acheived. Myxamoebae were then harvested from the liquid culture by centrifugation at 1500 rpm (455 x g) for 15 minutes and washed three times, centrifuging between each wash as above, with either phosphate buffer at 4 C for the sorocarp developmental pathway or Bonner's Salt Solution (BSS) at 4 C (Bonner and Frascella, 1953) for the macrocyst developmental pathway. At this point the final pellet was either frozen for future use as the zero hour sample or was resuspended in the appropriate buffer and diluted to a cell concentration of 3.0 x 10^8 myxamoebae/ml.

Sorocarp Development:

Previously prepared petri dishes containing an absorbant pad, a 47 mm GN-6 metricel filter and two milliliters of phosphate buffer with streptomycin (0.5 mg/ml) were inoculated with 0.5 ml of the 3.0 x 10^8 myxamoebae/ml suspension. The plates were incubated in the light at 23 C for three, six, or nine hours.

Macrocyst Development:

Previously prepared petri dishes containing an absorbant pad, a filter, and two milliliters of BSS with streptomycin (0.5 mg/ml) were inoculated with 0.5 ml of

the 3.0 x 10^8 myxamoebae/ml suspension. The inoculated filter was overlayed with a second filter and an additional two milliliters of BSS with streptomycin (Hanson, 1984). The plates were then placed in a light-proof canister and incubated at 23 C for three, six, or nine hours.

Cell Harvest:

Harvested cells were broken by vortexing with glass beads (500 um diameter). This was done by resuspending the pellet of harvested cells with two milliliters of the appropriate buffer (4 C) and adding an equal volume of glass beads. The suspension was vortexed in 15 second bursts and monitored every two minutes with phase contrast microscopy until sufficient breakage was attained. Cell pieces were suspended in the appropriate buffer and concentrated by centrifugation in a Sorvall RC-5B Refrigerated Centrifuge using an HB-4 swinging bucket rotor at 10,000 rpm (16,318 x g) for 20 minutes. The resulting pellet was utilized for plasma membrane isolation.

Membrane Isolation:

A dextran-polyethylene glycol aqueous two-phase polymer system described by Brunette and Till,(1971) was used for isolation of plasma membranes. This pro-

cedure was followed precisely except for centrifugation speeds during phase separation. Although the first centrifugation remained at 8,500 rpm (11,790 x g), the following two centrifugations were reduced to 7,500 rpm (9,180 x g) in order to provide higher membrane yield. The presence of alkaline phosphatase (a membrane marker) in the final pellet indicated that plasma membranes were being obtained through this procedure.

Protein Extraction and Separation:

Proteins were extracted from the plasma membranes by boiling for five minutes with a solution of 3% sodium dodecyl sulfate, 10% glycerol, and 5% mercaptoethanol in 0.0625 M tris-HCl (pH 6.8). After boiling, the extraction mixture was centrifuged in an Eppendorf microcentrifuge for 10 minutes. The supernatant was collected and protein concentration was determined by ultraviolet spectrophotometry. Using absorption readings at 260 and 280 nanometers, milligrams of protein per milliliter of sample was calculated (Schleif and Wensink, 1981).

Proteins were separated using SDS-PAGE with a discontinuous, vertical slab gel system. Each gel was 1.5 millimeters thick. A 12.5% separating gel and a 5%

stacking gel were used (Laemmli, 1970). Sample wells were loaded with either 10 or 100 micrograms of protein depending on which staining procedure, silver stain or coomassie-brilliant blue R-250 respectively, was used. Fifteen micrograms of a high molecular weight protein standard (Bethesda Research Laboratories) containing: myosin (H-chain), 200,000; phosphorylase B, 97,400; bovine serum albumin, 68,000; ovalbumin, 43,000; alpha-chymotrypsinogen, 25,700; beta-lactoglobulin, 18,400; lysozyme, 14,300; was utilized for molecular weight determinations. The gels were run at a constant current of 15 mA per gel, using a Heathkit regulated H.V. power supply, until the tracking dye reached the end of the gel (approx. seven hours).

Staining - Coomassie-Blue:

Gels were fixed with 12.5% trichloroacetic acid until the tracking dye turned yellow. They were then stained with coomassie-brilliant blue R-250 (0.2% in methanol: glacial acetic acid: distilled water, 50:10:40) overnight. The gels were destained in methanol: glacial acetic acid: distilled water, 250:75:675 (Atlas et al. 1977) until the background was clear and then stored in 7.5% glacial acetic acid.

Staining - Silver Stain:

The gels were fixed, washed, stained, and reduced according to the procedure outlined by Adams and Sammons (1981) with the following alterations: 1)To stop the reduction step a solution of 3% glacial acetic acid was agitated with the gels for a period of 30 minutes. 2)The color enhancement procedures were omitted.

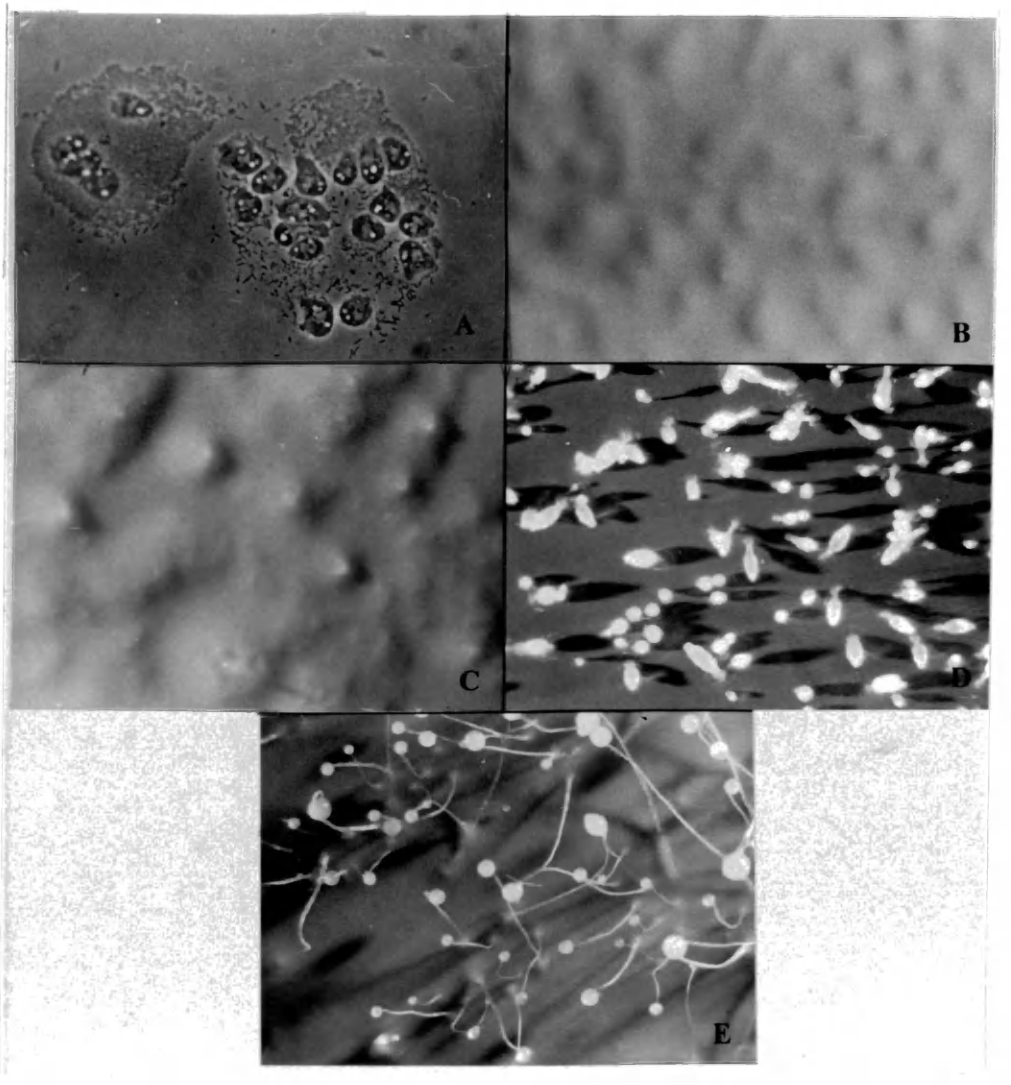
Results

Growth and Developmental Timing:

Wild type D. mucoroides, when grown in GYP medium, reached stationary phase (2.5-3.5 x 10^6 myxamoebae/ml) approximately 57 hours after inoculation. Microscopic examination of cells placed on filters, for synchronous development along the sorocarp pathway, showed cells in the individual, vegetative stage at zero hours. By three hours, cells began to migrate toward central collection points characteristic of early aggregation. At six hours, no sign of early stalk cells was evident, however, since aggregates markedly increased in size this stage was designated late aggregation. Filters, after nine hours incubation, exhibited myxamoebae in early culmination. Formation of a stalk mass in the center of the aggregates was characteristic of these cells. At 24 hours, filters showed immature sorocarps. Stalk elongation was not totally complete and the spore mass was still immature. By 48 hours, mature sorocarps covered the entire surface of the filter. (Fig. 2)

Cells, zero and three hours into development in the macrocyst pathway, were identical to those de-

Figure 2. The morphological stages of wild type D. mucoroides during development of sorocarps upon cellulose nitrate filters. (A) Vegetative myxamoebae, phase contrast, x 600; (B) three hours into development, x 40; (C) six hours into development, x 35; (D) nine hours into development, x 35; (E) mature sorocarps, x 25.


A
B
C
D
E

scribed in the sorocarp pathway. By six hours, cell aggregates grew in size and became tightly packed. At nine hours, cell aggregates were very dense and "rounded". At 24 hours, the primary and secondary macrocyst walls were clearly evident, however, the inside lacked the grainy appearance of the mature macrocysts observed at 48 hours. (Fig. 3)

Mutagenesis:

A variety of mutant strains with aberrant development were isolated. Those with abnormalities in the sorocarp pathway fell into two categories: 1)Two mutants were blocked in development; one of these aggregated but did not fruit (Fru-), while the second aggregated and produced a complete stalk but no sorus (Spo-)(Fig. 4). 2)Three mutants showed unusual morphology of the mature stalk. Two of these had stunted stalks (Mg-1 and Mg-2), while the third had a twisted stalk that was very fragile (Cur)(Fig. 5).

Two of the developmentally aberrant mutants described for the sorocarp pathway were also developmentally aberrant in the macrocyst pathway. The first, the non-fruiting mutant from the sorocarp pathway (Fru-) also stopped development after aggregation when under

Figure 3. The morphological stages of wild type D. mucoroides during development of macrocysts on cellulose nitrate filters. (A) Vegetative myxamoebae, x 600; (B) three hours into development, x 170; (C) six hours into development, x 170; (D) nine hours into development, x 170; (E) mature macrocysts, x 170 (all phase contrast).

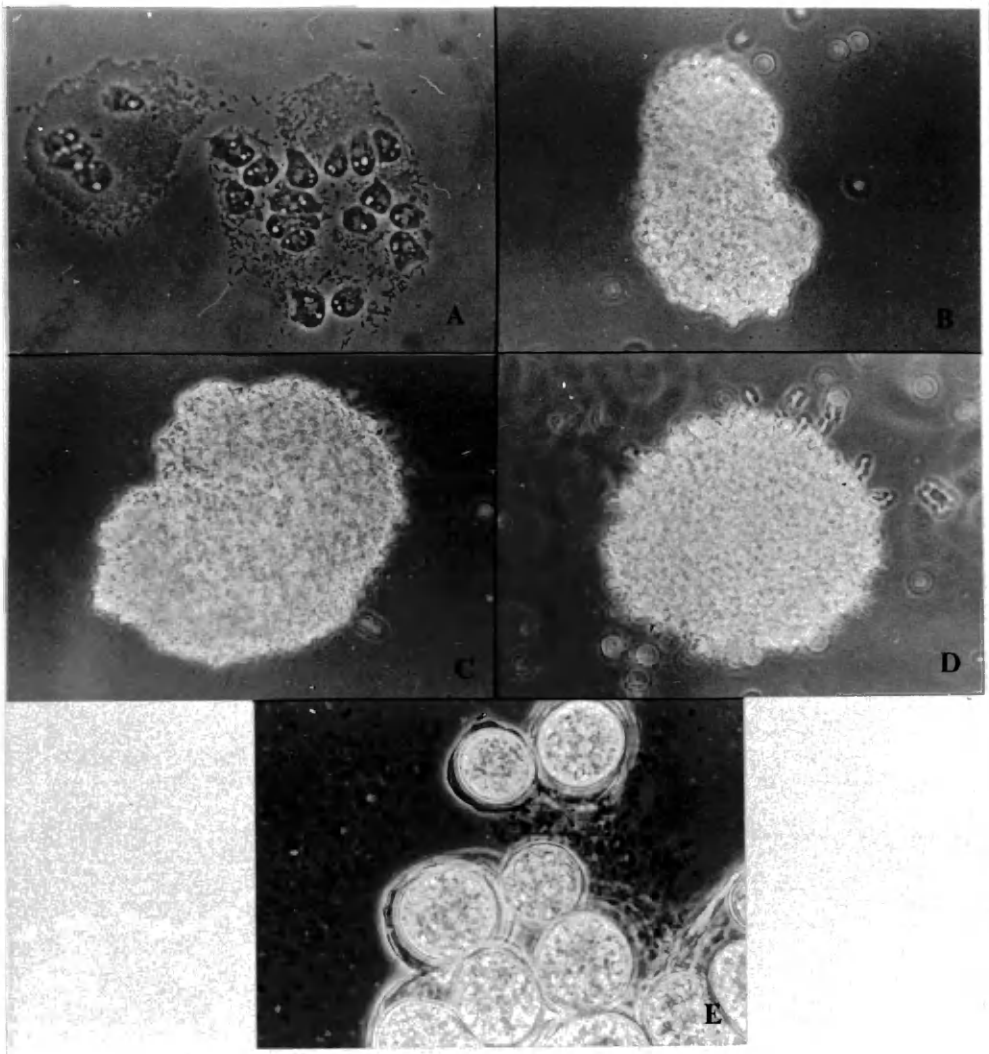

A
B
C
D
E

Figure 4. Final fruiting structures of wild type and developmentally blocked mutants of D. mucoroides in the sorocarp pathway. (A) Wild type, x 40; (B) Fru⁻, x 40; (C) Spo⁻, x 40.

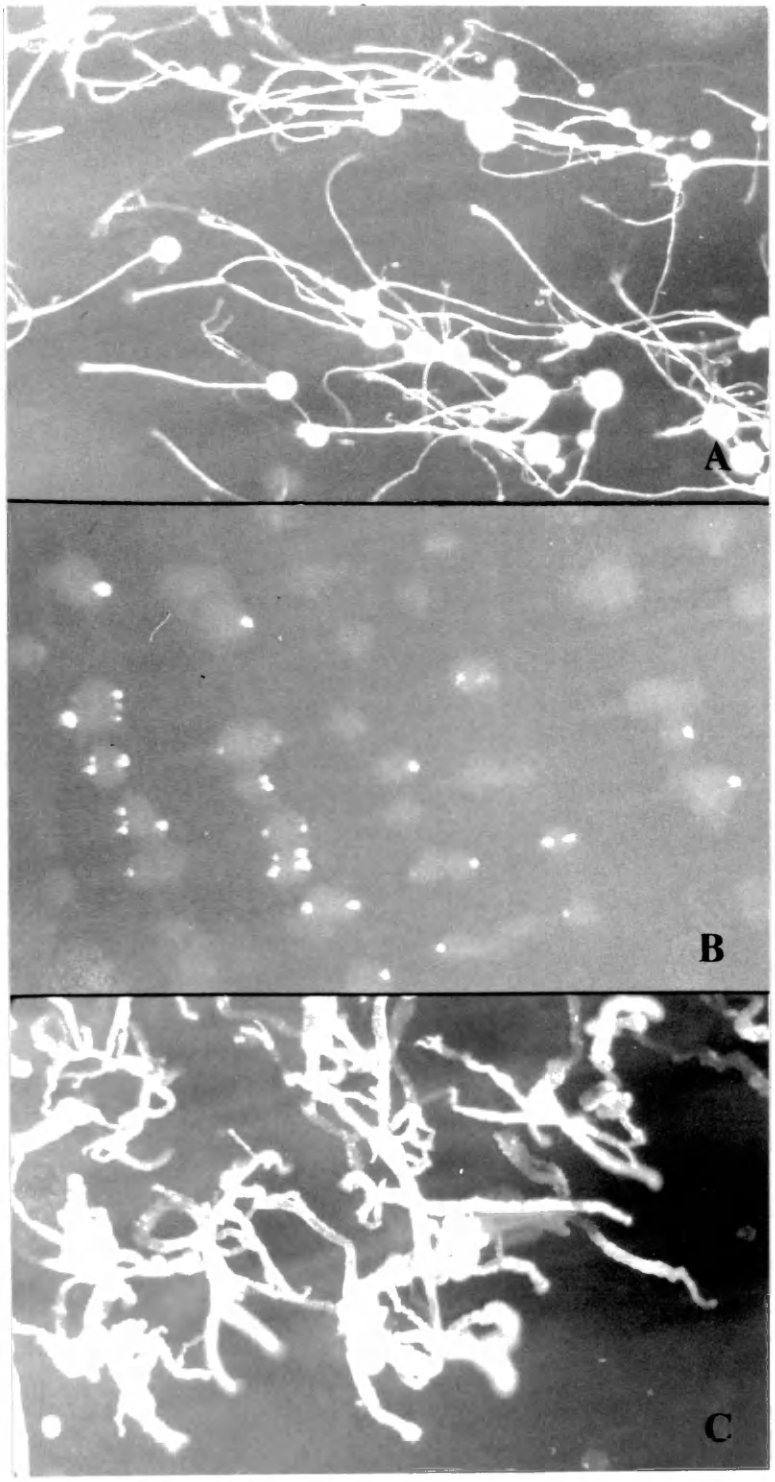
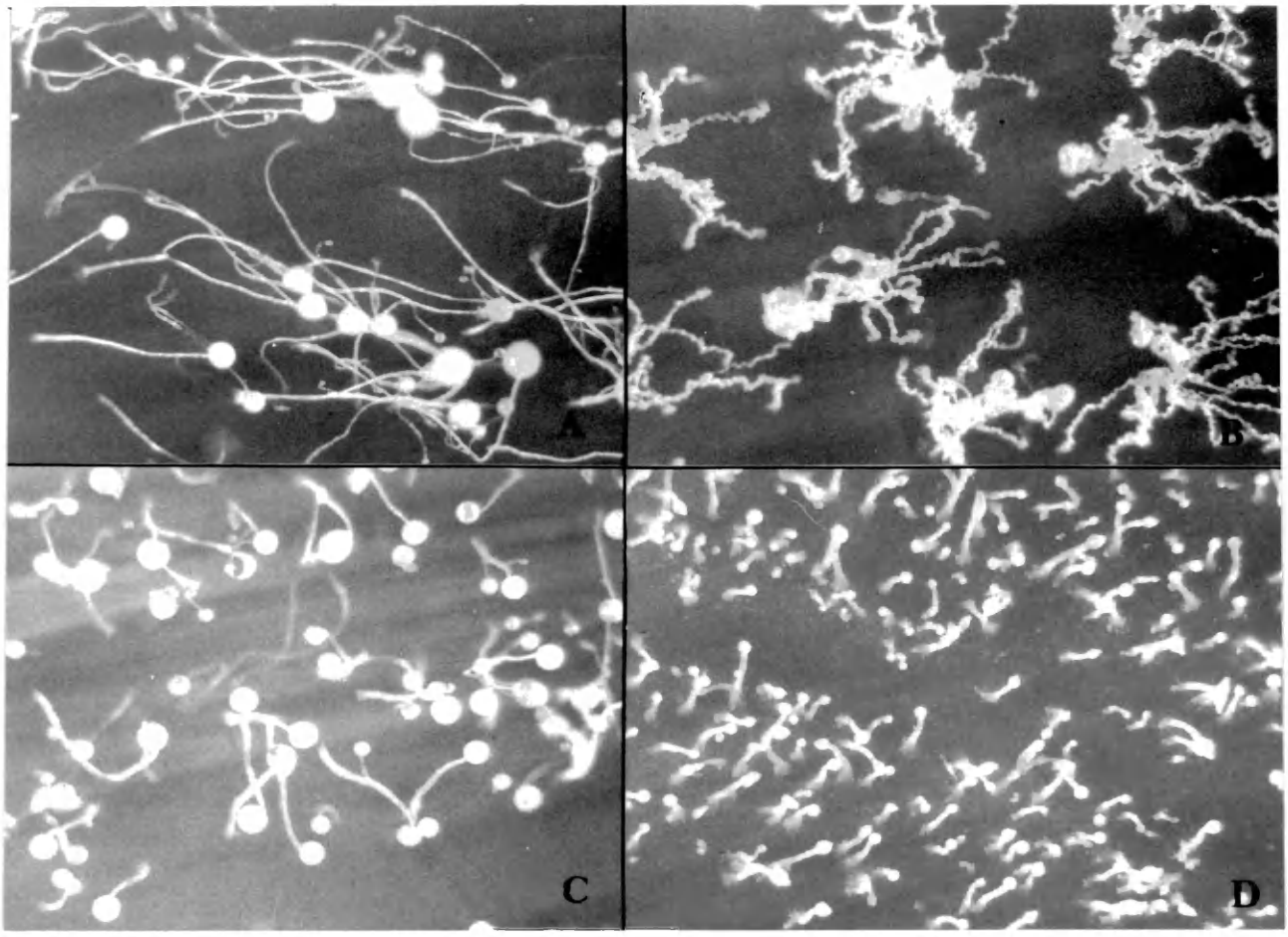

A
B
C

Figure 5. Final fruiting structures of wild type and morphologically aberrant mutants of *D. mucoroides* in the sorocarp pathway. (A) Wild type, x 40; (B) Cur, x 40; (C) Mg-1, x 40; (D) Mg-2, x 40.


A
B
C
D

conditions favoring macrocyst formation. The second, the twisted stalk mutant (Cur), produced macrocysts that lacked the primary wall and seemed to contain undigested myxamoebae (Fig. 6).

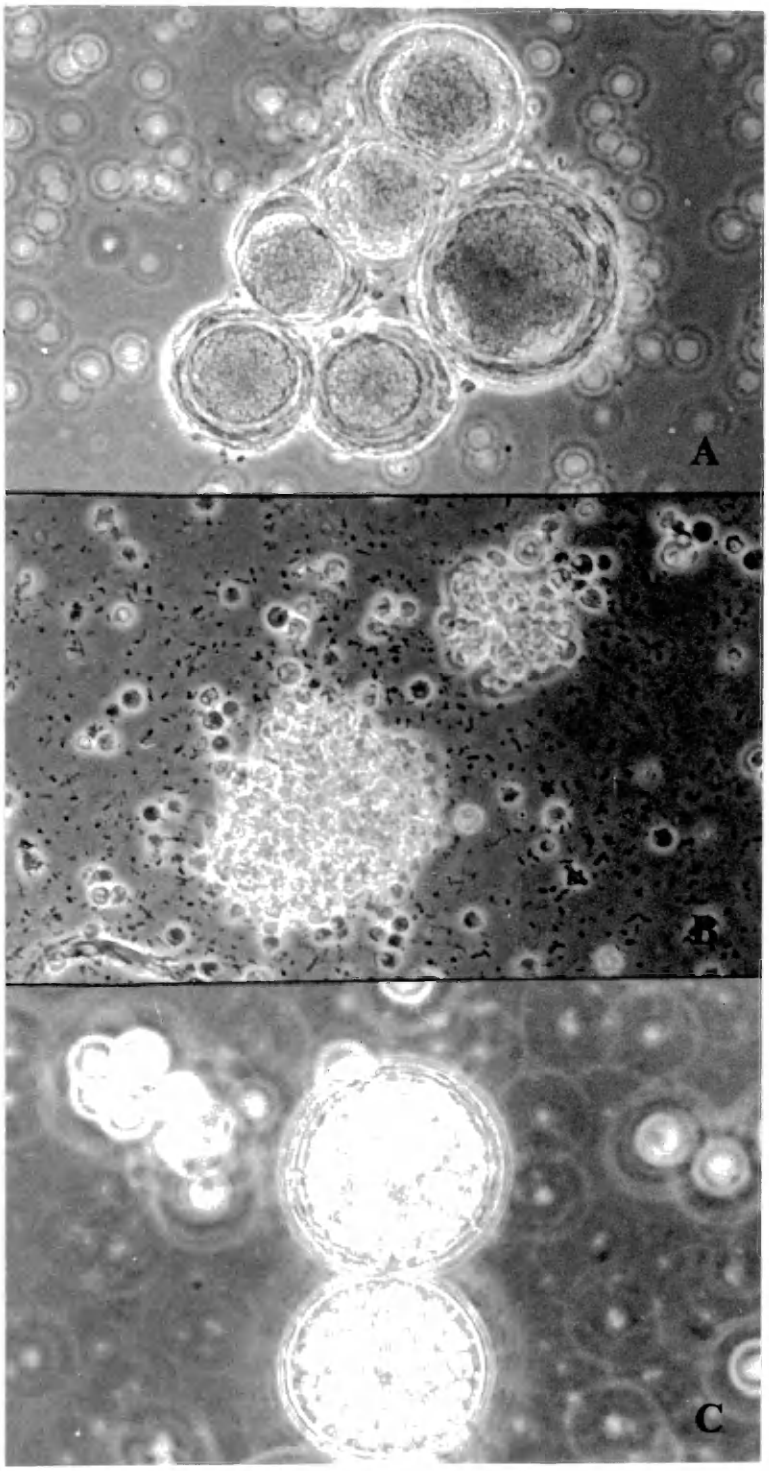
The following is a more detailed description of each developmentally aberrant mutant strain used in this investigation:

Fru-

Fru- was developmentally blocked after aggregation in both the sorocarp and macrocyst pathways. When myxamoebae were allowed to develop under environmental conditions favoring either sorocarp or macrocyst formation, normal aggregates were formed but no cell differentiation occurred therefore preventing mature fruiting body formation.

In GYP media, the growth rate of Fru- myxamoebae was much slower than that of wild type myxamoebae. Stationary phase was reached in approximately 67 hours, while wild type myxamoebae only reguired 57 hours to reach the same phase. This difference in the time required to reach stationary phase was actually an underestimate since the Fru- inoculation was performed with myxamoebae rather than spores. Spore germination typically requires four hours.

Figure 6. Final fruiting structures of wild type and developmentally aberrant mutants of D. mucoroides in the macrocyst pathway. (A) Wild type, x 350; (B) Fru^{-}, x 350; (C) Cur, x 860 (all phase contrast).


A
B
C

Morphogenesis of Fru- and wild type myxamoebae were comparable at zero, three, and six hours. However, at nine hours, while wild type myxamoebae were undergoing early culmination, Fru- was still in late aggregation. Observations at 24 and 48 hours indicated no further development of the late aggregation stage described for Fru- at nine hours.

Spo-

Spo- formed normal, mature macrocysts but was developmentally aberrant in spore formation under environmental conditions favoring sorocarp production. The developing myxamoebae completed aggregation and stalk formation but failed to differentiate into mature spores. Aggregates and stalks in Spo- were larger and thicker respectively, than in wild type.

Spo- myxamoebae in GYP medium reached stationary phase one to two hours faster than wild type myxamoebae. This was probably the result of using myxamoebae for Spo- inoculations as opposed to the spores used for wild type.

Developmental stages of Spo- were comparable with wild type at zero, three, and six hours. However, at nine hours, during early culmination, it was evident that the developing sorocarp of Spo- was missing a pre-

spore mass. This was confirmed when Spo- was observed at 24 and 48 hours. At those times, large, thick stalks were present with no evidence of a prespore or mature spore mass.

Cur

The developmentally aberrant mutant, Cur, completed development in both the sorocarp and macrocyst pathways. However, the morphology of both the mature sorocarps and macrocysts of Cur were abnormal.

In the sorocarp pathway, Cur differed from wild type in two characteristics: stalk structure and spore number. The maturing sorocarps in Cur possessed stalks which were very thin with a curled or twisted appearance. These stalks were very fragile and usually collapsed before the sorocarp reached full maturity. This fragility was also seen when Cur stalks were placed in water; the stalk cells readily broke apart giving the appearance of the stalk dissolving. Finally, there was a noticable reduction in the number of mature spores in Cur when compared with wild type. Cur macrocysts differed from wild type by being much smaller and also by lacking the outer, primary wall. They also appeared to contain undigested myxamoebae which was uncharacteristic of wild type macrocysts.

Growth of Cur myxamoebae in GYP medium was comparable to wild type. Developmental stages in both the sorocarp and macrocyst pathways were also comparable to wild type at zero, three, six, and nine hours. Not until the 24 and 48 hour observations did the developmentally aberrant characteristics previously described become evident.

Mg-1

Mg-1 formed normal, mature macrocysts. However, in the sorocarp pathway, instead of producing normal sorocarps of 7.5-9.0 mm in length, Mg-1 produced "midget" sorocarps which were only 4.0-6.0 mm in length. Spore masses were of normal size when compared to wild type.

Growth of Mg-1 myxamoebae in GYP medium was comparable to wild type. However, developmental stages in the sorocarp pathway were slightly behind those of wild type at zero, three, six, and nine hours. Not until 24 and 48 hour observations did the stunted sorocarp growth become evident.

Mg-2

Mg-2 was similar to Mg-1. It formed normal macrocysts but produced sorocarps which were only 1.0-3.0 mm in length compared to the 7.5-9.0 mm and 4.0-6.0 mm of wild type and Mg-1 respectively. Mg-2 spore masses were

smaller than in either wild type or Mg-1. Mg-2 aggregates were also much smaller and spaced more closely together than was observed for wild type or Mg-1.

Growth of Mg-2 in GYP medium was comparable to wild type. Developmental stages in the sorocarp pathway were also comparable to wild type at zero, three, six, and nine hours. Differences in sorocarp formation were not observed until the 24 and 48 hour observations.

Analysis of membrane proteins:

Membrane proteins from wild type and developmentally aberrant mutant strains were compared at zero, three, six, and nine hours into development during sorocarp and macrocyst formation. The molecular weights of the protein bands were determined by comparing the migration value (Rf) of an unknown protein band with a curve of the migration values of the molecular weight protein standards.

The stained profiles of the membrane proteins showed approximately twenty distinct bands with a molecular weight distribution between 18,000 and 200,000. Ten of these bands stained much darker than the others and together seemed to constitute the majority of the total membrane protein. Figures 7 through 18 show

protein profiles of wild type and developmentally aberrant mutant strains in the sorocarp and macrocyst developmental pathways at zero, three, six and nine hours. Tables I through IX give molecular weights of the major proteins in the gel profile of each strain and the change in the concentration of these proteins over time.

In the sorocarp pathway, the wild type gel profile remained very similar from the initiation of development (0 hr) to the culmination stage (9 hr)(Figs. 7 through 14, and Table I). However, a few prominent changes were seen. Two protein bands seen at 165,000, in the zero hour gel profiles, were no longer seen at three hours. At the same time, two new protein bands made an appearance, one at 200,000 and the other at 18,000. Protein bands at 87,000, 69,000, 51,000, and 42,000 increased in concentration (determined through visual observation) from zero to three hours while proteins at 34,000, 29,500, and 22,000 decreased in concentration during the same time. By six hours, the protein band at 29,500 had completely disappeared from the gel profiles while all other protein bands showed little change in concentration from three hours. The nine hour gel profile was unchanged from that at six hours with the exception of an increase of protein in

Figure 7. SDS-PAGE comparison of coomassie-blue stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides zero hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.

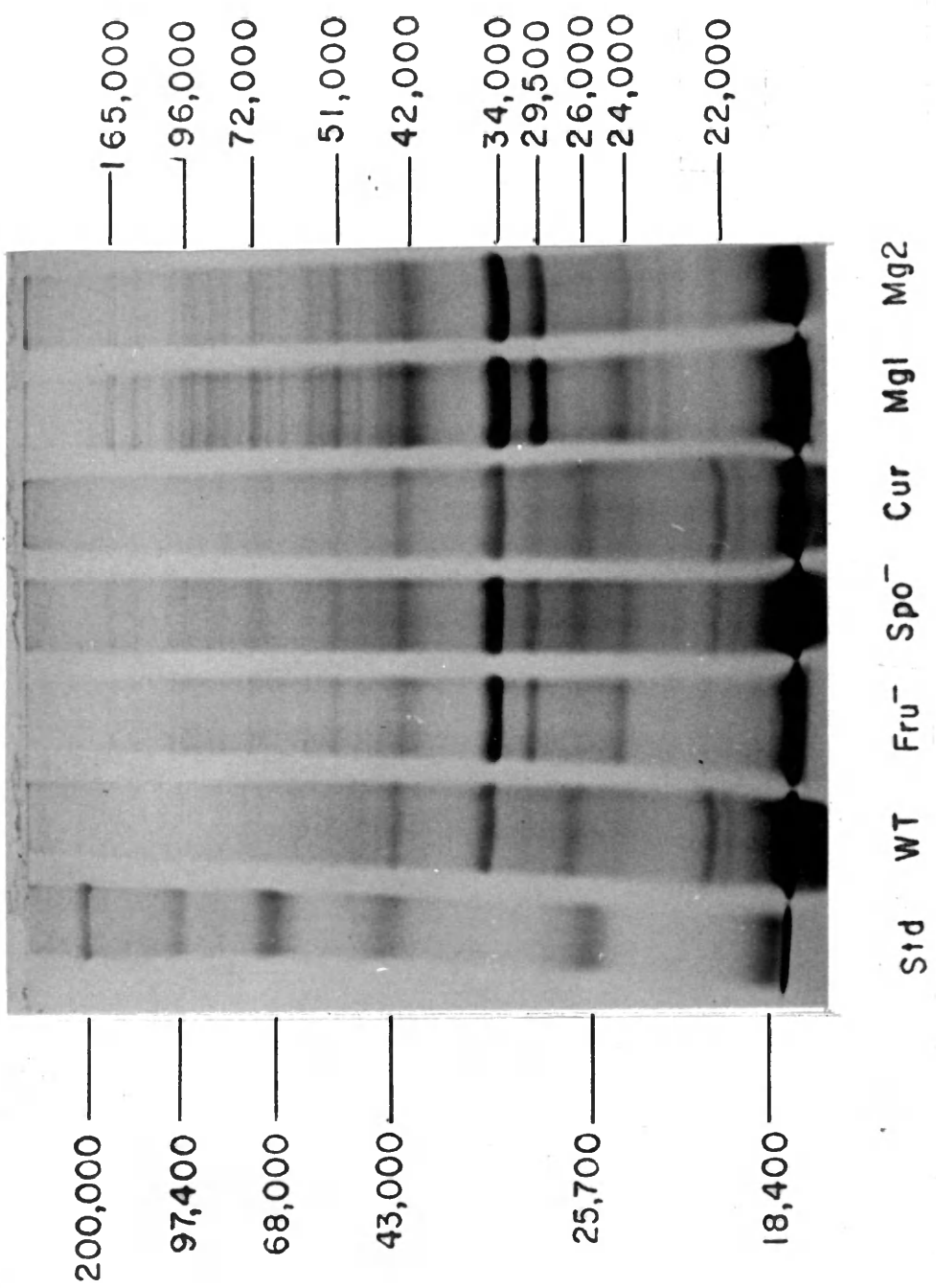
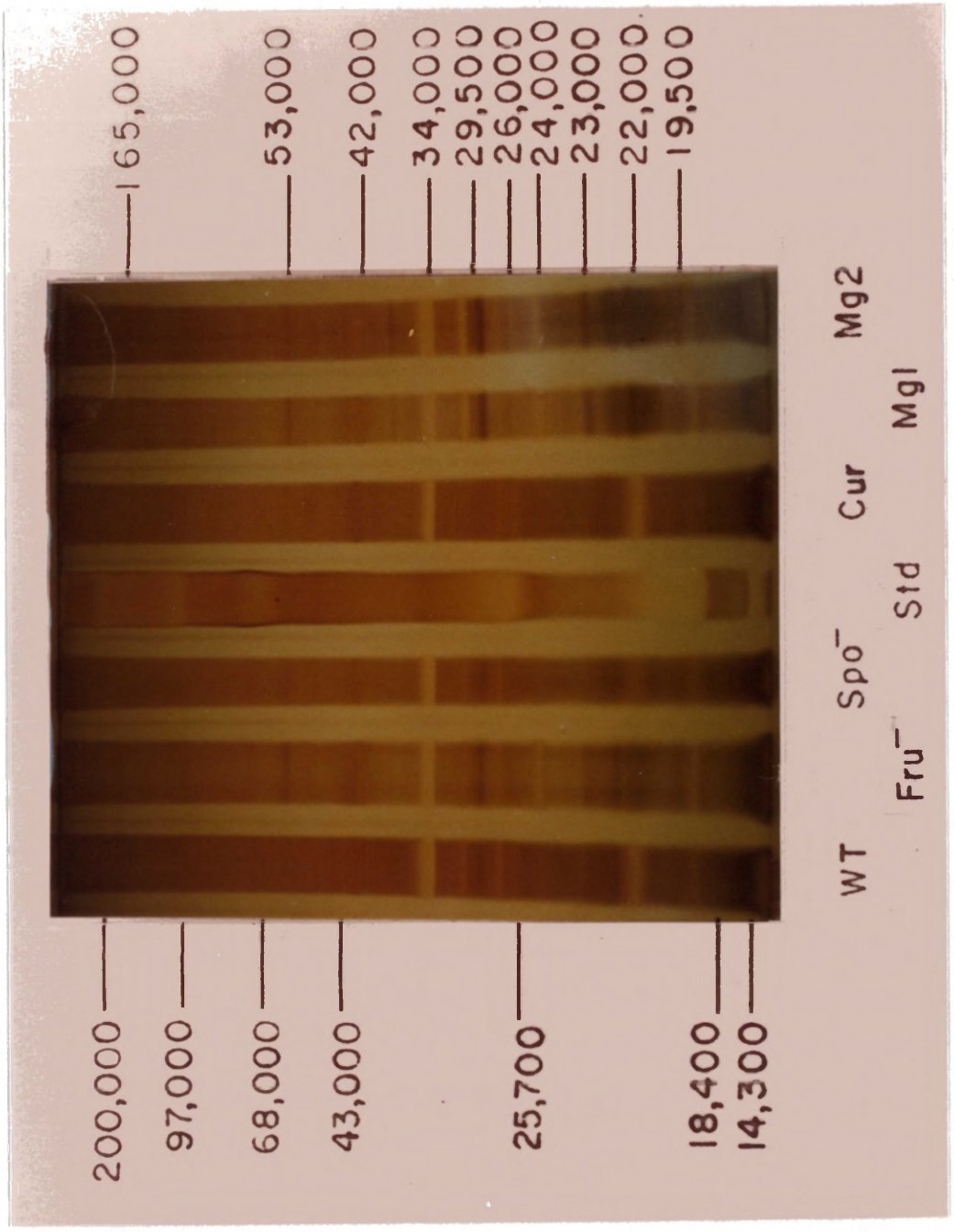

165,000
96,000
72,000
51,000
42,000
34,000
29,500
26,000
24,000
22,000
200,000
97,400
68,000
43,000
25,700
18,400
Std
WT
Fru⁻
Spo⁻
Cur
Mgl
Mg2

Figure 8. SDS-PAGE comparison of silver stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides zero hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while molecular weights of the protein standards are listed on the left.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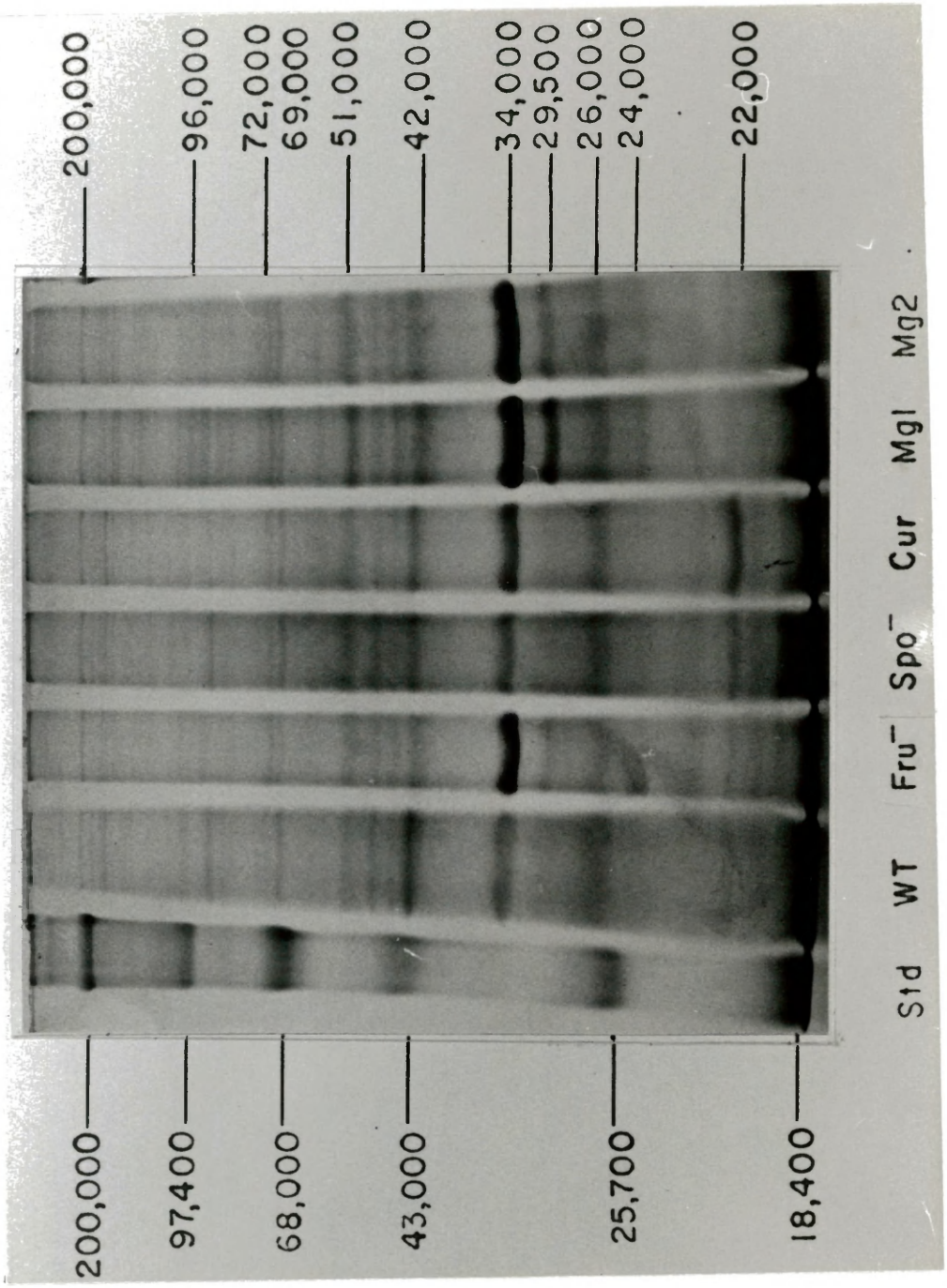
53,000
42,000
34,000
29,500
26,000
24,000
23,000
22,000
19,500
200,000
97,000
68,000
43,000
25,700
18,400
14,300
WT
Fru⁻
Spo⁻
Std
Cur
Mgl
Mg2

Figure 9. SDS-PAGE comparison of coomassie-blue stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides three hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.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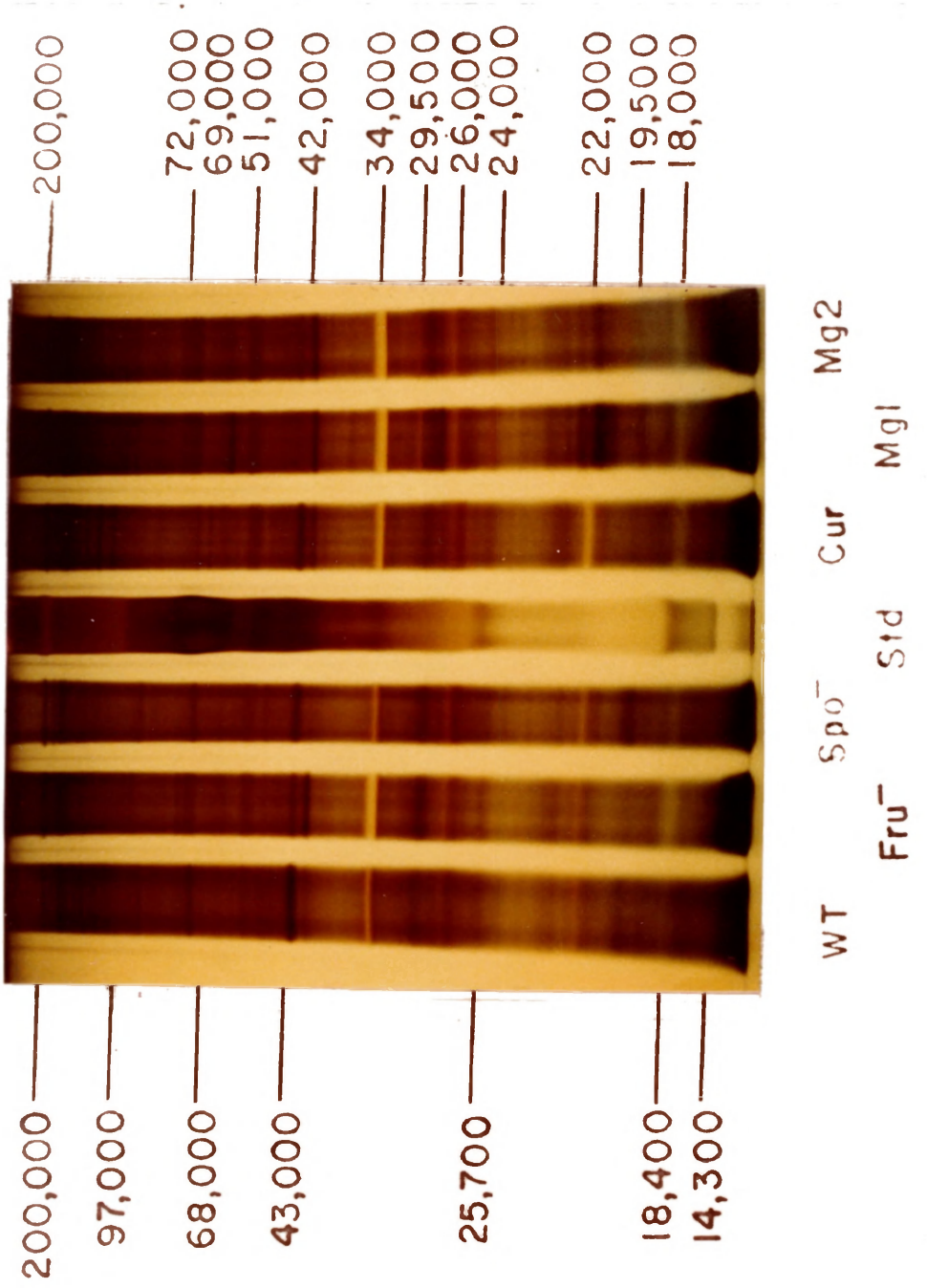
96,000
72,000
69,000
51,000
42,000
34,000
29,500
26,000
24,000
22,000
200,000
97,400
68,000
43,000
25,700
18,400
Std
WT
Fru⁻
Spo⁻
Cur
Mgl
Mg2

Figure 10. SDS-PAGE comparison of silver stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides three hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.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24,000
22,000
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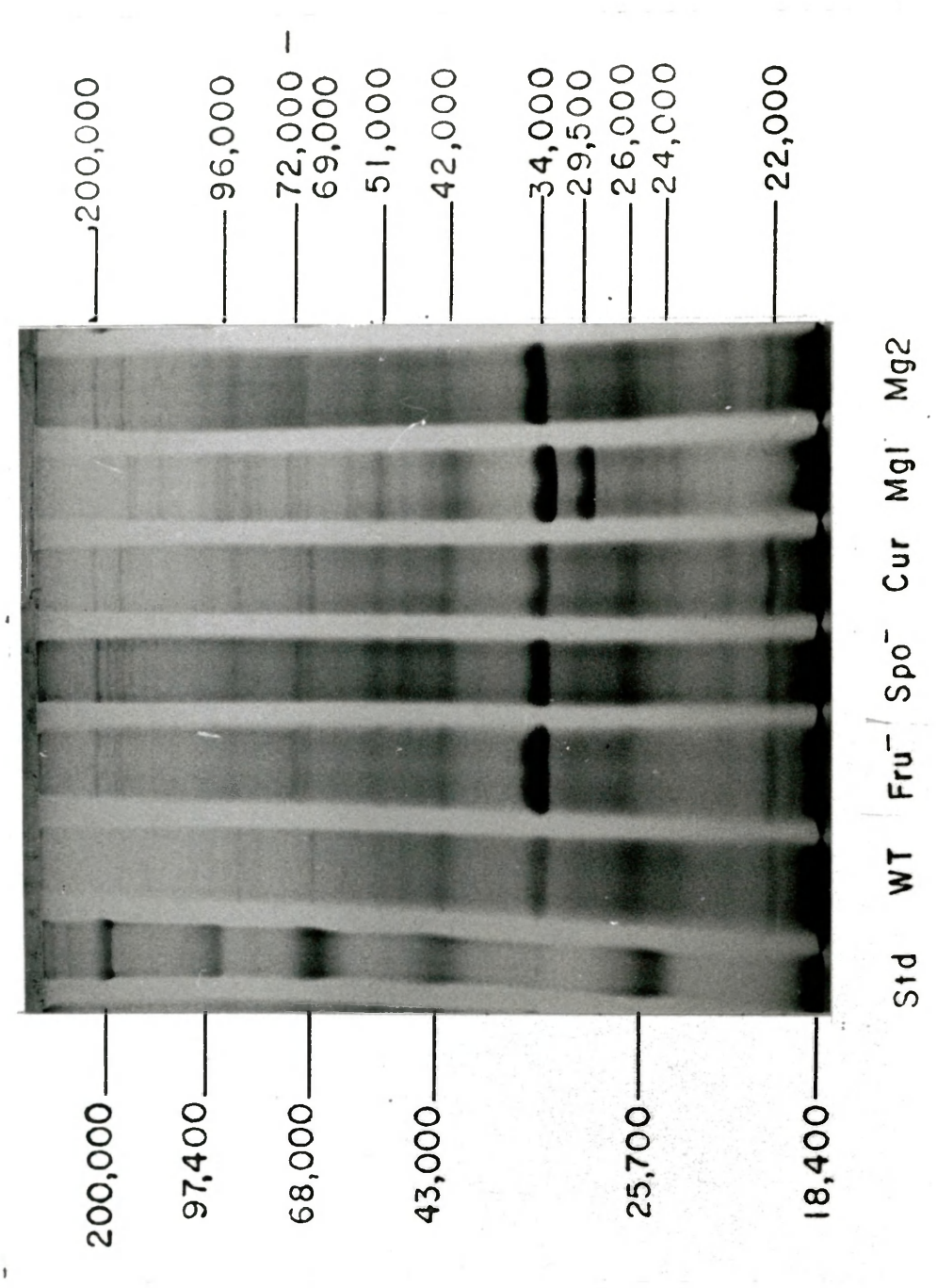
200,000
97,000
68,000
43,000
25,700
18,400
14,300
WT
Fru⁻
Spo⁻
Std
Cur
Mgl
Mg2

Figure 11. SDS-PAGE comparison of coomassie-blue stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides six hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.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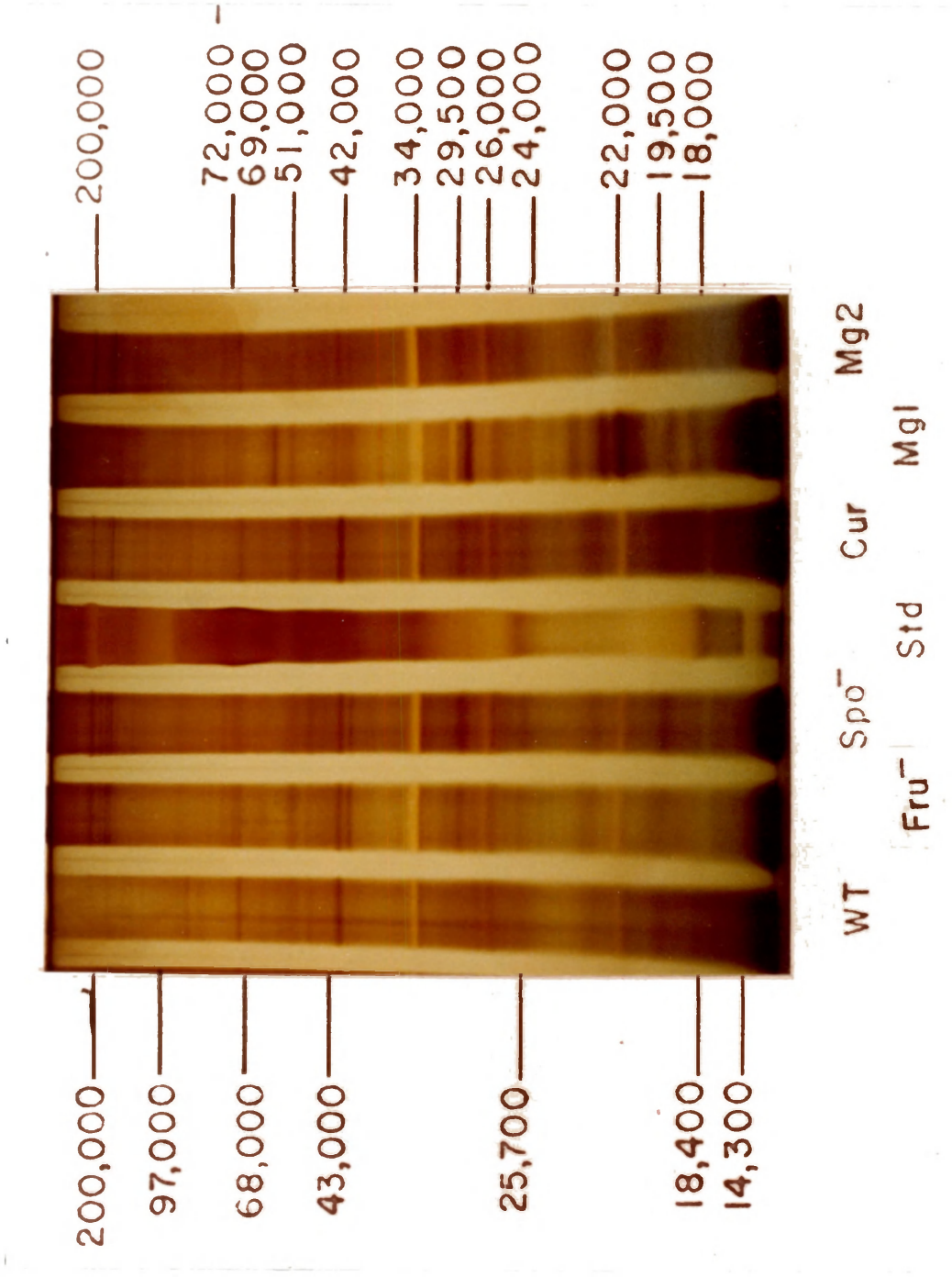
72,000
69,000
51,000
42,000
34,000
29,500
26,000
24,000
22,000
200,000
97,400
68,000
43,000
25,700
18,400
Std
WT
Fru⁻
Spo⁻
Cur
Mgl
Mg2

Figure 12. SDS-PAGE comparison of silver stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides six hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.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72,000
69,000
51,000
42,000
34,000
29,500
26,000
24,000
22,000
19,500
18,000
200,000
97,000
68,000
43,000
25,700
18,400
14,300
WT
Fru⁻
Spo⁻
Std
Cur
Mgl
Mg2

Figure 13. SDS-PAGE comparison of coomassie-blue stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides nine hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.

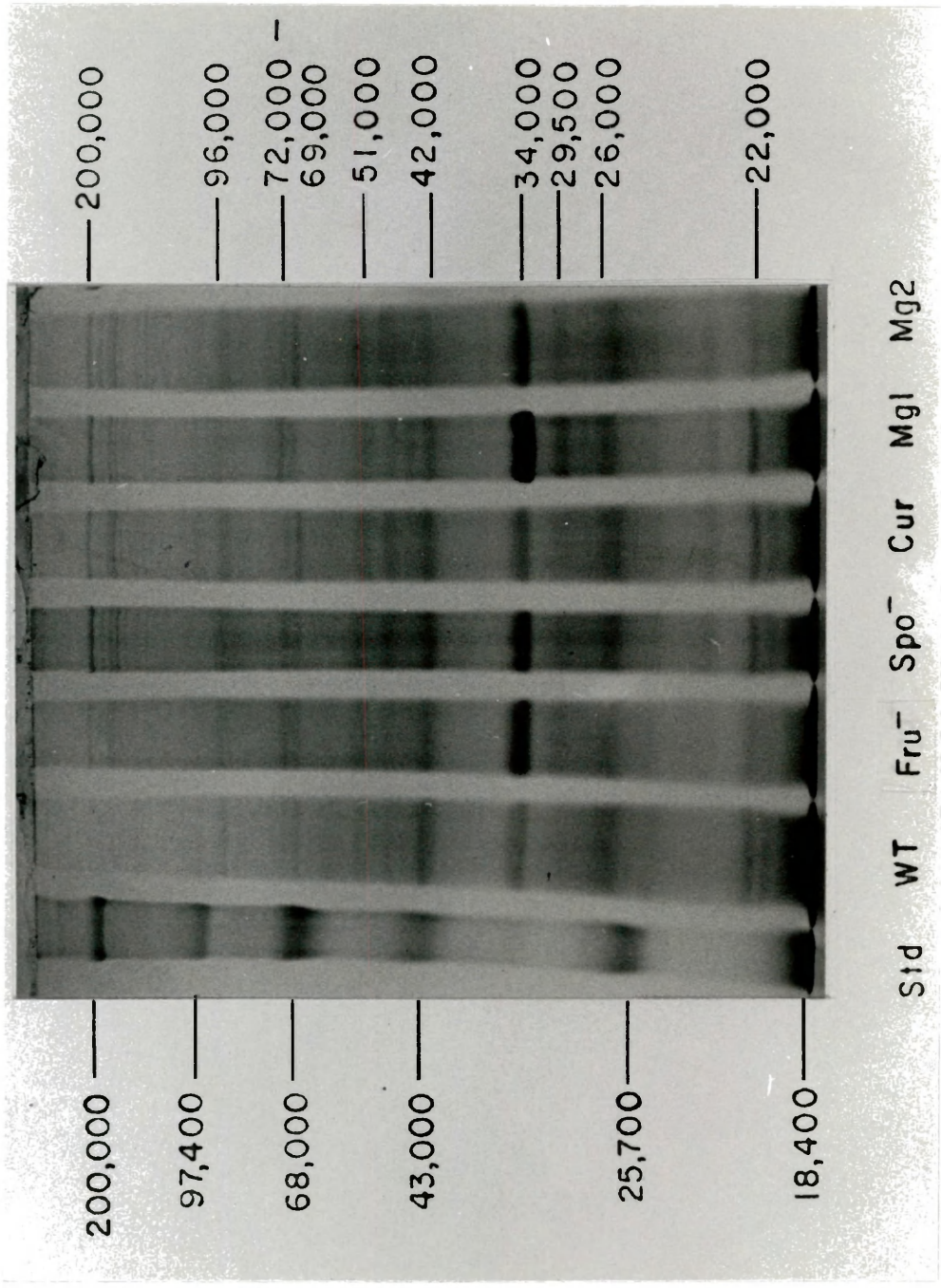

200,000
96,000
72,000
69,000
51,000
42,000
34,000
29,500
26,000
22,000
200,000
97,400
68,000
43,000
25,700
18,400
Std
WT
Fru⁻
Spo⁻
Cur
Mgl
Mg2

Figure 14. SDS-PAGE comparison of silver stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides nine hours into sorocarp development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.

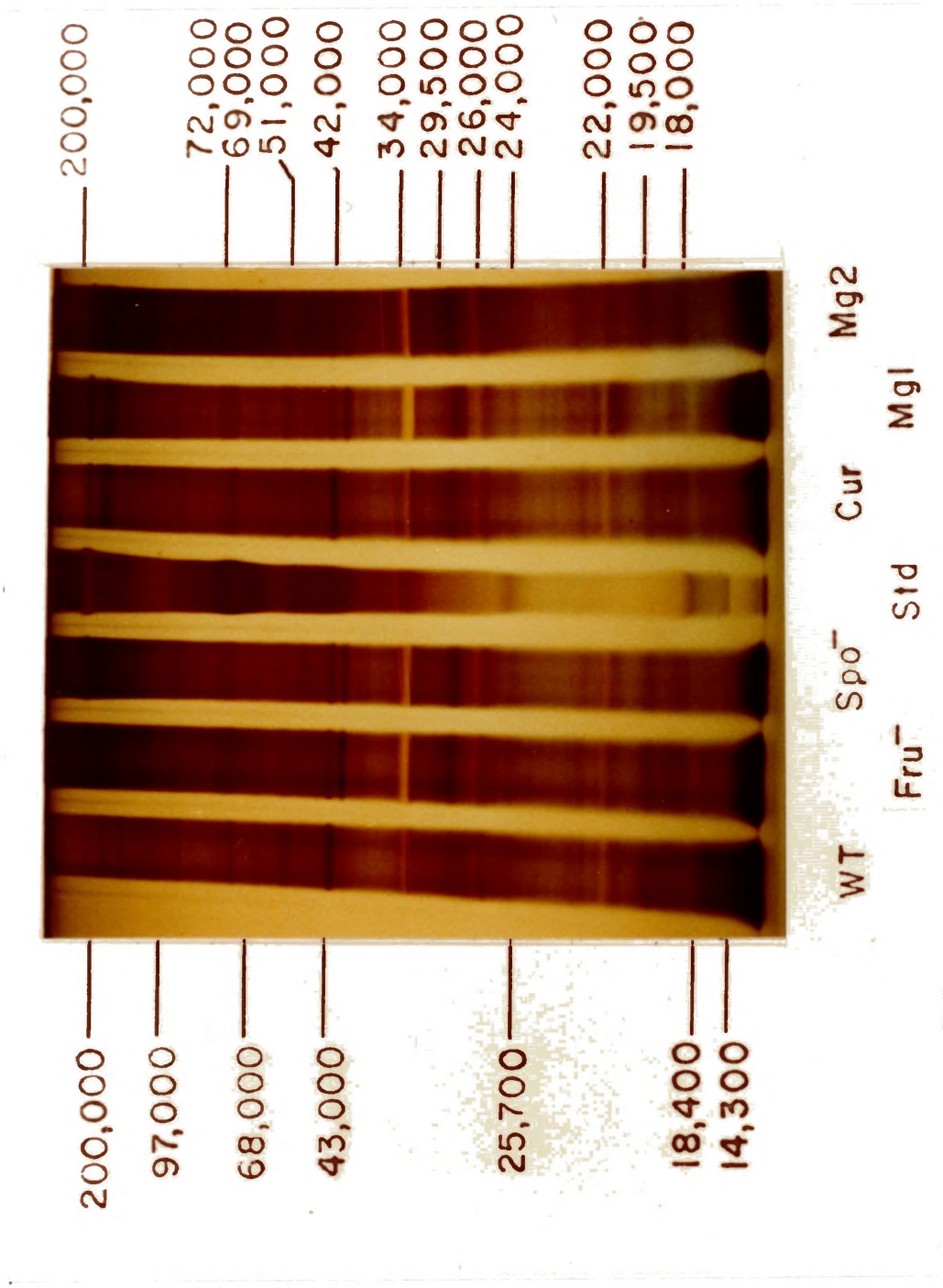

200,000
72,000
69,000
51,000
42,000
34,000
29,500
26,000
24,000
22,000
19,500
18,000
200,000
97,000
68,000
43,000
25,700
18,400
14,300
WT
Fru⁻
Spo⁻
Std
Cur
Mgl
Mg2

Table I. Variation in membrane proteins of wild type *D. mucoroides* at various times during sorocarp development[a]

Protein mol wt	Time of sample (hr) 0	3	6	9
200,000	O	X	→	→
165,000	X	O	O	O
96,000	X	→	→	→
87,000	X	↑	→	→
72,000	O	O	O	O
69,000	X	↑	→	↑
53,000	O	O	O	O
51,000	X	↑	→	→
47,500	X	→	→	→
44,500	O	X	→	→
42,000	X	↑	→	→
34,000	X	↓	→	→
29,500	X	↓	O	O
26,000	X	→	→	→
24,000	O	O	O	O
23,000	O	O	O	O
22,000	X	↓	→	→
19,500	X	→	→	→
18,000	O	X	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

the 69,000 band.

All of the developmentally aberrant mutants in sorocarp development, with the exception of Cur, had gel profiles with major differences from wild type at the initiation of development (0 hr)(Figs. 7 and 8). Although Cur possessed similar banding patterns with wild type (Table IV), gel profiles of Fru-, Spo-, Mg-1, and Mg-2 showed differences in a group of protein bands between 22,000 and 34,000 (Tables II, III, V, and VI). Fru-, Mg-1, and Mg-2 lacked bands at 26,000 and 22,000, while proteins in these bands were in high concentration in wild type and Cur. A 24,000 molecular weight protein present in Fru-, Mg-1, and Mg-2 was absent in wild type and Cur. Also, the protein bands at 34,000 and 29,500 were in much higher concentration in Fru-, Mg-1, and Mg-2 than in either wild type or Cur. Spo- was unique in possessing relatively high concentrations of all of the protein bands between 22,000 and 34,000.

As development progressed through three, six, and nine hours the gel profiles of Fru-, Spo-, Mg-1, and Mg-2 became increasingly similar to the gel profiles of wild type and Cur. The protein bands at 29,500 and 24,000 essentially disappeared in Fru-, Spo-, Mg-1, and Mg-2 while protein bands at 26,000 and 22,000 began to

Table II. Variation in membrane proteins of the developmentally aberrant mutant Fru$^-$ at various times during sorocarp development[a]

	Time of sample (hr)			
	0	3	6	9
Protein mol wt				
200,000	O	X	→	→
165,000	X	O	O	O
96,000	X	↓	→	→
87,000	O	X	→	→
72,000	X	↑	→	→
69,000	X	↑	→	→
53,000	X	↓	O	O
51,000	X	↑	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	↑	→	→
34,000	X	→	→	→
29,500	X	↓	↓	→
26,000	O	X	→	→
24,000	X	↓	↓	O
23,000	O	O	O	O
22,000	O	X	↑	→
19,500	X	→	→	→
18,000	O	X	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

Table III. Variation in membrane proteins of the developmentally aberrant mutant Spo$^-$ at various times during sorocarp development[a]

	Time of sample (hr)			
	0	3	6	9
Protein mol wt				
200,000	O	X	↑	↑
165,000	X	O	O	O
96,000	X	↓	→	→
87,000	O	X	→	→
72,000	O	O	X	→
69,000	X	↑	→	→
53,000	O	O	O	O
51,000	X	→	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	↑	→	→
34,000	X	↓	→	→
29,500	X	↓	↓	→
26,000	X	↑	→	→
24,000	X	↓	O	O
23,000	O	O	O	O
22,000	X	→	→	→
19,500	X	→	→	→
18,000	O	X	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

Table IV. Variation in membrane proteins of the developmentally aberrant mutant Cur at various times during sorocarp development[a]

Protein mol wt	Time of sample (hr) 0	3	6	9
200,000	O	X	→	→
165,000	X	O	O	O
96,000	X	↓	↓	→
87,000	O	O	X	↑
72,000	O	X	↓	O
69,000	O	X	↑	→
53,000	O	O	O	O
51,000	X	→	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	↑	↑	→
34,000	X	→	→	↓
29,500	X	↓	O	O
26,000	X	→	→	→
24,000	O	O	O	O
23,000	O	O	O	O
22,000	X	↑	→	↓
19,500	X	→	→	→
18,000	O	X	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

Table V. Variation in membrane proteins of the developmentally aberrant mutant Mg-1 at various times during sorocarp development[a]

Protein mol wt	Time of sample (hr) 0	3	6	9
200,000	O	X	→	→
165,000	X	→	→	O
96,000	X	→	→	↓
87,000	O	O	O	O
72,000	X	→	→	→
69,000	X	→	→	→
53,000	X	→	→	O
51,000	X	→	↓	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	→	→	→
34,000	X	→	→	→
29,500	X	→	→	↓
26,000	O	X	→	→
24,000	X	↓	↓	O
23,000	X	→	→	O
22,000	O	O	O	X
19,500	X	→	→	→
18,000	O	X	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

Table VI. Variation in membrane proteins of the developmentally aberrant mutant Mg-2 at various times during sorocarp development[a]

	Time of sample (hr)			
	0	3	6	9
Protein mol wt				
200,000	O	X	→	→
165,000	X	O	O	O
96,000	X	→	→	↓
87,000	O	O	X	→
72,000	X	→	→	→
69,000	O	X	→	→
53,000	X	↓	O	O
51,000	X	→	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	→	→	↓
34,000	X	→	→	↓
29,500	X	↓	O	O
26,000	O	X	→	→
24,000	X	↓	↓	O
23,000	X	O	O	O
22,000	O	O	X	→
19,500	X	→	→	→
18,000	O	X	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

accumulate. There were still, however, some protein differences between the developmentally aberrant mutants and wild type strain at the final sampling time (9 hr). In the coomassie-blue stained gels (Fig. 13) differences in the concentrations of protein bands between strains were: 1)The protein band at 34,000 in Fru-, Spo-, and Mg-1 was approximately twice the concentration of the corresponding band in wild type, Cur, and Mg-2. 2)In Fru-, Spo-, and Mg-1 three protein bands between 69,000 and 72,000 were distinct with the 72,000 protein band in highest concentration while in wild type, Cur, and Mg-1 only the 69,000 band was prominent. 3)The protein band at 200,000 in Spo- showed approximately twice the accumulation seen in any other strain. 4)In Fru- and Mg-2 the protein band at 22,000 showed approximately one-half the accumulation as seen in any other strain. In the silver stained gel at nine hours (Fig. 14) one other major difference became evident. In Fru-, Spo-, and Mg-1 the protein band at 42,000, which appeared as a singlet in the coomassie-blue stained gel, appeared as a protein doublet. The same 42,000 band was seen as a singlet in wild type, Cur, and Mg-2.

In the macrocyst pathway, the wild type gel profiles at initiation of development (0 hr)(Figs. 15 and 16)

Figure 15. SDS-PAGE comparison of coomassie-blue stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides zero and three hours into macrocyst development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.

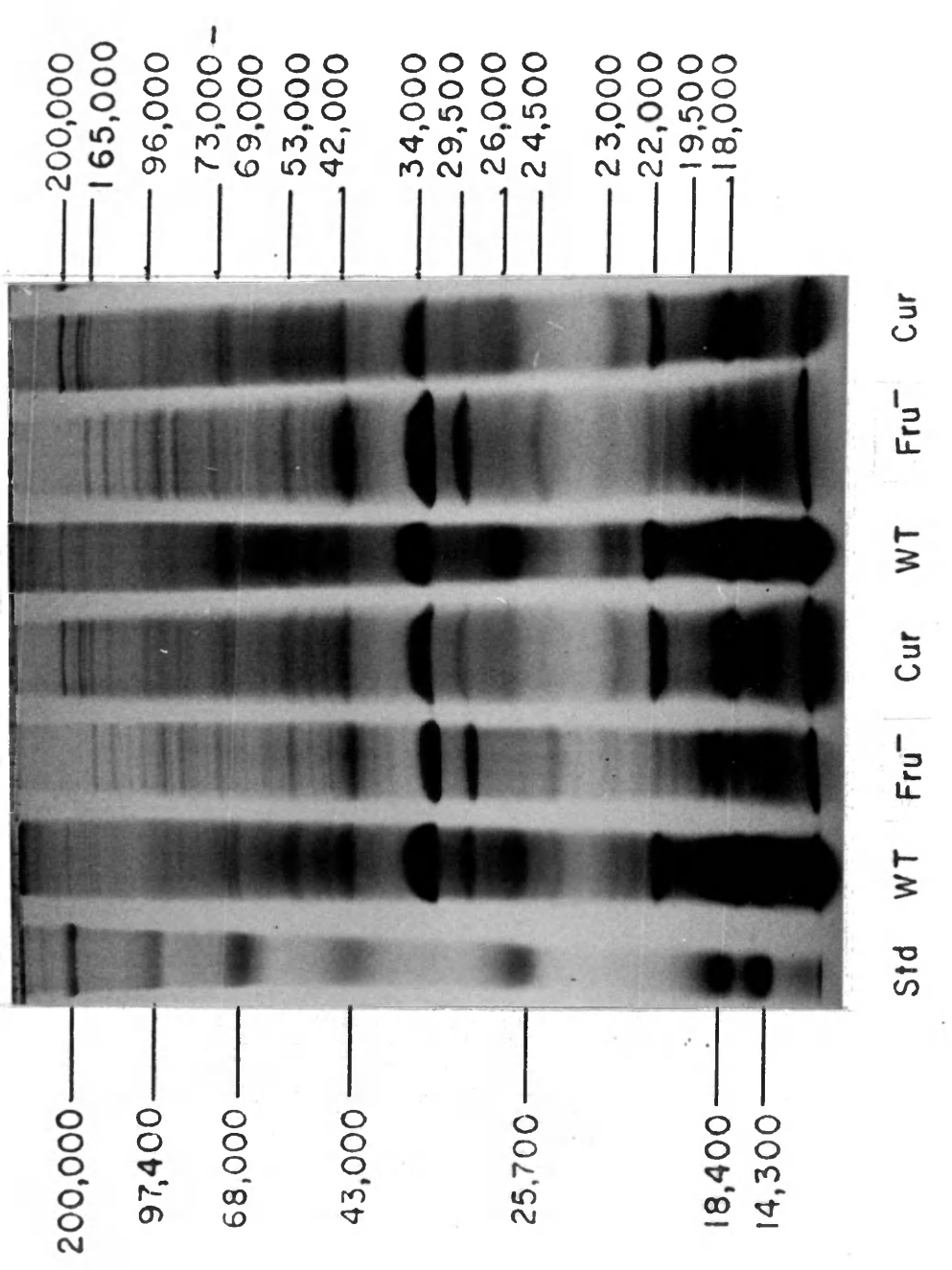
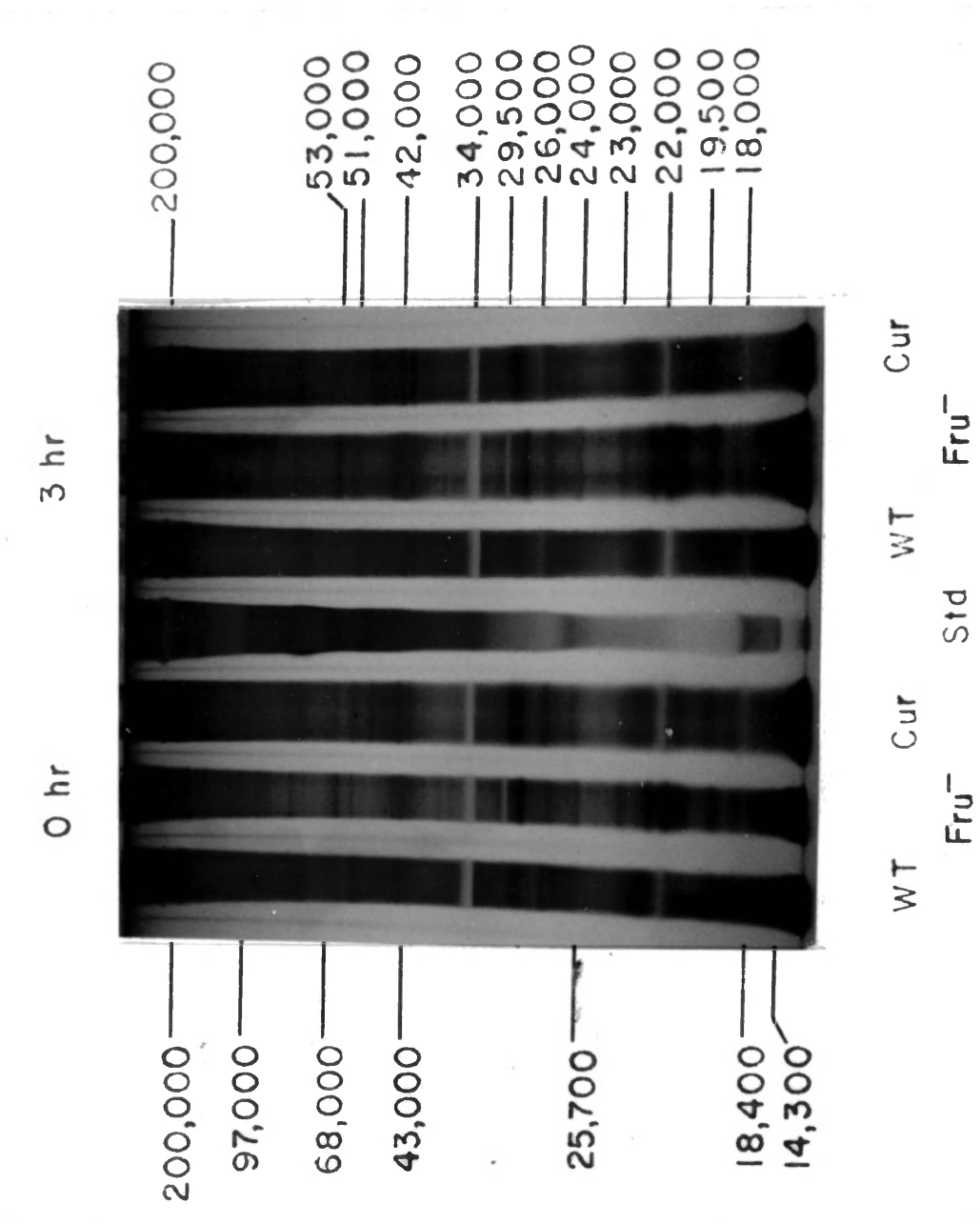

200,000
165,000
96,000
73,000 -
69,000
53,000
42,000
34,000
29,500
26,000
24,500
23,000
22,000
19,500
18,000
200,000
97,400
68,000
43,000
25,700
18,400
14,300
Std
WT
Fru⁻
Cur
WT
Fru⁻
Cur

Figure 16. SDS-PAGE comparison of silver stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides zero and three hours into macrocyst development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.


0 hr
3 hr
WT
Fru⁻
Cur
Std
WT
Fru⁻
Cur
200,000
97,000
68,000
43,000
25,700
18,400
14,300
200,000
53,000
51,000
42,000
34,000
29,500
26,000
24,000
23,000
22,000
19,500
18,000

were almost identical with the zero hour gel profiles of wild type from the sorocarp pathway (Figs. 7 and 8). Protein accumulation and disappearance as development proceeded in the macrocyst pathway was also very similar to that previously described for the sorocarp pathway (Table VII), however, there were a few notable exceptions. At three hours, a strong accumulation of a 23,000 protein band occurred and then began to disappear at six hours until it became barely visible by nine hours. Also, the protein bands at 87,000, 72,000 to 69,000, and 18,000 accumulated in much higher concentrations from three to nine hours than was evident in the sorocarp pathway.

Both of the developmentally aberrant mutants in the macrocyst pathway showed distinct differences in their gel profiles when compared to the wild type gel profiles at the corresponding developmental times (Figs. 15 through 18). Fru-, at zero, three, six, and nine hours, did not produce detectable protein bands at 200,000, 69,000, 26,000, or 22,000. Bands were present at all times in these locations in wild type and Cur gel profiles (Tables VII through IX). At zero and three hours of development, Fru- gel profiles showed higher concentrations of proteins at 165,000, 96,000, 72,000,

Figure 17. SDS-PAGE comparison of coomassie-blue stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides six and nine hours into macrocyst development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.

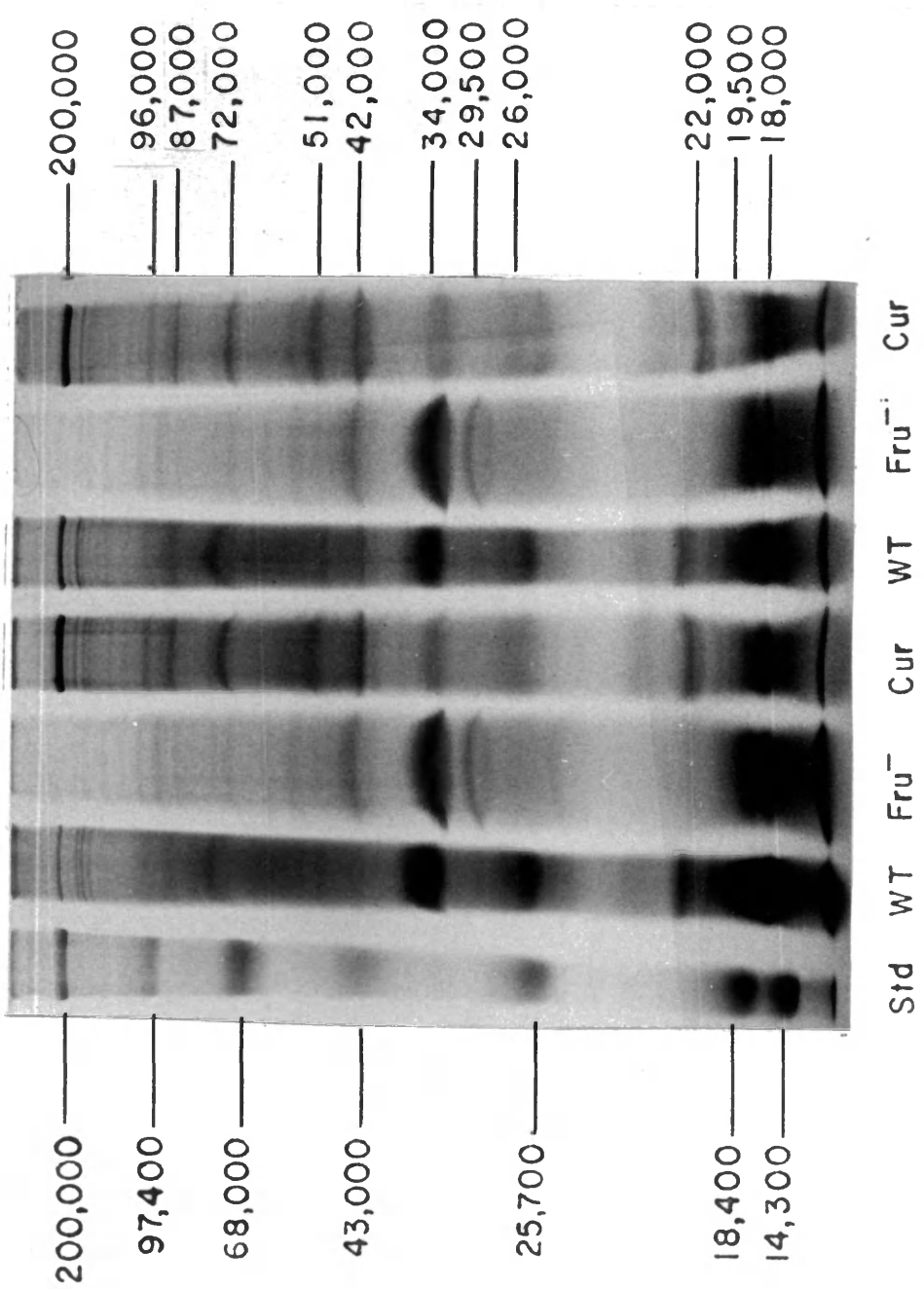

200,000
96,000
87,000
72,000
51,000
42,000
34,000
29,500
26,000
22,000
19,500
18,000
200,000
97,400
68,000
43,000
25,700
18,400
14,300
Std
WT
Fru⁻
Cur
WT
Fru⁻
Cur

Figure 18. SDS-PAGE comparison of silver stained membrane proteins from wild type and developmentally aberrant mutants of D. mucoroides six and nine hours into macrocyst development. Membrane proteins were separated with a 12.5% polyacrylamide gel. Molecular weights of protein bands which showed variation are indicated on the right while the molecular weights of the protein standards are listed on the left.

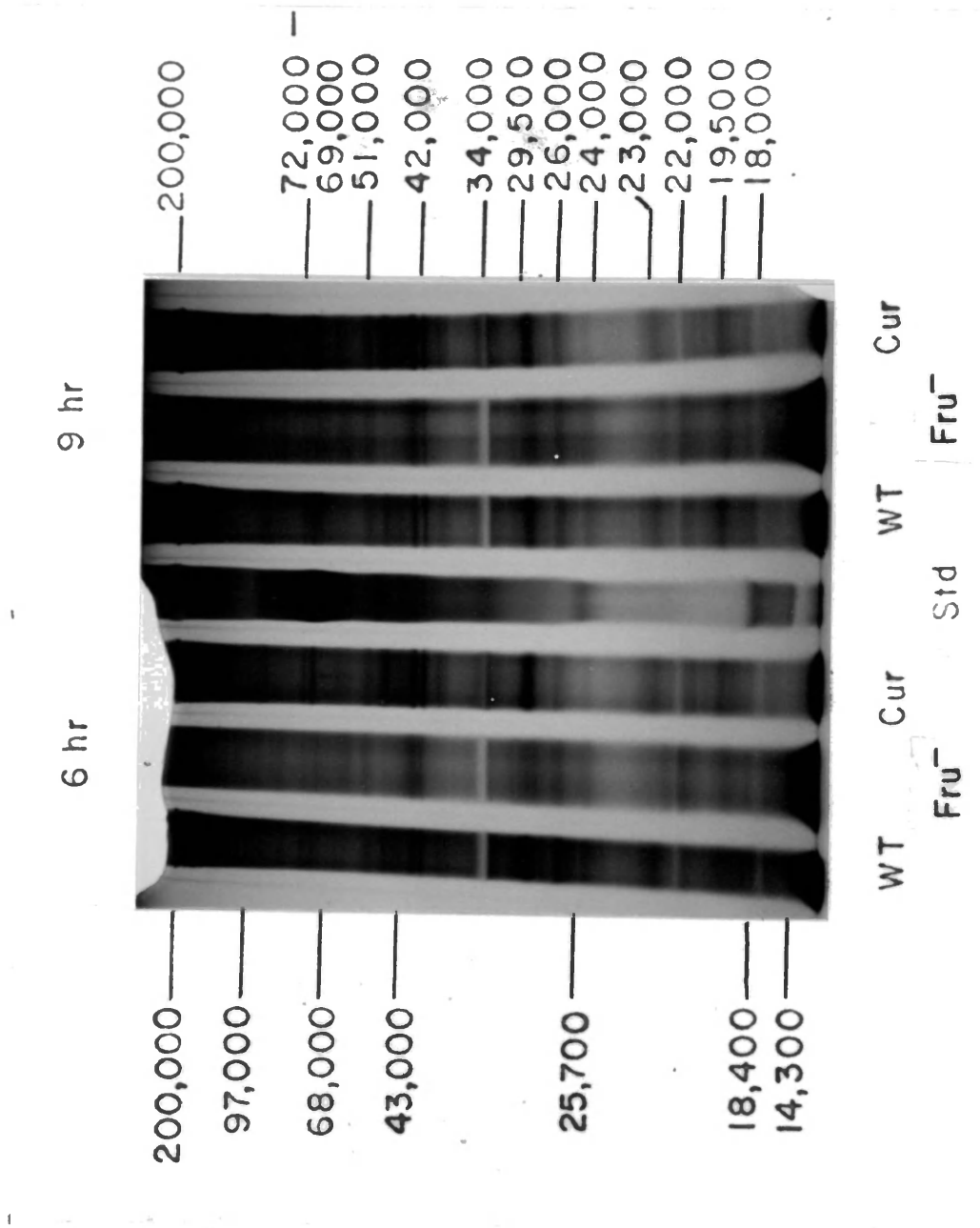

6 hr
9 hr
200,000
97,000
68,000
43,000
25,700
18,400
14,300
200,000
72,000
69,000
51,000
42,000
34,000
29,500
26,000
24,000
23,000
22,000
19,500
18,000
WT
Cur
Fru⁻
Std
WT
Cur
Fru⁻

Table VII. Variation in membrane proteins of wild type *D. mucoroides* at various times during macrocyst development[a]

	Time of sample (hr)			
	0	3	6	9
Protein mol wt				
200,000	X	↑	↑	↑
165,000	0	0	0	0
96,000	X	→	→	→
87,000	X	↑	↑	↑
72,000	X	↑	↑	→
69,000	X	↑	→	→
53,000	X	↓	0	0
51,000	X	↑	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	→	→	→
34,000	X	→	→	→
29,500	X	↓	→	→
26,000	X	→	→	→
24,000	X	↓	0	0
23,000	X	↑	↓	→
22,000	X	→	→	→
19,500	X	→	→	→
18,000	X	→	→	→

[a] 0-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

Table VIII. Variation in membrane proteins of the developmentally aberrant mutant Fru⁻ at various times during macrocyst development[a]

	Time of sample (hr)			
	0	3	6	9
Protein mol wt				
200,000	O	O	O	O
165,000	X	→	↓	→
96,000	X	→	↓	→
87,000	X	↓	↓	O
72,000	X	↓	↓	O
69,000	O	O	O	O
53,000	X	→	→	→
51,000	X	→	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	↑	→	↓
34,000	X	↑	↑	↑
29,500	X	→	↓	→
26,000	O	O	O	O
24,000	X	↓	↓	O
23,000	X	↓	O	O
22,000	O	O	O	O
19,500	X	→	→	→
18,000	X	→	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

Table IX. Variation in membrane proteins of the developmentally aberrant mutant Cur at various times during macrocyst development[a]

	Time of sample (hr)			
	0	3	6	9
Protein mol wt				
200,000	X	↑	↑	↑
165,000	O	O	O	O
96,000	X	→	→	→
87,000	O	O	X	→
72,000	X	→	→	→
69,000	X	→	→	→
53,000	X	↓	O	O
51,000	X	→	→	→
47,500	X	→	→	→
44,500	X	→	→	→
42,000	X	→	→	→
34,000	X	↓	↓	↓
29,500	X	↓	→	→
26,000	X	→	→	→
24,000	O	O	O	O
23,000	X	→	↓	↓
22,000	X	→	↓	→
19,500	O	O	X	→
18,000	X	↑	→	→

[a] O-indicates the absence of protein band.
X-indicates the presence of protein band.
↑-indicates an increase in protein concentration when compared with the corresponding protein band in the previous sample.
↓-indicates a decrease in protein concentration when compared with the corresponding protein band in the previous sample.
→-indicates the protein concentration remained the same when compared with the corresponding protein band in the previous sample.

29,500, 24,000, and 19,500 than did wild type and Cur profiles. Other notable differences were protein doublets at 19,500 and 18,000 in Fru- which appeared as singlets in wild type and Cur. By six and nine hours, protein bands at 34,000 and 29,500 in Fru- began to show a slight smile in the coomassie-blue stained gels while at the same time there appeared to be an overall drop in concentration of almost all of its membrane proteins. Although Cur had zero, three, six, and nine hour protein profiles that were similar to wild type (Figs. 15 through 18) there were two exceptional differences in protein accumulation and disappearance (Table IX): 1)The protein band at 200,000 in Cur accumulated much faster and to approximately three times the concentration as in wild type. 2)The protein band at 34,000 disappeared almost entirely from zero to nine hours in Cur while it remained relatively unchanged in wild-type.

Protein band color in silver stained gels:

Protein bands stained with the modified silver stain procedure appeared in a variety of different colors rather than in the single color characteristic of coomassie-blue. Tables X and XI give the color of the protein bands in the wild type and developmentally aber-

Table X. Protein band color in silver stained gels in the sorocarp pathway

Protein mol wt	Color
200,000	Brown
165,000	Brown
96,000	Brown
87,000	Brown
72,000	Brown
69,000	Brown
53,000	Brown
51,000	Brown
47,500	Brown
44,500	Brown
42,000[a]	Tan/Brown
34,000	Yellow
29,500[b]	Yellow/Brown
26,000	Yellow
24,000[c]	Yellow→Brown
23,000	Brown
22,000	Yellow
19,500[c]	Yellow→Brown
18,000	Blue-Gray

[a]In strains Fru$^-$, Spo$^-$, and Mg-1 the protein doublet is composed of a tan upper band and a brown lower band while in wild-type, Cur, and Mg-2 the protein singlet is a brown band.

[b]At zero hours appears as a doublet in all strains with a yellow upper band and a brown lower band. By six hours appears as a single brown band in all strains.

[c]Color before the arrow is how band appeared at the beginning of development while color after the arrow indicates how the band appeared at the end of development.

Table XI. Protein band color in silver stained gels in the macrocyst pathway

Protein mol wt	Color
200,000	Brown
165,000	Brown
96,000	Brown
87,000	Brown
72,000	Brown
69,000	Brown
53,000	Brown
51,000	Brown
47,500	Brown
44,500	Brown
42,000[a]	Tan/Brown
34,000[b]	Yellow
29,500[c]	Yellow/Brown
26,000	Yellow
24,000	Tan
23,000	Brown
22,000	Yellow
19,500	Brown
18,000	Blue-Gray

[a] In all strains appeared as a protein doublet with a tan upper band and a brown lower band.
[b] In Cur this band became orange by six and nine hours.
[c] Appears as a doublet in Fru$^-$ with a yellow upper band and a brown lower band while in wild-type and Cur it appears as a single brown band.

rant mutant strains in the sorocarp and macrocyst pathways. The majority of the protein bands from the different strains and pathways stained identically with the most common protein band color being brown. However, in both the sorocarp and macrocyst pathways protein bands at 34,000, 26,000, and 22,000 appeared bright yellow while at the same time an 18,000 molecular weight protein stained blue-gray.

Other staining differences in the sorocarp pathway were: 1)Protein bands at 24,000 and 19,500 that appeared yellow at zero hours became brown by nine hours. 2)In strains Fru-, Spo-, and Mg-1 the protein doublet at 42,000 was composed of a tan upper band and a lower brown band while in wild type, Cur, and Mg-2 the protein singlet at 42,000 was brown. 3)At zero hours, the protein bands at 29,500 appeared as a doublet (in all strains with the exception of Cur) with a yellow upper band and a brown lower band which by six hours changed to a single brown band in all strains.

In the macrocyst pathway: 1)The protein band at 24,000 was tan at all times rather than changing from yellow to brown as in the sorocarp pathway. 2)In wild type, Fru-, and Cur the protein at 42,000 was a doublet with a tan upper band and a brown lower band. 3)The

protein bands at 29,500 appeared as a doublet in Fru- with a yellow upper band and a brown lower band but appeared as a brown singlet in wild type and Cur. 4)The 34,000 protein band in Cur progressively changed from yellow at zero hours to orange by nine hours.

Discussion

Mutagenesis:

When isolating developmentally aberrant clones, ideally the morphogenetic defects arise from a single gene mutation. However, inducing mutations with a potent chemical mutagen such as N-methyl-N'-nitro-N-nitrosoguanidine can result in multiple mutational events (Liwerant and Dasilva, 1975). The induced mutagenesis of the vegetative myxamoebae of Dictyostelium mucoroides resulted in the isolation of five developmentally aberrant mutant strains. Three of the strains, Spo-, Mg-1, and Mg-2, showed different morphogenetic defects in the sorocarp pathway but were normal in the formation of mature macrocysts. The remaining two, Fru- and Cur, exhibited morphogenetic defects in both pathways. This suggested, while the overall developmental mechanisms were different between the sorocarp and macrocyst pathways, the two pathways shared certain components. The phenotypes of the two groups of mutants isolated in this investigation could be explained if the major developmental mechanisms which determined sorocarp and macrocyst formation differed or diverged at a point after aggregation, as suggested by Filosa et al.

(1975). Therefore, characteristics that were specific for sorocarp development, such as spore formation and stalk length, could be aberrant in Spo-, Mg-1, and Mg-2 while the macrocyst pathway was identical to wild type. Likewise, the mutant Cur, which showed a defect in the structure of the stalk in sorocarp formation and an absence of a primary wall in macrocyst formation, could have been aberrant due to the failure of a separate developmental process used in both pathways.

Fru- is blocked after the aggregation stage in both the sorocarp and macrocyst pathways. This argues that development proceeds along the same pathway through aggregation but then separates into alternative branches. Work by Filosa et al. (1975) supports this hypothesis. They determined that the aggregate and pseudoplasmodial stages of D. mucoroides produce mature macrocysts 78% and 21% of the time, respectively, when taken from environmental conditions favoring sorocarp formation and placed into conditions favoring macrocyst formation. Likewise, when myxamoebae at various stages of macrocyst development are placed into conditions favoring sorocarps, they found 100% of the precyst aggregates form fruiting bodies, whereas, after the precyst aggregates secrete a slime covering no sorocarps are formed. They proposed once cell differentiation begins to take place,

in either the sorocarp or macrocyst pathways, those cells are irreversibly committed to the formation of their respective fruiting structures. This implies that at the start of cell differentiation there are separate developmental mechanisms for each pathway. Therefore, if Fru- is blocked before the point where cell differentiation is induced then a fruiting structure could not be formed by either pathway.

While Spo- possessed a normal macrocyst cycle, its sorocarp pathway was abnormal in cell differentiation. Instead of forming both spore cells and stalk cells, the vegetative amoebae of Spo- differentiated into stalk cells exclusively. This ability of Spo- to differentiate entirely into stalk cells suggested that the process of cell differentiation in the sorocarp pathway may be controlled by two separate mechanisms. This idea of separate developmental branches controlling stalk and spore cell formation was first proposed by Francis (1969), and has been further supported by various investigations. For example, Town and Stanford (1979) isolated a Differentiation-Inducing Factor (DIF) in D. discoideum that, in the presence of cAMP, stimulates stalk cell but not spore cell differentiation. Brookman et al. (1982) also found that DIF had similar effects upon vegetative myxamoebae in D. mucoroides. Although

these studies are by no means conclusive, they strongly suggest that there are separate mechanisms which control stalk and spore cell differentiation. Therefore, a possible explanation for the Spo- phenotype was that while the developmental mechanism for stalk formation was normal, an essential component was either improperly produced or not perceived by the developmental mechanism controlling spore formation.

In Mg-1, although the spore mass was of normal size, the stalk length was approximately one-half the length of wild type. This phenotype was almost identical to that of a mutant isolated by MacWilliams (1982) in D. discoideum. Whereas, in wild type fruiting structures the stalk comprises approximately 10-20% of the sorocarp structure, the mutant isolated by MacWilliams forms fruiting bodies which contain only 8% stalk by volume. A corresponding alteration in the normal prestalk:prespore ratios led MacWilliams and David (1984) to propose a negative-feedback model for cell proportioning. In this system, they suggest that the proportion of prestalk cells is negatively controlled by an inhibitor of prestalk differentiation which is produced by the prestalk cells. Therefore, prestalk differentiation (and the accumulation of inhibitor) would continue until the inhibitor reaches the critical level

that will block further prestalk differentiation. Although alterations in the prestalk:prespore ratios were not determined for Mg-1 there was a reduction in the stalk length and presumably fewer prestalk cells were formed. Therefore the Mg-1 phenotype could be explained using this negative-feedback model. If Mg-1 had a higher sensitivity level than the wild type to the prestalk inhibitor then fewer prestalk cells would be formed and a shorter stalk would be produced. Alternatively, if the prestalk cells of Mg-1 were producing higher concentrations of inhibitor, then the critical concentration blocking prestalk differentiation would be reached much faster and would result in a stalk which was reduced in length.

Mg-2, like Mg-1, formed stalks which were much shorter than in wild type. However, since both the stalk length and spore mass size were reduced in Mg-2 it was unlikely that the Mg-2 phenotype was due to a mutation in the cell proportioning mechanism suggested for Mg-1. Instead, the Mg-2 phenotype may be determined by a mutation in an inhibitor system which controls aggregation size. As previously described, aggregates are formed during development when certain myxamoebae establish themselves as aggregation centers and begin to send out pulses of the attractant cAMP. Shaffer (1963)

in experiments with Polysphondylium violaceum demonstrated that these "founder" cells also release a substance which inhibits other "founder" cells from being formed. Therefore, it could be that the small, closely spaced aggregates which were observed in Mg-2 were caused by a mutation in the "founder" cell inhibitor. If less inhibitor was being produced, or if the myxamoebae were less sensitive to the inhibitor, then a larger number of aggregation centers could be established. When more aggregation centers are established then each aggregate would contain fewer myxamoebae and smaller fruiting structures will be formed as was seen in Mg-2.

Of the five developmentally aberrant mutants used in this study, Cur was the most interesting. The absence of a primary wall in the mature macrocysts and the fragile, twisted stalks formed in the sorocarp pathway suggested that some structural component(s) was either blocked in its production or was produced but nonfunctional. One possible component, cellulose, which comprises the majority of the stalk structure as well as much of the macrocyst wall composition (Harrington and Raper, 1968), was assayed in Cur by staining with the fluorescent brightener, calcofluor white, and was found to be present in normal levels. Other structural components have yet to be identified, so no other assays

were performed.

Cur was the first macrocyst mutant isolated and its unusual morphogenetic pattern seemed to contradict previous descriptions of macrocyst development. Erdos et al. (1972) and Filosa and Dengler (1972) suggested the meeting of the primary wall and the "giant" cytophagic cell is necessary before the secondary wall can be secreted between the two. Cur, however, secreted the secondary wall without the presence of a primary wall or a cytophagic cell. This indicated that the secretion of the secondary wall may actually be an independent event.

Analysis of membrane proteins:

The overall pattern of accumulation and disappearance of membrane proteins in the sorocarp pathway of wild type and developmentally aberrant mutant strains seemed to be consistent with the concept suggested by Loomis et al. (1978) that a linear dependent pathway controls the temporal expression of membrane protein synthesis and accumulation. Although mutants Fru-, Spo-, Mg-1, and Mg-2 appeared to be temporally behind wild type and Cur, the overall pattern of membrane protein synthesis and accumulation was virtually the same for all strains. Thus, a possible order of protein accumulation and disappearance in D. mucoroides was

suggested.

Analysis of the zero hour protein profiles of each strain revealed large differences in the membrane proteins of the preaggregate myxamoebae even though the myxamoebae from all strains were harvested at similar cell densities. Although wild type and Cur accumulated similar proteins in preparation for development, Fru-, Spo-, Mg-1 and Mg-2 appeared to be temporally behind. This was confirmed when the zero hour protein profiles of Fru-, Spo-, Mg-1 and Mg-2 were compared with the protein profiles of exponential phase myxamoebae reported by Hanson and Weber (1985) for wild type myxamoebae of D. mucoroides. The delay in the temporal accumulation of proteins was ascertained for Fru-, Spo-, Mg-1 and Mg-2 by determining the time at which their gel profiles matched those of the wild type zero hour profiles. It was found that Spo-, Fru- and Mg-2 were approximately three hours behind while Mg-1 was approximately seven hours behind the wild type.

A large number of membrane proteins were seen throughout development. The more obvious of these proteins had molecular weights of 96,000, 51,000, 47,500, 44,500, 42,000, 34,000 and 19,500 and possibly were structural components of the membrane. The rest of the isolated proteins appeared or disappeared during devel-

opment. Between late exponential growth phase and the initiation of development, the first band to disappear was a protein with a molecular weight of 23,000. It was followed by proteins at 53,000, 29,500, and 24,000. As these bands disappeared, proteins with molecular weights of 26,000 and 22,000 began to accumulate. Also, there was a concentration change in the protein band at 34,000. Between late exponential phase and the initiation of development this band initially dropped in concentration, however, after development began it remained relatively unchanged. This protein behaved similarly to a 34,000 molecular weight protein reported by Siu et al. (1977) in D. discoideum.

Between the initiation of development and early aggregation (3 hrs) a second group of proteins began to change. A protein doublet at 165,000 disappeared while proteins at 200,000, 87,000, 69,000, and 18,000 all began to accumulate. Gilkes et al. (1979) described proteins at 220,000 and 91,000 that accumulated during aggregation in D. discoideum. These could be analogous to the 200,000 and 87,000 proteins observed in this study. The overall protein patterns remained relatively unchanged between aggregation and early culmination (9 hrs) with the exception of a reduction in the concentration of the 22,000 molecular weight protein. A

22,000 molecular weight protein isolated by Gilkes et al. (1979) in D. discoideum also dropped in concentration after aggregation.

In the macrocyst pathway the overall pattern of protein accumulation was similar to that described for the sorocarp pathway. However, there were some notable exceptions. First, the protein band at 42,000, which was unchanged in the sorocarp pathway, began to drop in concentration in the macrocyst pathway after aggregation. Second, the 53,000 molecular weight protein, which disappears during late exponential phase in the sorocarp pathway, was present throughout aggregation in macrocyst development. Finally, the protein band at 23,000, which also disappeared during late exponential phase in the sorocarp pathway, continued to accumulate throughout development in the macrocyst pathway.

Only a few membrane bound proteins have been identified in the cellular slime molds. Some of these are: 1)Actin, which was reported to have a molecular weight of 42,000 (Spudich, 1974). 2)Myosin, which has two heavy chains, each having a molecular weight of 210,000, and two classes of light chains with molecular weights of 18,000 and 16,000 (Clarke and Spudich, 1974). 3)Discoidin I and II, two surface lectins discovered in D. discoideum, which accumulate at aggregation and are

thought to participate in cell to cell adhesion (Rosen et al., 1973). Discoidin I and II are reported to have molecular weights of 26,000 and 24,000 respectively (Frazier et al., 1975).

The gel profiles of wild type and developmentally aberrant mutants of D. mucoroides had protein bands with molecular weights similar to those reported for actin, mysosin, and discoidin I and II. The 42,000 molecular weight protein found in each strain may very well have been the actin protein described in D. discoideum. Also, since the protein bands at 200,000 and 18,000 appeared in the gel profiles of each strain at the same time I propose that these two proteins are the heavy and light chains of myosin previously described for D. discoideum. Finally, each strain of D. mucoroides accumulated proteins with molecular weights of 26,000 and 22,000 during early aggregation. These proteins were very similar in molecular weight to the 26,000 and 24,000 molecular weight proteins of discoidin I and II and may also have been lectins which function in cell to cell adhesion.

The majority of the membrane proteins were very similar by nine hours in wild type and the developmentally aberrant mutant strains in both sorocarp and macrocyst development. It was possible that the phenotypic

differences seen in the the mutant strains were caused by aberrations in the cytoplasmic proteins rather than differences in the membrane proteins. Also some differences in the membrane proteins may have gone unnoticed since simple substitutions, insertions or deletions may not have altered the molecular weight of a protein enough to be resolved with SDS-PAGE. So, even though a protein was nonfunctional and contributed to the aberrant phenotype it might still have appeared to be normal in the gel profile.

Some of the differences in the membrane proteins from wild type and mutant strains in the sorocarp pathway were: 1)The 200,000 molecular weight band in Spo-, the 34,000 molecular weight band of Mg-1, and the 22,000 molecular weight band in Fru- and Mg-2 differed in concentration from the corresponding bands in wild type. 2)A prominent protein with a molecular weight of 69,000 in wild type, Cur and Mg-2 appeared as three protein bands with molecular weights between 69,000 and 72,000 in Fru-, Spo- and Mg-1, with the 72,000 molecular weight protein being the most prominent. 3)In the silver stained gels, the protein band at 42,000 appeared as a singlet in wild type, Cur and Mg-2 but was a protein doublet in Fru-, Spo- and Mg-1.

Differences in the membrane proteins of wild type,

Fru-, and Cur were more distinct in the macrocyst pathway. For example, Fru- appeared to be blocked in the synthesis and accumulation of new membrane proteins throughout development. In fact it appeared that many of the proteins began to disappear during the later sample times. Since Fru- was blocked soon after aggregation its protein profile may indicate which membrane proteins were either produced during or necessary for later stages of development. One problem with this was if Fru- was blocked at the same point in both sorocarp and macrocyst development and if development proceeded in a linear fashion through cell differentiation, as previously suggested, then Fru- would not be expected to have different protein patterns in the two pathways. One explanation may be that Fru- was mutated in some cytoplasmic factor that blocked development in both pathways and was also necessary to initiate membrane protein synthesis in the macrocyst pathway but not the sorocarp pathway.

The mutant Cur also showed distinct differences in the membrane proteins when compared to wild type. As development proceeded the 200,000 molecular weight protein accumulated to almost three times the concentration of the corresponding protein in the wild type. If this protein were a subunit of the myosin protein then

it might have been expected to accumulate the 18,000 molecular weight band at the same rate as the 200,000 molecular weight band. However, because of the darkness of the proteins at the gel front this could not be determined. The 34,000 molecular weight protein also behaved much differently in Cur than in wild type. As development proceeded it almost totally disappeared from the gel profiles. Because these two proteins accumulated and disappeared at approximately the same rate it was possible both had a role in primary wall formation.

In the silver stained gels, there were variations in the colors of the protein bands. While the majority of the bands were brown, proteins with molecular weights of 34,000, 29,500, 26,000, 24,000, 22,000 and 19,500 were yellow either throughout development or at times during development. One other protein, with a molecular weight of 22,000, appeared as a blue-gray band. It has been suggested that color might be used to characterize protein groups (Adams et al., 1981). The colors produced in silver stained gels are due largely to the physical effects of a protein and its ability to combine metallic silver. Although, little work has been done in this area Goldman et al. (1980) have reported in their study of human cerebrospinal fluid proteins that some lipoproteins stained blue while some glycoproteins ap-

peared as yellowish-brown and red bands.

In this investigation a series of developmentally aberrant mutants were isolated from the sorocarp and macrocyst pathways of D. mucoroides. Inferences drawn from the observation of development in these mutants suggested that: 1)Commitment to development along either the sorocarp or macrocyst pathway occured after aggregation in D. mucoriodes. 2)Separate mechanisms controlled stalk and spore formation in the sorocarp pathway. 3)The primary wall was not required for secondary and tertiary wall formation during macrocyst development. Further, variations in the membrane proteins between wild type and developmentally aberrant mutants were also demonstrated, certain proteins from the mutants accumulated and disappeared at different times and rates than the corresponding proteins from wild type.

Summary

Membrane proteins from five developmentally aberrant mutants were compared with membrane proteins from wild type Dictyostelium mucoroides at four different times during development toward sorocarps and macrocysts. The developing myxamoebae were harvested from filters and their membranes isolated using a two-phase polymer system of dextran and polyethylene glycol. Proteins were extracted with sodium dodecyl sulfate and separated on 12.5% polyacrylamide gels. Myxamoebae of one mutant, Fru-, aggregated but failed to differentiate into mature fruiting structures in either pathway. Mutant Cur produced sorocarps with aberrant stalks and small macrocysts that lacked the primary wall. Mutants Spo-, Mg-1 and Mg-2 all formed normal macrocysts but produced aberrant sorocarps. The vegetative myxamoebae of Spo- differentiated into stalk cells but failed to form spore cells. Mg-1 and Mg-2 showed different degrees of stunted sorocarps. Although the majority of the membrane proteins were similar between strains there were some differences. Certain proteins from the mutants accumulated and disappeared at different times and rates than the cooresponding proteins from the wild type.

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