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MEMBRANE PROTEIN VARIATION IN *DICTYOSTELIUM MUCOROIDES* DURING DEVELOPMENT ALONG ALTERNATIVE PATHWAYS

A Thesis

Presented to the

Department of Biology

and the

Faculty of the Graduate College

University of Nebraska at Omaha

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Nancy Jo Dohse Hanson

December, 1984

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THESIS ACCEPTANCE

Accepted for the faculty of the Graduate College, University of Nebraska, in partial fulfillment of the requirements for the degree Master of Arts, University of Nebraska at Omaha.

Committee

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Nov 28, 1984
Date

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Many people have helped me to accomplish the research in this thesis as well as the writing itself.

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A special thanks to my committee, Dr. Barbara Hayhome, Dr. Ann Antlfinger, Dr. Eric Manley and Dr. Bob Ramaley, for the use of lab space and equipment as well as time and energy during the research and writing.

I would like to thank Dave Kelly for helping develop and mount the pictures for this thesis.

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Introduction

Dictyostelium is a genus of cellular slime mold which is important in developmental research. The first cellular slime mold to be discovered was *Dictyostelium mucoroides*. It was discovered by Brefeld in 1869. However, the cellular slime mold most widely used in developmental research today is *Dictyostelium discoideum*, discovered by Raper in 1935.

The asexual life cycle, or sorocarp pathway, of both organisms is similar. Both begin with spore germination, each spore producing a single uninucleate amoeba. The amoebae enter the vegetative phase during which they multiply by binary fission and use bacteria as the main source of food. Upon starvation the amoebae aggregate and form a pseudoplasmodium, or slug. In *D. discoideum* the slug migrates freely while in *D. mucoroides* the slug produces a stalk as it migrates. After migration ceases, culmination, the formation of the final fruiting body, begins. In this stage the slug stops all forward movement and begins an upright motion which allows the beginning of upright stalk formation. Stalk cells are formed by a progressive vacuolization of cells, during which time the cells deposit cellulose walls. Culmination results in the

production of the sorcarp which consists of a cellular stalk with a mass of spore cells at its apex. The sorocarp pathway is favored by certain environmental conditions such as light, low humidity, high phosphate concentration, and low calcium concentration (Loomis, 1982). It is the sorocarp pathway that is more easily produced in the lab and thus it has been the pathway of choice for developmental experiments.

In 1957 Blaskovics and Raper discovered a second resistant form of the cellular slime molds, which they called macrocysts (Blaskovics and Raper, 1957). It was suggested that macrocyst formation was part of the sexual cycle of Dictyostelium (Erdos et al, 1972). In D. discoideum two mating types were required for macrocyst formation, thus demonstrating its heterothallic nature, whereas D. mucoroides was found to be homothallic (Clark et al, 1973).

Macrocyst development is favored by environmental conditions such as darkness, high humidity, low phosphate concentration, high population density, and high calcium concentration (Nickerson and Raper, 1973). Upon starvation and under conditions favoring macrocyst formation the amoebae form loose aggregates. Within each aggregate a "giant" cell appears. This giant cell is formed by the fusion of two cells which then become one large uninucleate cell. As development continues the giant cell begins to

engulf the surrounding amoebae in the aggregate. While engulfment is taking place the aggregate forms a primary wall which will surround the mature macrocyst. Upon engulfment of the amoebae a secondary cellulosic wall is formed on the surface of the giant cell, followed by the appearance of a tertiary wall inside the cellulosic wall. During formation of the secondary wall the ingested amoebae, now known as endocytes, are reduced to smaller bodies. After most of the reduction of the endocysts takes place there is a meiotic and several mitotic divisions (Erdos, 1972). The macrocyst is mature when the endocysts appear granular (Loomis 1982).

Dictyostelium makes an excellent developmental research organism for four reasons: 1) Growth and development take place rapidly compared to other organisms. Depending on the environmental conditions, the organism can reach maturity within twelve hours. 2) The organism is small and requires little space. 3) The overall developmental process is relatively simple. Dictyostelium develops from one cell type, vegetative amoebae, into two different cell types, vacuolated stalk cells and spore cells. This should make investigating the developmental sequence less complicated than in an organism which differentiates into many different cell types. 4) The processes of growth and development in Dictyostelium are completely separate in time. This allows developmental

processes to be studied without the complications of growth.

As mentioned previously, _D. discoideum_ is the most extensively studied cellular slime mold. Some work has been done on membrane bound proteins isolated from various developmental stages in the sorocarp pathway of _D. discodeum_. For example, Siu et al (1977) showed 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 from the plasma membrane after twelve hours. Later in development several new high molecular weight proteins are synthesized. The most prominent being a 60,000 molecular weight protein.

I have chosen to look at variation in membrane bound proteins of _D. mucoroides_ for two reasons. First, the events of development in _D. mucoroides_ have not been investigated at the molecular level. Second, because _D. mucoroides_ requires no other mating type for macrocyst development, differences in membrane bound proteins may be attributed to overall developmental events rather than to differences between mating types. As with other cellular slime molds, _D. mucoroides_ must have specific regulatory mechanisms which cause it to develop by one of the two pathways, sorocarps or macrocysts. I chose to look at membrane bound proteins in particular because these proteins may be the most crucial in regulation. The

environment and cellular cohesion are two important factors in development. Both of these are associated with the cell membrane. The environment seems important in initiating development along a particular pathway and it is the plasma membrane which is the site of contact with the environment. Cell adhesion is a necessary step in aggregation. Signals must be sent and relayed in order for aggregation and orderly development to begin. The site at which these signals are sent and received is the membrane.

The purpose of this research was to determine if membrane protein variation existed in D. mucoroides during development along alternate pathways. In this study, membrane proteins extracted from amoebae developing toward sorocarps and amoebae developing toward macrocysts were separated using sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the resulting protein bands were evaluated. Protein variation was found between developmental times in the same pathway as well as between pathways.

Material and Methods

Organism: D. mucoroides strain Dm7 was the organism used in this investigation. The amoebae were grown in

association with <u>Escherichia</u> <u>coli</u> B/r as their source of nutrient. Stock cultures of <u>D</u>. <u>mucoroides</u> were maintained on either 0.1% lactose-peptone agar plates or glucose-salt agar plates (Adam, 1959).

<u>Growth</u> <u>Conditions</u>: Growth flasks (250 ml erlenmeyer) containing 40ml of glucose-yeast-peptone medium (GYP) (Weber and Raper, 1971) were inoculated with 1.0 x 10^5 spores and a loopful of <u>E</u>. <u>coli</u> B/r. Incubation took place in a shaking water bath at 23 C for 56 hours. After incubation the amoebae were harvested from the liquid culture by centrifugation at 250 x g for 12 minutes and washed three times in cold (4 C) Bonner's Salt Solution (BSS) (Bonner and Frascella, 1953). The pellet was suspended in cold buffer appropriate for the desired pathway. For the sorocarp pathway a phosphate buffer (0.025M, pH 6.5) was used and for the macrocyst pathway BSS was used.

<u>Vegetative</u> <u>Stages</u>: Two vegetative stages were sampled, 48 and 56 hours after inoculation. These times corresponded to late exponential growth and stationary phase respectively. After washing the amoebae, they were concentrated by centrifugation at 250 x g and the resulting pellet was frozen until the membranes could be isolated.

Sorocarp Development: Petri dishes (48 x 8.5mm) containing absorbant pads, filters (GN 6 metricel, 0.45um) and 2 ml of the phosphate buffer with streptomycin (0.5 mg/ml) were inoculated by placing 5 spots of 0.1 ml on each filter. Each spot contained 3.5×10^8 amoebae/ml. The plates were incubated in the light at 23 C for 3 hours, 6 hours, or 9 hours.

Macrocyst Development: Petri dishes containing pads, filters and 2 ml of BSS with streptomycin (0.5 mg/ml) were inoculated with 0.5 ml of a suspension of amoebae containing 3.5×10^8 amoebae/ml. A second filter was placed on each inoculated filter and 2 additional milliliters of BSS plus streptomycin were added. The plates were wrapped in foil to exclude light and placed in a 23 C incubator for 3 hours, 6 hours or 9 hours.

Cell Harvest and Breakage: Developing cells were washed from the filters with the appropriate cold buffer and concentrated by centrifugation at 450 x g for 15 minutes. The supernatant was discarded and the pellet frozen. Cell breakage was accomplished by agitation with glass beads 450-500 micrometers in size. The pellet of harvested cells was resuspended in 3-4 ml of appropriate buffer and an equal volume of beads was added. The cells were vortexed using 15 second bursts. Cell breakage was monitored at 2

minute intervals using phase microscopy until sufficient breakage had been achieved, usually within 6 minutes. Broken cells were suspended in the appropriate buffer and concentrated by centrifugation in a refrigrated Sorvall centrifuge using a HB4 swinging bucket rotor for 20 minutes at 16,300 x g. The supernatant was discarded and the pellet was now used for membrane isolation.

Membrane Isolation: An aqueous two-phase polymer system of polyethylene glycol and dextran was used to isolate the membranes as described by Brunette and Till (1971) with one modification. The centrifugation velocity used to move the membranes to the interface was reduced from 11,800 x g to 8,000 x g. The amount of membrane collected at the interface increased with this reduction of velocity.

Protein Extraction: Proteins were extracted by boiling the sample for 5 minutes in a solution of 3% SDS, 10% glycerol and 5% mercaptoethanol in 0.0625 M Tris-HCl (pH 6.8). After boiling, the sample was centrifuged in an Eppendorf microcentrifuge for 10 minutes. The supernatant was collected and the pellet discarded.

Alkaline Phosphatase Assay: Alkaline phosphatase was used as an enzyme marker for the membrane (Green and Newell, 1974) and was assayed as described by Loomis (1969). The

fractions obtained using the two-phase aqueous polymer system showed the presence of alkaline phosphatase, thus demonstrating that cell membranes were present.

Protein Determination: Protein determination was achieved by two methods, Lowry (Hanson and Phillips, 1981) and UV spectrophotometry (Schleif et al, 1981). The Lowry method was used to determine the protein concentration of the broken cell pieces and the membrane pellet. The samples were read in a Bausch and Lomb Spectronic 20. Globulin (Bovine) fraction II and III (40% Beta, 30% Gamma) was used as the standard. A UV spectrophotometer (Gilford model 252) was used to determine the amount of protein in the extracted sample. The sample was read at 280nm and 260nm.

Protein Separation: SDS-PAGE was used for protein separation (Laemmli, 1970). A discontinuous system was employed using a tube gel apparatus. A 10% separating gel (5mm x 60mm) was used along with a 3% stacking gel (5mm x 7mm). To each gel a volume of 100ul containing 46.5ug of protein was applied. Fifteen micrograms of a high molecular weight protein standard consisting of myosin (H-chain), 200,000; phosphorylase B, 97,400; bovine serum albumin, 68,000; ovalbumin, 43,000; α-chymotrypsinogen, 25,700; β-lactoglobulin, 18,400; lysozyme, 14,300 (Bethesda Research Laboratories) was used for molecular weight

comparisons. Electrophoresis was carried out at a constant voltage, 100 volts, using a Heathkit regulated H.V. power supply model lP-17, for 3 hours or until the tracking dye was at the end of the gel. The gels were then removed and fixed in 12.5% trichloroacetic acid until the tracking dye turned yellow. The gels were stained with 0.2% Coomassie-brilliant blue R-250 in methanol:glacial acetic acid:water, 50:100:40 for 24 hours. The gels were then destained in methanol:glacial acetic acid:water, 250:75:675 (Ramaley, R. F., personal communication) and stored in 7.5% acetic acid.

Results

Developmental Timing

The exact developmental stage reached by amoebae in the sorocarp pathway, zero, three, six and nine hours into development, was determined by observing the cells microscopically. Zero hour cells consisted of individual amoebae while at 3 hours the cells were beginning early

aggregation. At six hours no sign of stalk formation or migration was visible so development was determined to be in the late aggregation stage. Nine hours into development culmination had begun as indicated by stalk cell differentiation and the upright orientation of the stalk. The morphological stages of development reached at the various sample times are shown in Figure 1. Development of amoebae along the sorocarp pathway was found to be well synchronized.

Developmental timing of the macrocyst pathway was also determined. Amoebae were observed at the same times as in the sorocarp pathway, namely zero, three, six and nine hours into development. At three hours the cells had begun to aggregate as in the sorocarp pathway. At six hours the cells appeared more tightly aggregated and at nine hours they appeared more rounded and very densely aggregated. Photographs of amoebae developing toward macrocysts, along with corresponding developmental times, are shown in Figure 2. As in the sorocarp pathway, development of amoebae along the macrocyst pathway was found to be well synchronized.

Protein Variation

Proteins in the cell membrane fractions were separated by SDS-PAGE. Variation in protein concentration was found between the developmental times of each pathway, sorocarp

Figure 1. The morphological stages of *D. mucoroides* during development of sorocarps on filters. (A) Vegetative amoebae, phase contrast, x 400; (B) 3 hours into development, x 40; (C) 6 hours into development, x 40; (D) 9 hours into development, x 30; (E) mature sorocarps, x 30.

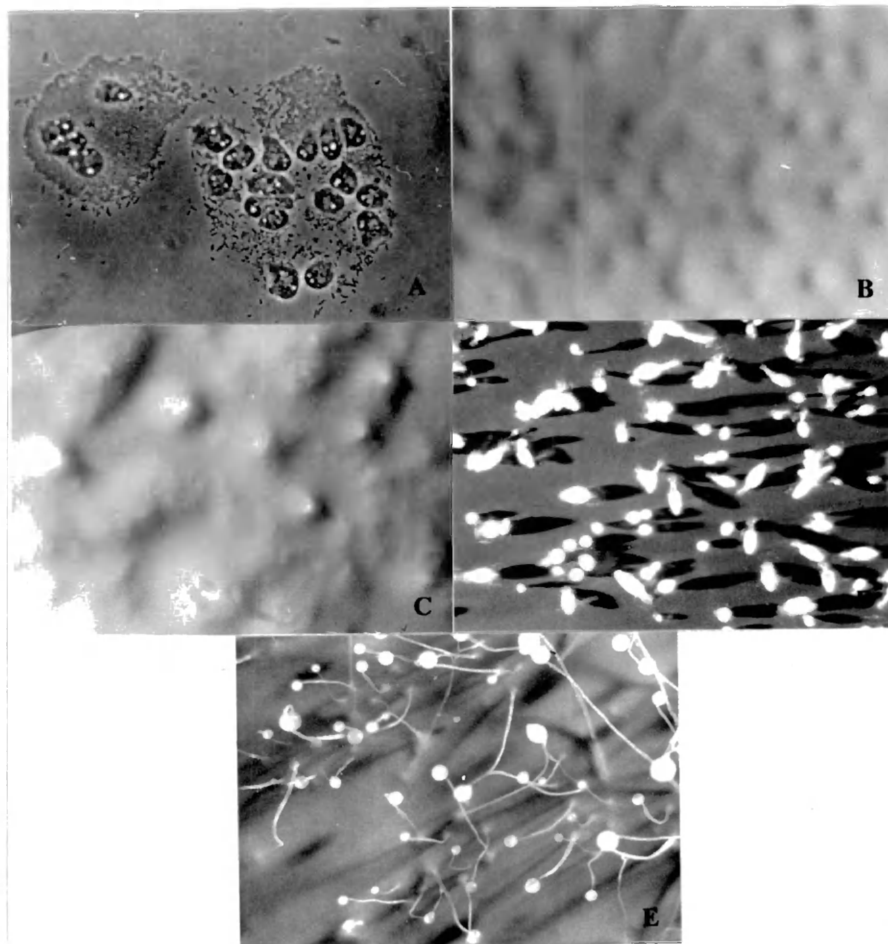
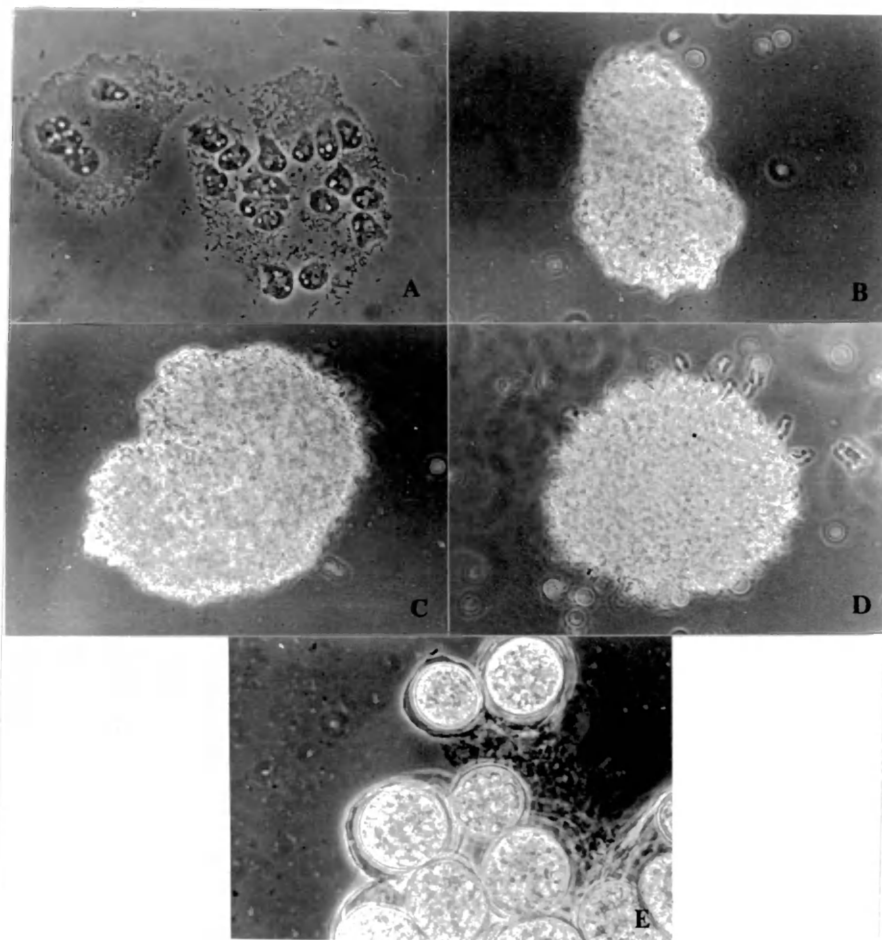

A
B
C
E

Figure 2. The morphological stages of *D. mucoroides* during development of macrocysts on filters. (A) Vegetative amoebae, x 400; (B) 3 hours into development, x 160; (C) 6 hours into development, x 160; (D) 9 hours into development, x 160; (E) mature macrocysts, x 160 (all phase contrast).


A
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D
E

and macrocyst, and between the two pathways at the same developmental times. Variation was defined as an observable change in the concentration of the protein in the bands found on the gel. Some protein bands disappeared. This could indicate a loss of the protein or a decrease in the concentration of the protein below one ug, the minimal amount of protein normally detected by coomassie blue (Hoefer,1983 and Fazekas, 1963).

The variation that was observed is presented in three tables. Table I shows variation at different developmental times for the sorocarp pathway. Table II shows the data for the macrocyst pathway and Table III is a comparison of the two developmental pathways at the same developmental times. Included in the tables of the sorcarp and macrocyst pathways are comparisons of results from samples taken during vegetative growth at 48 hours (exponential phase) and 56 hours (stationary phase).

Variation was found in the sorocarp pathway in proteins with molecular weights of 14,000 to 87,000 (Figure 3). Prominant bands which showed variation had approximate molecular weights of 42,000, 35,000, 26,600, 20,400 and 14,600. The minor bands in which variation occurred corresponded to the approximate molecular weights of 87,100, 67,000, 53,700, 42,660 and 30,200. Table I correlates the protein bands of various molecular weights in which variation occurred with the developmental times.

Table I. Variation in membrane proteins of *D. mucoroides* during growth and development toward sorocarps[a]

	Time of Sample (hr)			
	56[b]	3	6	9
Protein mol wt				
87,100	S	S	I	I
67,600	S	I	D	D
53,700	I	I	D	D
42,660	S	I	S	S
42,000	I	I	D	S
35,000	D	I	I	D
30,200	D	S	S	D
26,600	I	S	S	S
20,400	I	I	D	D
14,600	I	S	S	D

[a] I-indicates an increase in protein concentration compared with the corresponding protein band from the previous sample.
D-indicates a decrease in protein concentration compared with the corresponding protein band from the previous sample.
S-indicates the protein concentration remained the same compared with the corresponding protein band from the previous sample.

[b] Concentration comparisons for protein bands in the 56 hour vegetative sample are relative to the vegetative sample of 48 hours.

Table II. Variation in membrane proteins of D. mucoroides during growth and development toward macrocysts[a]

	Time of sample (hr)			
	56[b]	3	6	9
Protein mol wt				
53,700	I	S	S	I
42,000	I	S	S	S
35,000	D	I	I	D
30,200	D	S	I	D
26,600	I	S	S	S
20,400	I	I	D	I
19,500	I	D	D	I[c]

[a]I-indicates an increase in protein concentration compared with the corresponding protein band from the previous sample.
D-indicates a decrease in protein concentration compared with the corresponding protein band from the previous sample.
S-indicates the protein concentration remained the same compared with the corresponding protein band from the previous sample.

[b]Concentration comparisons for protein bands in the 56 hour vegetative sample are relative to the vegetative sample of 48 hours.

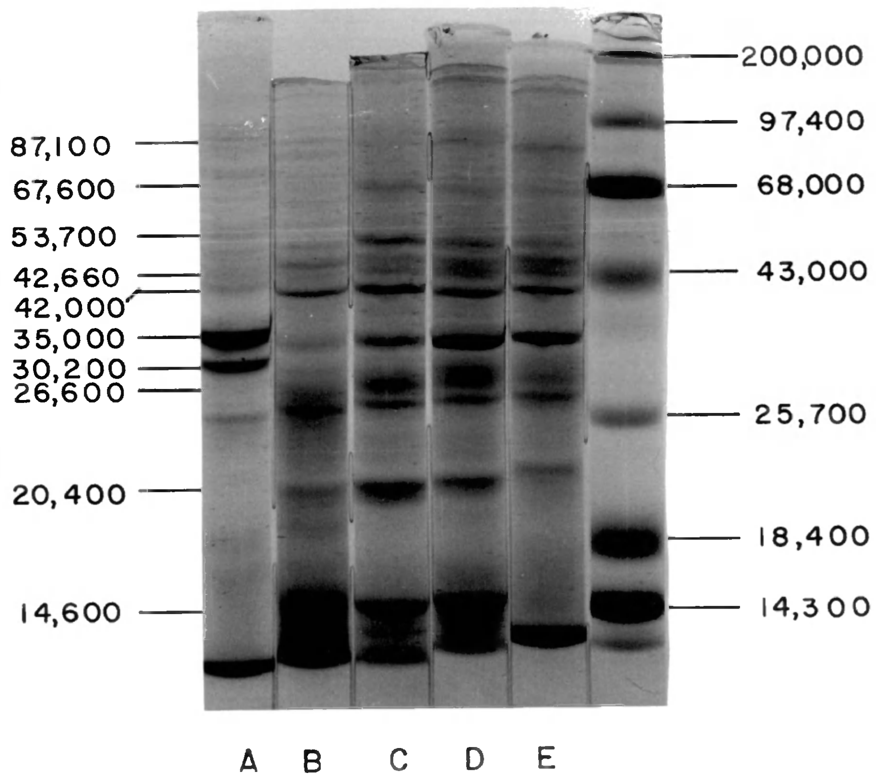
[c]This band is present on the original gel but did not photograph well (Figure 4).

Table III. *D. mucoroides* membrane protein variation between the sorocarp and macrocyst pathways[a]

	Time of Sample (hr)		
	3	6	9
Protein mol wt			
87,100		S	S
67,600	S*	S	S
53,700	S*	S	N
44,700	S	S	S
42,000	S	N	N
35,000	M	N	N
21,000	S	S	M

[a]S-indicates the band in the sorocarp pathway was more concentrated
M-indicates the band in the macrocyst pathway was more concentrated.
S*-indicates that the corresponding band appeared to be absent in samples from the macrocyst pathway.
N-indicates no obvious difference between the two pathways at that particular developmental time.

Figure 3. SDS-PAGE comparison of membrane proteins from vegetative cells and cells developing toward sorocarps. Plasma membrane proteins were separated using a 10% acrylamide gel and stained with coomassie blue R-250. Molecular weights are indicated for bands that changed during development. (A) 48 hour, exponential phase; (B) 56 hour, stationary phase; (C) 3 hours into development; (D) 6 hours into development; (E) nine hours into development. Molecular weight standard proteins are in the gel on the right (Myosin (H-chain), 200,000; Phosphorylase B, 97,400; Bovine serum albumin, 68,000; Ovalbumin, 43,000; α-Chymotrypsinogen, 25,700; β-Lactoglobulin, 18,400; Lysozyme, 14,300).

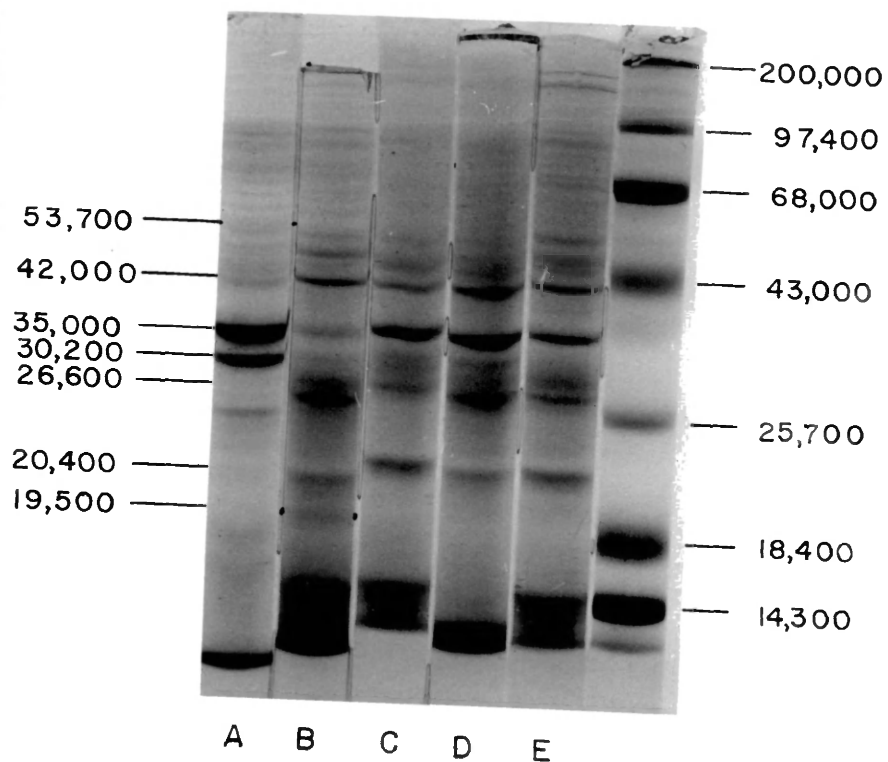

87,100
67,600
53,700
42,660
42,000
35,000
30,200
26,600
20,400
14,600
200,000
97,400
68,000
43,000
25,700
18,400
14,300
A
B
C
D
E

The range of molecular weights for the membrane proteins that varied in the macrocyst pathway was 19,500 to 53,700 (Figure 4). The prominant bands which showed variation had proteins with the approximate molecular weights of 42,000, 35,000, 26,600 and 20,400. The minor bands in which variation occurred corresponded to proteins with molecular weights of 53,700, 30,200 and 19,500. Table II correlates the protein bands of various molecular weights in which variation occurred with the developmental times.

Several major protein bands appeared in both developmental pathways. Two of these bands behaved in the same manner in both pathways. The protein band corresponding to a molecular weight of 35,000 decreased in concentration from the exponential phase to stationary phase. This band increased in concentration at both three and six hours then decreased by nine hours into development. The second band which behaved the same in both pathways had an approximate molecular weight of 26,000. This protein increased during stationary phase and then remained at that concentration throughout the nine hours of development.

Four major bands seen in both pathways behaved differently during development. These bands corresponded to proteins with molecular weights of 53,700, 42,000, 30,200 and 20,400. During the sorocarp pathway, the 53,700

Figure 4. SDS-PAGE comparison of membrane proteins from vegetative cells and cells developing toward macrocysts. Plasma membrane proteins were separated using a 10% acrylamide gel stained with coomassie blue R-250. Molecular weights are indicated for bands that changed during development. (A) 48 hour, exponential phase; (B) 56 hour, stationary phase; (C) 3 hours into development; (D) 6 hours into development; (E) nine hours into development. Molecular weight standard proteins are in the gel on the right (standards as in Figure 3).


53,700
42,000
35,000
30,200
26,600
20,400
19,500
200,000
97,400
68,000
43,000
25,700
18,400
14,300
A
B
C
D
E

molecular weight protein increased in concentration during stationary phase and three hours into development then decreased after six and nine hours of development. In the macrocyst pathway the concentration of this protein increased in stationary phase, remained the same through the first six hours of development and then increased again nine hours into development. The 42,000 molecular weight band in the sorocarp pathway increased during stationary phase and three hours into development, decreased by six hours of development, and then remained the same through nine hours of development. In the macrocyst pathway this same protein increased in concentration during stationary phase and then remained the same throughout the nine hours of development. The protein corresponding to a molecular weight of 30,200 decreased in concentration in the stationary phase and then remained at the same concentration throughout the nine hours of development in the sorocarp pathway. The protein of identical molecular weight in the macrocyst pathway behaved similarly except at six hours into development the protein increased in concentration instead of remaining the same. The protein of molecular weight 20,400 also behaved similarly in both pathways. In each pathway this protein increased in concentration during stationary phase as well as three hours into development. The protein then decreased in concentration after six hours of development. However,

nine hours into development the protein decreased in concentration in the sorocarp pathway and increased in the macrocyst pathway.

Both pathways exhibited protein bands which did not have corresponding bands in the other pathway. In the sorocarp pathway bands that showed variation, but did not appear as bands in the macrocyst pathway, were those with molecular weights of 87,100, 67,600, 42,660 and 14,600. The macrocyst pathway showed one band with a molecular weight of 19,500 which did not have a corresponding band in the sorocarp pathway.

Protein variation was also seen between the two pathways at the same developmental time (Table III). After three hours of development the major bands which showed differences corresponded to proteins with the approximate molecular weights of 67,000, 53,700, 44,700, 42,000 and 35,000 (Figure 5). All of the proteins, with the exception of the 35,000 molecular weight protein, were present in greater concentration in the sorocarp pathway. The bands which corresponded to the 67,600 and 53,700 molecular weight protein seemed to be absent in the macrocyst pathway. This could have been due to very low concentrations or the fact that the proteins were truly missing. All the major protein variation seen at six hours of development showed the sorocarp pathway had the greater concentration of protein (Figure 6). These proteins had

Figure 5. SDS-PAGE comparison of membrane proteins from cells in the sorocarp pathway with those from cells in the macrocyst pathway three hours into development. Plasma membrane proteins were separated using a 10% acrylamide gel and stained with coomassie blue R-250. Molecular weights are indicated for bands that changed during development. (A) sorocarp pathway; (B) macrocyst pathway. Molecular weight standard proteins are in the gel on the right (standards as in Figure 3).

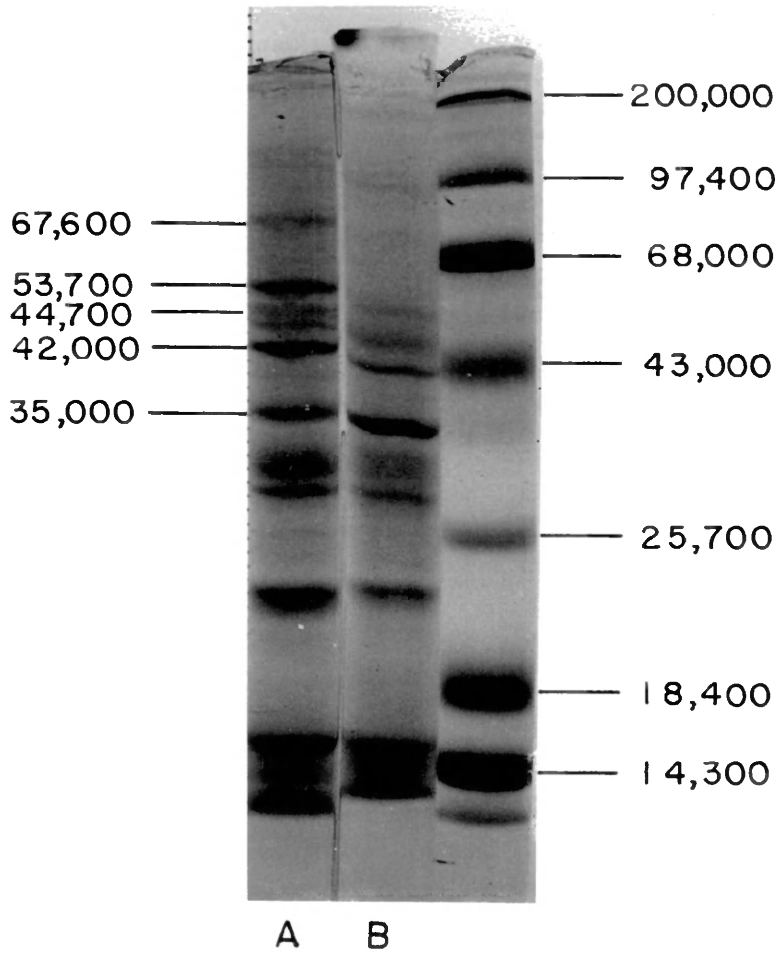
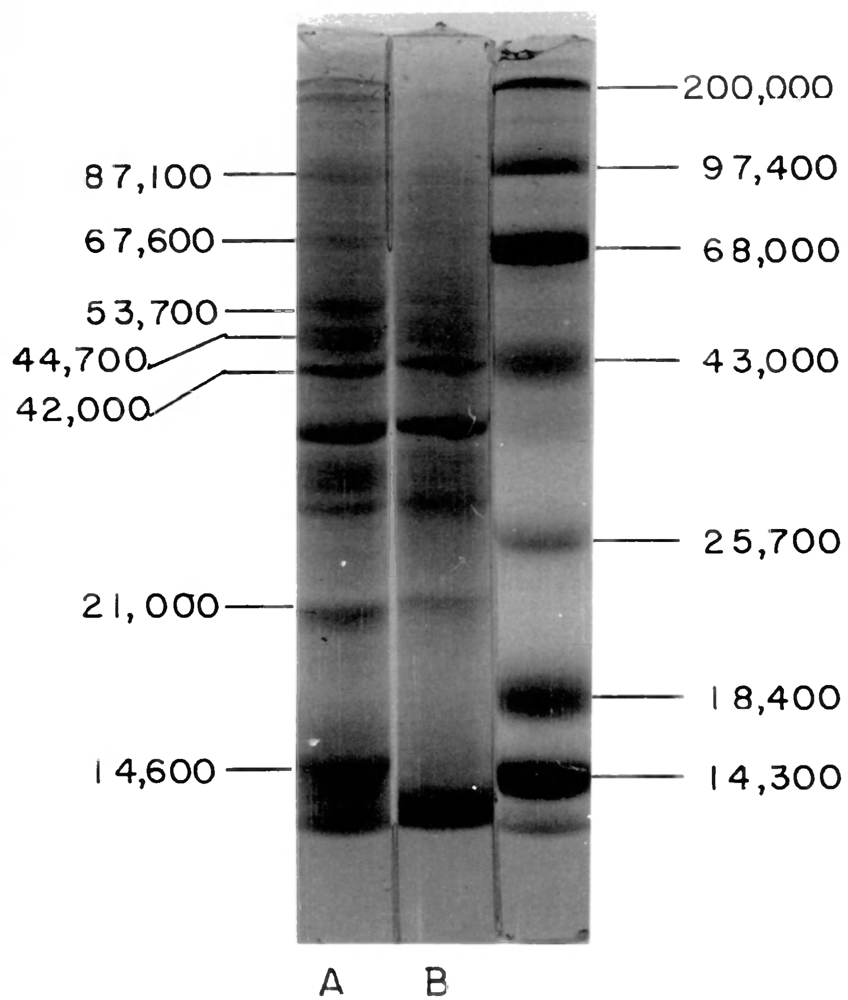

67,600
53,700
44,700
42,000
35,000
200,000
97,400
68,000
43,000
25,700
18,400
14,300
A
B

Figure 6. SDS-PAGE comparison of membrane proteins from cells in the sorocarp pathway with those from cells in the macrocyst pathway six hours into development. Plasma membrane proteins were separated using a 10% acrylamide gel and stained with coomassie blue R-250. Molecular weights are indicated for bands that changed during development. (A) sorocarp pathway; (B) macrocyst pathway. Molecular weight standard proteins are in the gel on the right (standards as in Figure 3).

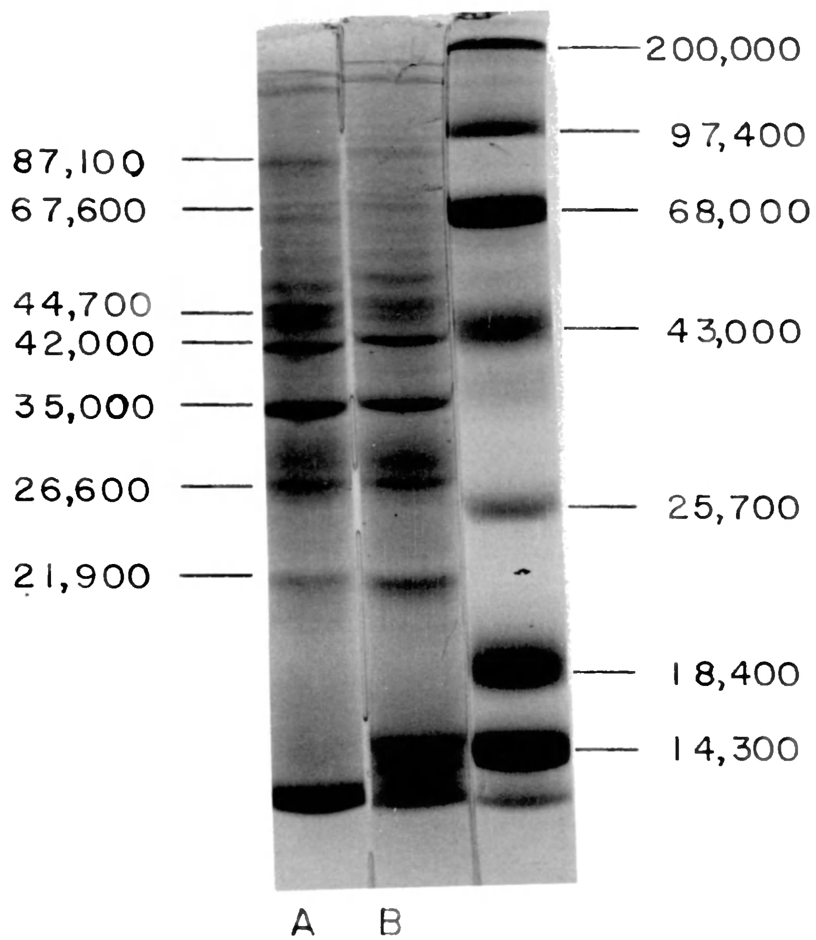

87,100
67,600
53,700
44,700
42,000
21,000
14,600
200,000
97,400
68,000
43,000
25,700
18,400
14,300
A
B

approximate molecular weights of 87,100, 67,600, 53,700, 44,700 and 21,000. Four major bands at nine hours of development showed variation (Figure 7). The bands at 87,100, 67,600 and 44,700 were more concentrated in the sorocarp pathway than in the macrocyst pathway. The band found at molecular weight 21,000 was more concentrated in the macrocyst pathway than in the sorocarp pathway.

Discussion

The plasma membrane must play an important role in initiating a specific developmental pathway in *Dictyostelium*. Two factors which are critical during development of *Dictyostelium* are directly related to the plasma membrane, these are 1) the environment and 2) intercellular cohesion. Selection of one of the alternative developmental pathways is dramatically influenced by environmental factors such as the presence of calcium or phosphate ions (Loomis, 1982). The membrane is the site of calcium receptors (Gardner and Hanna, 1982) and therefore must have some mechanism for signaling the cell to initiate development toward sorocarps or macrocysts.

Figure 7. SDS-PAGE comparison of membrane proteins from cells in the sorocarp pathway with those from cells in the macrocyst pathway nine hours into development. Plasma membrane proteins were separated using a 10% acrylamide gel and stained with coomassie blue R-250. Molecular weights are indicated for bands that changed during development. (A) sorocarp pathway; (B) macrocyst pathway. Molecular weight standard proteins are in the gel on the right (standard as in Figure 3).


87,100
67,600
44,700
42,000
35,000
26,600
21,900
200,000
97,400
68,000
43,000
25,700
18,400
14,300
A
B

Establishment of cell to cell contact is another important process in development. The signals for chemotaxis during aggregation are sent and received by membrane components (Gerish, 1982). The cell to cell contact which results from aggregation is required for initiation and continuation of development specific metabolism (Loomis, 1982).

Several methods have been employed to show that changes in the plasma membrane were associated with differentiation in Dictyostelium. Agglutination of Dictyostelium amoebae using Concanavalin A (Con A) was demonstrated by Weeks, (1973). He showed that cells of D. discoideum harvested in exponential phase agglutinated much more readily with Con A than did cells harvested during stationary growth phase or during early differentiation. He suggested that the inability of Con A to agglutinate these differentiating cells reflected changes in the cell surface during development.

Saito and Yanagisawa (1978) reported finding differences in surface properties of D. purpureum amoebae developing as sorocarps or macrocysts in this heterothallic species. In their study Con A was shown to have no affect on sorocarp formation but it inhibited macrocyst formation. Agglutination of cells showed that the macrocyst cells were more readily agglutinated with Con A during all stages of development than were sorocarp cells. Proteases were also

used to determine differences in the surface properties. After proteolysis, one mating type, when cultured alone, produced no macrocysts while the other mating type produced a few normal macrocysts. The results of Saito and Yanagisawa's experiments showed that the surface properties of amoebae developing as sorocarps or macrocysts differ. They also suggested that some specific Con A binding sites were involved in macrocyst formation.

In another investigation, surface properties of D. discoideum cells were studied by selectively labeling the outer cell surface using a Na ^{125}I-lactoperoxidase system (Smart and Hynes, 1974). Using this method the plasma membrane of D. discoideum was observed to contain two major surface proteins (molecular weights of 135,000 and 55,000) during vegetative growth but once aggregates were firmly established a new major protein (molecular weight of 130,000) appeared.

Another way to determine if the composition of the plasma membrane changes during differentiation is to use SDS-PAGE to examine proteins from cells at different stages of development. Several reports show that membrane proteins vary during development. Changes in the protein and glycoprotein composition of cell membranes were studied in D. discoideum developing toward sorocarps (Gilkes et al, 1979). Using SDS-PAGE Gilkes and associates found several proteins (approximate molecular weights of 220,000, 91,000,

63,000, 59,000 and 56,000) that increased in concentration during aggregation while two proteins (molecular weights of 82,000 and 22,000) decreased in concentration. Several glycoproteins (approximate molecular weights of 285,000, 150,000, 137,000, 53,000, 50,500 and 34,500) were also found to increase during aggregation while one glycoprotein (molecular weight 125,000) decreased in concentration.

Siu et al (1977) found proteins with molecular weights of approximately 38,000, 36,000 and 10,000 to 12,000 accumulated rapidly during the first six hours of sorocarp development in *D. discoideum* and then disappeared from the membrane after twelve hours. Synthesis of new higher molecular weight proteins later on in development were also reported by Siu. These proteins had molecular weights of 280,000, 103,000, 96,000, 85,000, 63,000 and 51,000. While changes in protein concentration during development were exciting, it was also noted that the majority of the protein components of the membrane in vegetative cells were conserved during development (Siu et al, 1977).

Results presented here demonstrate that the concentration of some plasma membrane proteins of *D. mucoroides* are developmentally regulated in the sorocarp and macrocyst pathways. This not only extends the SDS-PAGE results to another species of *Dictyostelium* but provides the first comparison of membrane proteins in cells developing toward sorocarps with those developing toward

macrocysts.

In a comparison of protein variation one would expect to find some similarities between D. mucorodies and D. discoideum. Indeed some similarities are observed in the early developmental stages. One similarity occurs in the three low molecular weight bands which appear during stationary phase in D. mucoroides. These are bands with molecular weights of 20,400, 19,500 and 14,600. It is possible that these proteins correspond to those in the low molecular weight bands which are found to accumulate rapidly during the first six hours of development in D. discoideum (Siu et al, 1977). It is likely that these proteins in D. mucorodies are synthesized somewhere between exponential and stationary phase. Because different growth and developmental conditions were used in the two experiments, developmental times are not easily compared. D. mucoroides development progressed much faster in this research than the corresponding development described by Siu for D.discoideum. Perhaps the time period between exponential growth and stationary phase corresponds to early developmental times in the work done by Siu. To determine if this is correct, an increased number of samples of D. mucoroides obtained between exponential and stationary phase could be studied.

Two proteins described by Gilkes et al (1979) in D. discoideum have similar molecular weights, and behaved

similarly during development, to proteins found in D. mucoroides. Gilkes describes a protein with a molecular weight of approximately 34,500 which increases during aggregation. This protein is identified as a glycoprotein. A protein with an approximate molecular weight of 35,000 increases during early and late aggregation in D. mucoroides. It is possible that these two proteins are the same. Staining for glycoproteins in D. mucoroides would help to determine if this were true. A protein with an approximate molecular weight of 53,000 is also seen to increase during aggregation in D. discoideum (Gilkes et al, 1979). This may correspond to a 53,700 molecular weight band which increases during exponential phase as well as in early aggregation in D. mucoroides. According to Gilkes this protein is not a glycoprotein.

An external surface protein with the molecular weight 34,000 is described for D. discoideum at zero hours and then is not seen after five hours (Siu et al, 1977). A very intense band with a molecular weight of 35,000 is present during the exponential growth phase of D. mucoroides. This protein then almost disappears in stationary phase but reappears during development in both pathways. The reappearance of the band does not correspond with what is found in D. discoideum. It is possible that this 35,000 molecular weight protein obscures the 34,000 molecular weight band resulting in a single, very

concentrated band during exponential phase. The 34,000 molecular weight protein may disappear during development while the 35,000 molecular weight protein remains. It is also possible that this protein is a similar external protein but one which behaves differently due to species differences. Labeling by lactoperoxidase radioiodination would help to determine if the 35,000 molecular weight protein of *D. mucoroides* is an external protein.

Actin is one of the few proteins to be identified in *Dictyostelium* membrane preparations. It has a molecular weight of 42,000 (Spudich, 1974). A membrane protein corresponding to a molecular weight of 42,000 was found in *D. mucoroides*. I suggest that this 42,000 molecular weight protein is actin.

Variation of membrane bound proteins during development in *D. mucoroides* was evident based on the results presented. Variation, some of which corresponded to variation seen in *D. discoideum*, was found in at least ten proteins within the sorocarp pathway of *D. mucoroides*. Membrane protein variation was also found within the macrocyst pathway where seven different proteins that change in concentration could be identified. A comparison of membrane proteins seen in the two pathways at the same developmental times also revealed considerable variation. This membrane bound protein variation found in *D. mucoroides* during development demonstrated that the

membrane was being modified, if only slightly, perhaps to allow or cause differentiation to continue. Without these modifications differentiation may cease or be altered in some manner.

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