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Echinostelium minutum (Myxomycetes) Amoebal Phase

2 Illustrations

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INSTITUT FÜR DEN WISSENSCHAFTLICHEN FILM

Film E 1816

Echinostelium minutum (Myxomycetes) Amoebal Phase

E. F. HASKINS, Seattle (Wash.)

General Remarks

Systematic Position and Occurrence

The myxomycetes or true slime molds have long attracted the attention of biologists because of their position at the boundary of the plant and animal kingdoms. *Echinostelium minutum* DE BARY is recognized (MARTIN and ALEXOPOULOS [10]) as a member of the Order Echinosteliales, Class Myxomycetes, Division Mycota. ALEXOPOULOS [2] and OLIVE [12] have suggested recently that this species possesses a number of characteristics which indicate that it is one of the most primitive myxomycetes. This study was undertaken to document the developmental stages of its amoebal phase in an attempt to clarify the phylogenetic position of this species within the Class Myxomycetes.
The isolate used for this film, a white form of *E. minutum*, was obtained by the author from a mixed collection of lodged, dead stems and leaves of *Poa pratensis* and *Agropyron repens*. It has been used in a number of investigations (HASKINS [5], [6], [7], [8]) and is currently available for distribution from the American Type Culture Collection, Rockville, Md., U.S.A. (ATCC 22345).

Life Cycle

There have been few developmental studies on myxomycetes belonging to orders other than the Physarales (GRAY and ALEXOPOULOS [3]), so the reports by ALEXOPOULOS [1], HASKINS [4], [5], REINHARDT [13], and OLIVE [11] on *E. minutum* are of great interest. In this species the germinating spore releases one uninucleate myxamoeba, which in the presence of a food supply of bacteria or yeast undergoes a number of

[1] Film data and summary of the Film (English, German, French) see p. 13.

binary fissions producing a large population of cells. When exposed to a liquid environment a myxamoeba can differentiate reversibly into a flagellate cell (swarm cell). When a myxamoeba or swarm cell encounters unfavorable environmental conditions a cyst (microcyst) is formed. Under favorable conditions, at the cessation of exponential growth,

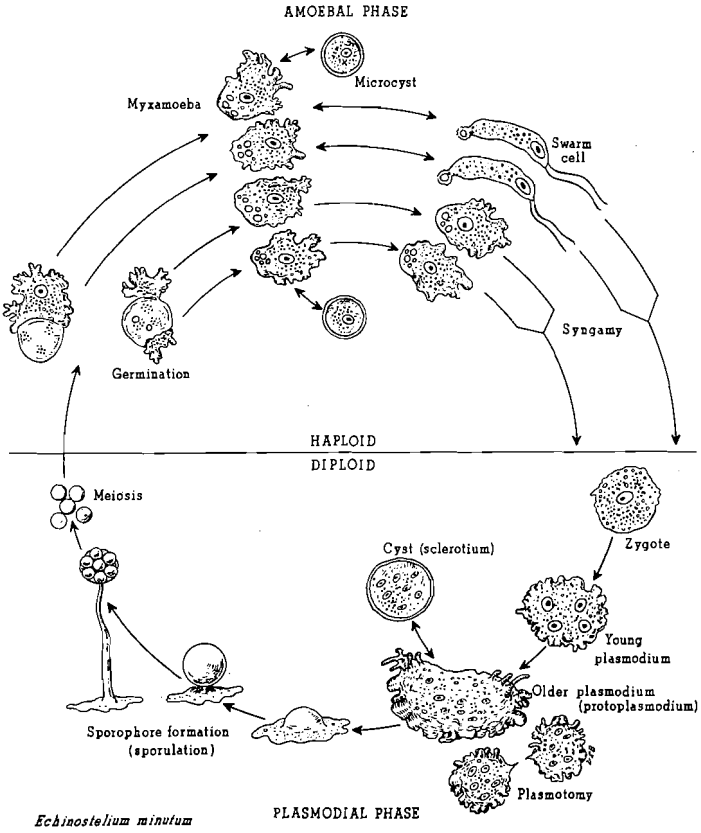

Fig. 1. Life history diagram of *E. minutum*

myxamoebae form multinucleate, reniform masses of protoplasm termed plasmodia (protoplasmodia) 20—250 μm in width. In the presence of bacteria or yeast protoplasmodia multiply by binary fission and upon differentiation each gives rise to a single fruiting body which typically contains less than 75 spores. If a protoplasmodium encounters

unfavorable environmental conditions it forms a cyst or sclerotium (Fig. 1).

The Spore

The spore of *E. minutum* is nearly spherical (ALEXOPOULOS [1]) and measures 8—14 μm in diameter (HASKINS, HINCHEE and CLONEY [8]). The wall of the mature spore is covered with several prominent patches of granules which apparently represent the contact points of adjacent spores during their formation. HASKINS, HINCHEE and CLONEY [8] postulate that the two nuclear divisions observed within the spore represent meiosis. This contention is supported by the observation of normal and anomalous synaptonemal complexes in the early stages of the first division. Germination occurs subsequent to nuclear division. The studies by ALEXOPOULOS [1] and HASKINS [4] indicate that spore germination begins with the emergence of a bleb of the protoplast through a pore in the spore wall. A break often develops in the wall as the protoplast emerges.

The Myxamoeba, Swarm Cell, and Microcyst

The trophic myxamoeba of *E. minutum* has a single nucleus containing a relatively large, central nucleolus. The cytoplasm possesses the usual complement of organelles including endoplasmic reticulum, Golgi material, contractile and food vacuoles, mitochondria, and at least two pairs of juxtanuclear centrioles. The light microscopical studies by HASKINS [4] and REINHARDT [13] indicate that the myxamoebal mitosis of this taxon is a classical, spindle type of division. The electron microscopical study by HASKINS (unpublished) on this species has shown that mitosis is astral according to the terminology of ROTH [16]. As has been documented by the studies of HASKINS [5] and REINHARDT [13], a myxamoeba of *E. minutum* readily develops 1—4 flagella in the presence of a suitable liquid environment. The present study has demonstrated that a low percentage of cells develop more than 4 flagella. The flagellate myxamoeba or swarm cell is typically pyriform in shape and swims through the medium with a corkscrew rotation. The electron microscopical study by HASKINS (unpublished) indicates that the pointed conformation of the anterior of the swarm cell is maintained by one or more cones of microtubules. Each cone encloses a pair of basal bodies. The microtubular apparatuses, which often ramify to the posterior of the cell, cap the anterior of the nucleus giving it a "beaked" appearance. Under unfavorable environmental conditions a refractile membrane forms around the cell. In the case of the swarm cell, this is preceded by resorption of the flagella. That this refractile boundary represents an actual wall is indicated by an electron microscopical study made on microcysts (HASKINS, unpublished).

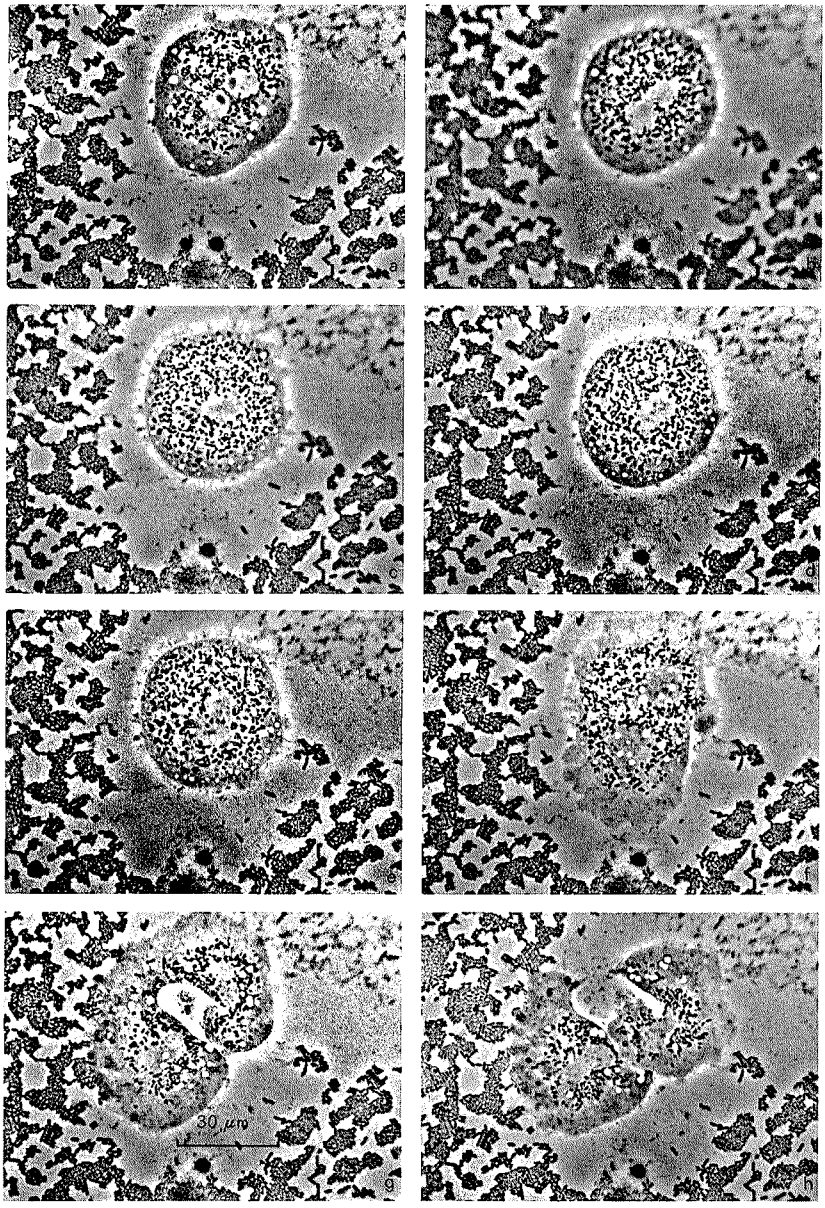

Fig. 2. Myxamoebal nuclear fusion and subsequent cytokinesis
a: Two nuclei side by side in a myxamoebal cell — b: Nuclear division begins in both nuclei — c: The dividing nuclei fuse and form a common spindle — d: The fusion nucleus begins division — e: A later stage in the division of the fusion nucleus — f: Division of the fusion nucleus has been completed — g: Asymmetric cytokinesis begins — h: Two uninucleate daughter cells have been produced by cytokinesis

Nuclear fusion

HASKINS [5] reported that fusion of pairs of myxamoebae and swarm cells occurred. However, the connection between this observation and the actual production of protoplasmodia was not presented in detail. In the present investigation it has been observed that approximately 1% of the myxamoebae were binucleate. Not uncommonly, nuclear fusion was observed in the binucleate cells. This was followed by cytoplasmic division and subsequent separation of the daughter cells (Fig. 2a—h). Although the origin of the binucleate condition is unknown and the significance of this type of nuclear fusion is open to question, it is tempting to suggest that this is a phenomenon of syngamy. ROSS [15], however, has made a similar observation in a study on *Didymium iridis* and has suggested that this is an abnormal cellular phenomenon leading to the formation of diploid myxamoebae which, consequently, may fuse and form tetraploid plasmodia.

Methods

Myxamoebae, in association with a bacterial food source *Enterobacter aerogenes*, were grown in test tubes containing GPY/10 broth[1] or on GPY/10 agar. Differentiation of swarm cells was accomplished by suspending the cells in SS buffer[2].

The myxamoebae were photographed on a thin layer of agar pressed against a coverslip in a chamber described by HEUNERT [9] or were recorded photographically in a Ross microchamber (ROSS [14]). Photographs were taken on 35 mm film (Eastman Double X, London) using an Askania-Z-camera. A Zeiss WL microscope equipped with bright-field, Zernike phase-contrast, or Nomarski interference-contrast optics was used.

The technical assistance of BRIGITTE MILTHALER, IWF, is gratefully acknowledged.

Film Contents[3]

Spore Germination

Phagocytosis

1 f/s to 8 f/min

1. Spore germination. Protoplasmic movement including contractile vacuole activity is seen in a cluster of five ungerminated spores. The first

[1] GPY = 0.1% Difco yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$, 1% Difco peptone, 1% glucose. GPY/10 is a 1 : 9 dilution of GPY.

[2] SS = 0.01M phosphate, pH 6.8. GPY/10 agar was solidified with 1.5% Difco Bacto agar.

[3] The headlines in *italics* correspond with the subtitles in the film.

sign of germination is the emergence of an amoebal pseudopodium through a pore in the spore wall. Five uninucleate myxamoebae ultimately emerge, migrate around the empty spore cases and occasionally re-enter and exit the empty spores.

Frame width 160 μm, exposure frequency 8 f/min, interference-contrast elapsed time 1 h. 38 min.

2. Spore germination. Germination commences in two spores as pseudopodia project and retract from pores in the spore walls. In one spore, pseudopodia project simultaneously from two pores. Subsequently this uninucleate myxamoeba emerges through a break in the spore case. The recently germinated myxamoeba often possesses a c-shaped nucleus with multiple nucleoli.

Frame width 125 μm, exposure frequency 8 f/min, phase-contrast, elapsed time 1 h. 51 min.

3. Myxamoeba with prominent cell center. The central myxamoeba exhibits a prominent juxtanuclear cell center consisting of a circle of granules. The cell center containing the complement of centrioles, is the site of flagellar formation.

Frame width 98 μm, exposure frequency 30 f/min, phase-contrast, elapsed time 11 min. 26 sec.

4. Migrating myxamoebae. Two uncompressed myxamoebae migrate through an aqueous medium. The uninucleate myxamoebae display active cyclosis including contractile vacuole activity. Peripherally, the cells form numerous filose pseudopodia while migrating.

Frame width 120 μm, exposure frequency 2 f/sec, water immersion phase-contrast, elapsed time 2 min. 44 sec.

5. Phagocytosis. This myxamoeba, which has been strongly compressed, is polarized. The cell has an advancing front of feeding pseudopodia, a central nucleus surrounded by a corona of granules, and a posterior cluster of contractile vacuoles. As phagocytosis proceeds, bacteria are ingested. Their phase density decreases as they are digested and moved by cyclosis into the endoplasm of the myxamoebal cell.

Frame width 39 μm, exposure frequency 1 f/sec, phase-contrast, elapsed time 7 min. 33 sec.

6. Phagocytosis. An uncompressed myxamoeba advances on a group of bacteria and ingests them. This myxamoeba is polarized.

Frame width 80.5 μm, exposure frequency 1 f/sec, interference-contrast, elapsed time 6 min. 28 sec.

Mitosis

Cytokinesis

30 f/min

7. Myxamoebal cell division. At the beginning of the scene the nucleolus has begun to fade from the myxamoebal nucleus. Note the presence of numerous small contractile vacuoles and granules which are evenly dispersed in the cytoplasm. The nuclear membrane breaks down, the spindle appears, and the chromosomes arrange themselves on the metaphase plate. Anaphase and telophase follow rapidly. Cytokinesis begins with a constriction in the mid-region of the cell. As it reaches completion, the nuclear membrane reappears and the cytoplasmic granules migrate from the periphery of the cell and cluster around the nucleus. During late cytokinesis the movement of granules increases in tempo and the daughter cells begin to feed on bacteria.
Frame width 98 μm, exposure frequency 30 f/min, phase-contrast, elapsed time 1 h. 5 min.

8. Myxamoebal cell division. Cell division is observed in an isolated myxamoeba. The nuclear membrane fades and contractile vacuole activity and movement of cytoplasmic granules slows as division proceeds. The chromosomes form a metaphase plate on the mitotic spindle. Separation of chromosomes to the opposite poles occurs, followed by reconstitution of the daughter nuclei during telophase. Several nucleoli are visible in each nucleus. During cytokinesis the cytoplasmic granules migrate from the ectoplasm of the daughter cells and congregate around the nuclei. Concurrently the cells begin to ingest bacteria.
Frame width 120 μm, exposure frequency 30 f/min, phase-contrast, elapsed time 1 h. 12 min.

Swarm Cells

Morphogenesis

16 f/s to 2 f/s

9. Flagellar formation. As this sequence begins a myxamoeba becomes polyflagellate. The flagellate myxamoeba assumes the polarized conformation of a swarm cell with the flagellar apparatus located at the anterior and the contractile vacuoles and filose pseudopodia at the posterior.
Frame width 120 μm, exposure frequency 4 f/s, phase-contrast, elapsed time 2 min. 9 sec.

10. Flagellar migration. An uncompressed mature swarm cell is polarized and has an ellipsoidal conformation. The flagella and nucleus are located

at the anterior and the contractile vacuoles are confined to the posterior. The cell shows active cyclosis as well as anterior to posterior peristalsis. Note that one flagellar apparatus remains attached at the anterior as another migrates around the periphery of the cell.
Frame width 120 μm, exposure frequency 2 f/sec, phase-contrast, elapsed time 4 min. 22 sec.

11. Swarm cell. A solitary swarm cell shows flagellar activity, active cyclosis, and contractile vacuole activity.
Frame width 120 μm, exposure frequency 4 f/sec, phase-contrast, elapsed time 1 min.

12. Swarm cell. Under high magnification a solitary swarm cell clearly exhibits a "beaked" nucleus at the anterior of the cell. Basal bodies are present at the base of the flagella. The posterior of the cell contains a complex of contractile vacuoles and is covered with numerous filose pseudopodia.
Frame width 51 μm, exposure frequency 16 f/sec, phase-contrast, elapsed time 40 sec.

13. Flagellar resorption. A compressed polyflagellate cell is shown migrating over the agar substratum. Flagellar resorption occurs progressively in this cell. Resorption takes place as an individual flagellum is whipped against the swarm cell and fuses with it. A momentary bleb forms at the site of flagellar fusion. At the end of the sequence all flagella have been resorbed.
Frame width 120 μm, exposure frequency 2 f/sec, phase-contrast, elapsed time 9 min. 34 sec.

Encystment

Cyst Germination

8 f/min to 2 f/min

14. Overview of myxamoebal encystment. A large population of myxamoebae is observed migrating over the substratum feeding on a layer of bacteria. Progressively, as the food supply is exhausted, the myxamoebae cease their movement, lose their pseudopodia, shrink in size, and develop refractile cyst walls. Some of the myxamoebae ingest newly formed cysts and in turn encyst.
Frame width 600 μm, exposure frequency 2 f/min, phase-contrast, elapsed time 9 min. 57 sec.

15. Myxamoebal encystment. The scene commences as a single myxamoeba begins to encyst. It ceases its migration, retracts its pseudopodia, shrinks to about ½ of its former size, and develops a refractile wall.

Frame width 89 μm, exposure frequency 8 f/min, phase-contrast, elapsed time 36 min. 8 sec.

16. Myxamoebal excystment. A group of myxamoebal cysts excyst. Note that no cyst walls remain upon the completion of germination.

Frame width 245 μm, exposure frequency 4 f/min, phase-contrast, elapsed time 1 h. 31 min.

17. Myxamoebal excystment. Five myxamoebal cysts are observed during excystment. As germination proceeds the movement of cytoplasmic granules and pulsation of contractile vacuoles is seen. The cyst walls disappear and pseudopodia are reformed. Large inclusion vacuoles are present within some of the myxamoebae.

Frame width 120 μm, exposure frequency 8 f/min, phase-contrast, elapsed time 50 min, 23 sec.

Nuclear Fusion

8 f/min

18. Nuclear fusion in a myxamoebal cell. At the beginning of the sequence a myxamoeba with two nuclei side by side is in the center of the field. The prominent, central nucleoli in both nuclei fade and the nuclear membranes disappear. As the nuclei proceed into mitosis they organize into a single spindle apparatus. Anaphase and telophase follow rapidly and a binucleate cell is formed. Cytokinesis is accomplished by a constriction in the mid-region of the cell. This constriction is asymmetric in that it occurs on only one side of the cell. Two uninucleate daughter cells of equal cytoplasmic volume are produced by this division (Fig. 2a—h).

Frame width 120 μm, exposure frequency 8 f/min, phase-contrast, elapsed time 2 h. 10 min.

Amoeba-Protoplasmodium

Transformation

15 f/min

19. Mitosis in a uninucleate protoplasmodium. Whether the uninucleate protoplasmodium in the center of the field is a product of nuclear fusion, or has developed by apogamy remains unclear. The large central nucleolus begins to fade and the chromosomes become arranged on a prominent metaphase plate. Anaphase and telophase occur and as nucleolar fragments reappear the daughter nuclei become more distinct. At the termination of mitosis the binucleate protoplasmodium begins to feed upon bacteria. A uninucleate protoplasmodium is distinguished from a myxa-

moeba by the occurrence of intranuclear mitosis and the absence of cytokinesis following nuclear division.

Frame width 155 μm, exposure frequency 15 f/min, phase-contrast, elapsed time 2 h.

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Film Data

The film was published in 1973 for use in research and university education. Silent, 16 mm, black and white, 130 m, 12 min (running speed 24 f/s).
It was taken in March and April 1971 at the Institut für den Wissenschaftlichen Film, Göttingen. Published by the College of Arts and Sciences, Department of Botany, University of Washington, Dr. E. F. HASKINS and the Institut für den Wissenschaftlichen Film, Göttingen, Dr. H.-K. GALLE; photography: H. H. HEUNERT.
With financial support by N. S. F. grants GB-20517 and GB-31243.

Summary of the Film

Spore germination, myxamoebal cell division, phagocytosis, encystment, and excystment are presented. The transformation of a myxamoeba into a swarm cell as well as flagellar resorption are illustrated. Nuclear fusion, which may be related to protoplasmodial formation, is demonstrated.

Inhalt des Films

Sporenkeimung, Zellteilung der Myxamöben, Phagozytose, Encystierung und Excystierung werden im Film gezeigt. Die Umwandlung einer Myxamöbe in eine Schwarmzelle sowie die Resorption der Geißel werden dargestellt. Es wird eine Kernfusion demonstriert, die mit der Bildung von Protoplasmodien verbunden sein dürfte.

Résumé du Film

Germination des spores, division cellulaire "myxamoébalique", phagocytose, enkystement et dékystement sont présentés. La transformation d'une "myxamoeba" en zoospore ainsi que la résorption des flagelles sont illustrés. La fusion nucléaire, liée à la formation de protoplasmodies, est démontrée.