Closed Spindle Nuclear Division in the Plasmodial Phase of the Acellular Slime Mold *Echinostelium minutum*

A. A. Hinchey*1 and E. F. Haskins

Department of Botany, University of Washington, Seattle, Washington

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Summary

Mitosis in the plasmodium of *Echinostelium minutum* is intranuclear (closed spindle) and centrioles are not present at the spindle poles. The nuclear envelope remains essentially intact throughout mitosis with polar fenestrae appearing in anaphase and persisting through telophase. During anaphase there is a shortening in the distance of the chromosomes to the poles followed by a further separation of the poles. Zippering of microtubules may be the basis for these two anaphasic movements. During telophase the polar MTOCs are extruded into the cytoplasm through the polar fenestrae prior to reconstitution of the nuclear envelope. It is proposed that during sporulation such MTOCs are responsible for the differentiation of the centrioles which subsequently persist in the myxamoebal phase of this species.

Keywords: Closed spindle mitosis; *Echinostelium*; Myxomycete; Plasmodial mitosis.

1. Introduction

Nuclear division in a plasmodium was first observed by A. Lister (1893) who reported that in *Badhamia utricularis* the nuclei underwent simple division or constriction rather than mitosis. In this report he contrasted his observations with those of his son J. J. Lister who had on two occasions made preparations of *B. utricularis* plasmodia in which each nucleus was clearly undergoing mitosis. The observations of Howard (1932) on *Physarum polycephalum* supported the earlier suggestion of J. J. Lister (1909) and that the nuclear envelope persisted through mitosis. Howard (1932) further stated that centrosomes and asters were not present during plasmodial mitosis. To the best of our knowledge the first complete ultrastructural study of plasmodial nuclear division was done on *P. polycephalum* by Kessler (1964).

* Correspondence and Reprints: Department of Botany, University of California, Davis, CA 95616, U.S.A.
1 Based on the doctoral dissertation of the first author presented to the Department of Botany, University of Washington, Seattle, WA 98195, U.S.A.
He confirmed that centrioles and asters were lacking during plasmodial mitosis. His study showed that the nuclear envelope remained essentially intact during division although gaps did appear in the interzonal and polar regions, especially during telophase. The study by Aldrich (1969) on mitosis in the myxamoebal and sporulating phase of *Physarum flavicominum* was the first comparative ultrastructural investigation that showed the presence of extranuclear (open spindle) and intranuclear (closed spindle) mitosis in the same species.

The present study on plasmodial mitosis complements an antecedent report on myxamoebal mitosis in *Echinostelium minutum* (Hinchee and Haskins 1980 a) and represents the conformation at an ultrastructural level of dimorphic mitosis in a second myxomycete. Problems studied during the course of this investigation included the behavior of the nuclear envelope during division, kinetochore and non-kinetochore microtubular interaction relating to chromosomal movements, and the behavior of the MTOCs found at the spindle poles.

2. Materials and Methods

Plasmodia of *E. minutum* (American Type Culture Collection 22345) were grown in association with the bacterium *Klebsiella pneumoniae* on a dilute glucose-peptone-yeast extract agar medium at pH 6.8 (Haskins 1968) incubated at 22 °C. The procedures used for electron microscopy have been presented elsewhere (Hinchee and Haskins 1980 a).

3. Results

By prometaphase, the nucleolus disperses while the chromosomes become more apparent and appear in scattered array throughout the nucleoplasm. Microtubules have also appeared within the nucleus by this time. During metaphase the chromosomes form an equatorial metaphase plate on the spindle (Fig. 1). The nuclear envelope remains intact with small horn-like projections protruding short distances into the surrounding cytoplasm. Bundles of microfilaments occasionally abut the nuclear envelope. Microtubules of the metaphase spindle are largely arranged at oblique angles to one another. Unlike myxamoebal mitosis in this species, there are no centrioles at the poles at this stage, although we have observed on occasion electron-dense amorphous regions that may be the precursors of the anaphase/telophase spindle MTOCs.

Anaphase begins with a shortening of the distance between the chromosomes and the poles (Fig. 2). Interzonal microtubules are sparse at this stage. The nuclear envelope remains intact and the horn-like projections of the nuclear envelope observed in metaphase are not as prevalent. The second anaphasic movement consists of a further separation of the respective spindle poles (Fig. 3). The nucleus consequently takes on an ovoidal appearance in contrast to the nearly spherical shape it possessed during metaphase and early ana-