trees included oak, hickory, maple, beech, and dogwood. In Monroe Co., nets were placed across or in proximity to a pond which appeared shallow and was about 40 m in greatest length and 25 m in greatest width. Dominant trees included buckeye, birch, maple, and hemlock. No information is available concerning the specific day and site of collection for the Shelby Co. specimen. The *L. seminolus* reported are housed as museum specimens in the Memphis State University Museum of Zoology (MSUMZ 4990, 10554, 10555).

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INTRANUCLEAR MICROFILAMENTS AND CELLULOSE SYNTHESIS IN *ACANTHAMOEBA*

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ABSTRACT

Intranuclear bundles of microfilaments are visible by transmission electron microscopy in *Acanthamoeba castellanii* when this soil amoeba is induced to form cyst walls. The bundles may be very large (10 um X 0.85 um) and are ultrastructurally similar to giant bundles of actin microfilaments which occur in the slime mold, *Dictyostelim mucoroides* after dimethyl sulfoxide (DMSO) treatment. DMSO is not used in this experimental procedure. Instead Ca^{++} accumulation and subsequent cellulose synthesis are correlated with the appearance of the intranuclear microfilaments.

INTRODUCTION

Fukui (1978) reported giant bundles of intranuclear microfilaments 3 um long and 0.85 um wide in nuclei of cellular slime molds, *Dictyostelium mucoroides* and *D. discoideum* when cultures of these organisms were harvested at aggregation, dissociated by treatment of cellulose-macerozyme mixture and induced to form macrocysts synchronously in Bonner's salt solution. Aggregates of microfilaments were identified by Fukui as bundles of the contractile protein, actin, on the basis of their ability to bind rabbit skeletal muscle heavy meromyosin and reversal of such binding by magnesium-adenosine triphosphate. Fukui concluded that the actin microfilaments were specifically induced in *Dictyostelium* in response to dimethyl sulfoxide (DMSO) treatment which immediately preceded fixation for transmission electron microscopy.

Tomlinson (1982) reported that bundles of intranuclear microfilaments, ultrastructurally similar to those which were observed in *Dictyostelium,* occur in a small soil amoeba, *Acanthamoeba castellanii* without DMSO treatment. Tomlinson noted that the appearance of intranuclear microfilaments in *Acanthamoeba* correlated in time with initiation of cyst wall formation and might be related to the induction of cellulose synthesis from endogenous carbon sources.

This report documents the sequential appearance of intranuclear microfilaments and cellulose fibrils in newly-formed *Acanthamoeba* cyst walls in the absence of any contact or use of DMSO.

MATERIALS AND METHODS

Log-phase *Acanthamoeba castellanii* were induced to encyst synchronously by suspending them in sterile encystment medium (EM) consisting of a buffered inorganic saline solution which was adjusted to pH 6.8 and aerated (Tomlinson, 1962). Observations were made on aliquots which were collected from a single culture of synchronously encysting cells. Samples were collected at the time the culture was induced to encyst (T_0) and at 2 hour intervals until completion of encystmen 30 hours later. The period T_0 to T_{12} is defined as the preencystment period since it preceeds cyst wall formation as viewed by phase contrast microscopy. Immediately after collection, each sample was washed in 0.1 M phosphate buffer pH 6.8 which had been rendered isotonic with potassium chloride. Cells were then
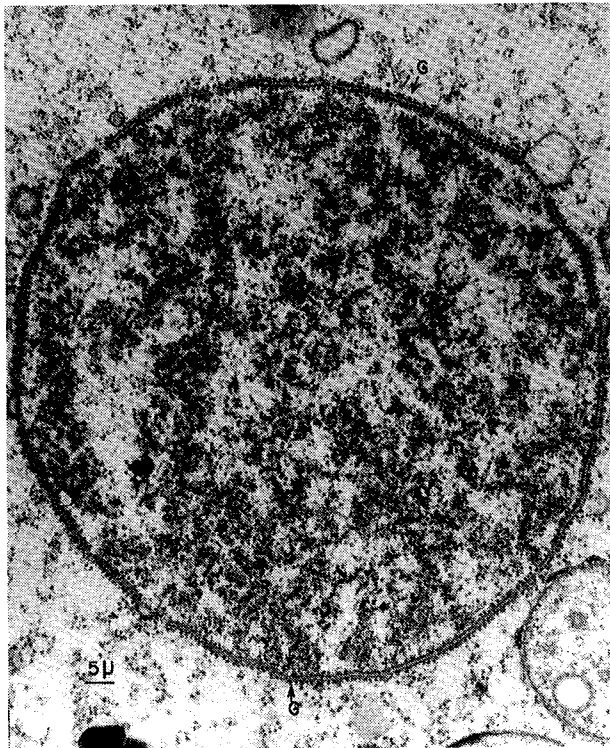
FIG. 1. *A section of **Acanthamoeba** 8 hours after induction to encyst showing the nucleus with granules on both inner and outer nuclear membrane. G, granules X 70,000.*
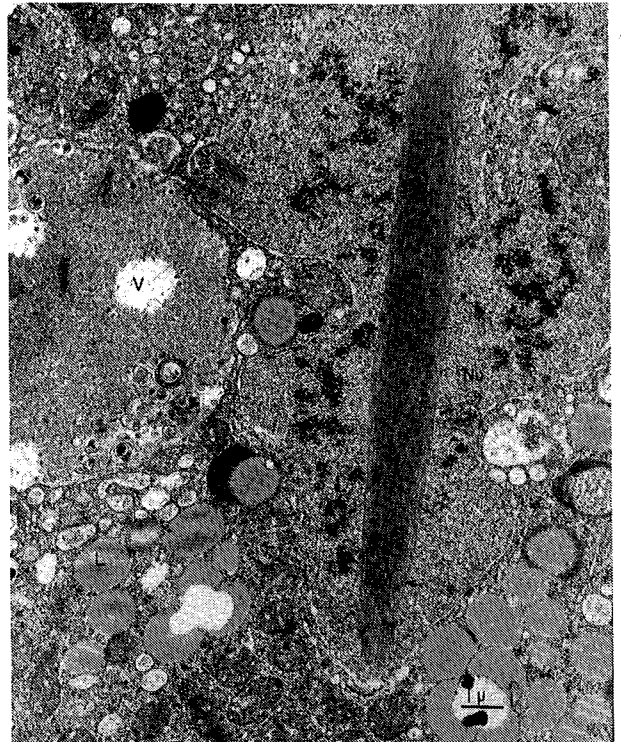

FIG. 2. *A section of **Acanthamoeba** during 12 hour stage of preencystment. Arrows point to Intranuclear mcirofilament. V, Vacuole; L, lipid: Nu, nucleus X 70,000.*
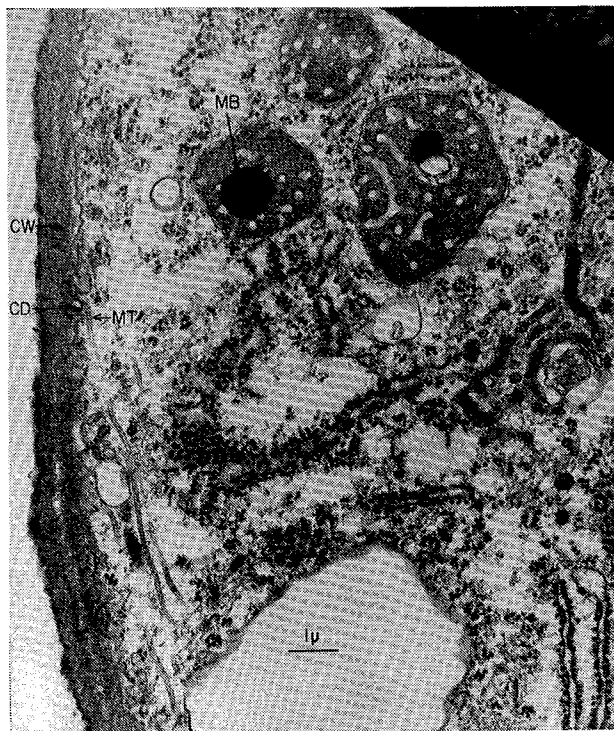

FIG. 3. *A section of **Acanthamoeba** during 16 hour stage of preencystment showing cellulose deposition in the developing cyst wall. CW, cyst wall; CD, cellulose deposition; MT, microtubule; MB, mitochondrial body; X 70,000.*
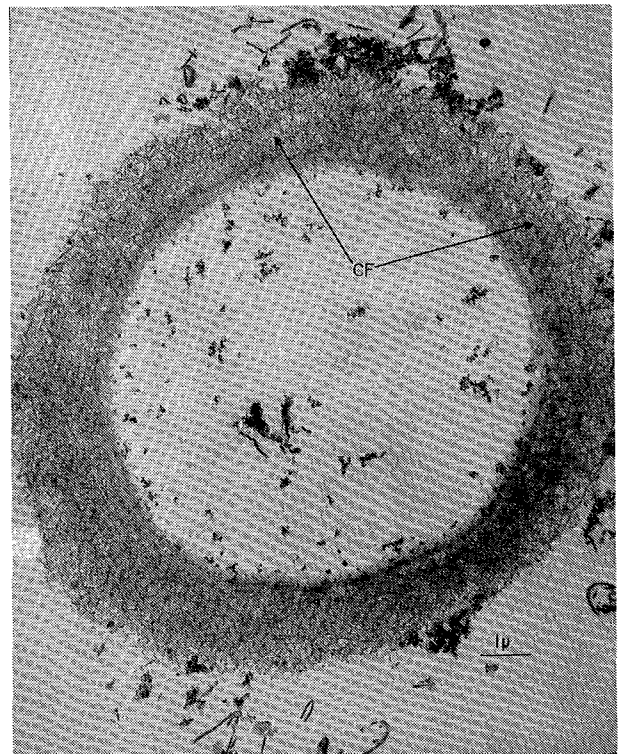

FIG. 4. *A section through alkail-treated cyst wall of **Acanthamoeba** showing cellulose fibrils and their arrangement. CF, cellulose fibrils; X 35000.*

concentrated by centrifuging 3 minutes at 500 X g in an HR-1 centrifuge.

Isolation of cyst walls from whole cysts was carried out by ultrasonic oscillation and Mickle disintegrator treatment according to the method of Tomlinson and Jones (1962). Isolated cyst walls were treated with 1M potassium hydroxide for 12 hrs at 20 °C to remove alkali soluble components of the cyst wall and expose newly formed cellulose fibrils.

Acanthamoeba were fixed for electron microscopy in 2% gultaraldehyde for 1 hour at 4°C and postfixed in 1% osmium tetroxide for 1 hour at 4°C. Specimens were dehydrated in ethanol using two washes of 50%, 70%, 95% and absolute, embedded in Epon 812 and sectioned on an LKB Ultratome with DuPont diamond knives, Specimens were then stained on grids with 1% uranyl acetate for 10 minutes and examined in a Philips 200 electron microscope operated at 40 KV to 100 KV with double condensors and 20 micrometer molybdenum apertures in the objective lens.

RESULTS

Cyst wall formation in *Acanthamoeba* is characterized by the transformation of an irregularly shaped, active, amoeboid cell into a rounded, apparently quiescent cell enclosed by a cyst wall. The cyst wall appears double under phase contrast microscopy and mature cysts range in size from 10 to 30 um in diameter. The major structural component of the cyst wall is cellulose (Tomlinson and Jones, 1962). When cells are induced to encyst, the nuclear membrane is porous but without any attached particles and each nucleus contains one centrally-placed, highly dense nucleolus (Tomlinson, 1982).

By the T_8 period of preencystment, prior to any visible cyst wall formation, nucleolar mass is greatly dispersed and granules with the dimensions and staining properties of ribosomes are often attached to both the inner and outer surface of the nuclear membrane (Fig. 1).

As preencystment activity reaches the T_{12} stage, *Acanthamoebae* have rounded up to a near-spherical shape, and large intranuclear bundles of microfilaments are visible by transmission electron microscopy. Such bundles may reach dimensions of 10 um X 0.85 um and almost completely traverse the nucleus (Fig. 2). The intranuclear microfilaments are ultrastructurally indistinguishable from the giant bundles of action microfilaments which have been reported in *Dictyostelium mucoroides* after specific induction by dimethyl sulfoxide treatment (Fukui, 1978). As noted previously by this investigator (Tomlinson, 1982), *Acanthamoeba* has no contact whatsoever with DMSO in this experimental procedure.

By T_{16}, cyst wall is morphologically visible and microtubules and cellulose fibrils are shown in close proximity to the inner cyst wall. Large, electron dense deposits with diameters up to 170 nm appear in mitochondria at this time (Fig. 3).

When mature cyst wall is isolated from whole cysts and treated with dilute alkali to remove other cyst wall constituents, cellulose fibrils appear cross-linked rather than arranged in a lamellar manner (Fig. 4). This cellulose fibrillar network serves as the chief structural component of the cyst wall in *Acanthamoeba*.

DISCUSSION

The slime mold, *Dictyostelium* and the soil amoeba,[1] *Acanthamoeba* share the common characteristic that large intranuclear bundles of microfilaments occur in their nuclei under certain conditions. In the case of *Acanthamoeba*, it has been clearly demonstrated in these studies that treatment with DMSO is not necessary for induction of the intranuclear mcirofilaments. It has not been determined in this study whether DMSO treatment *could* induce formation of such microfilaments in *Acanthamoeba*. Experiments designed to answer this question are currently in progress.

Dictyostelium and *Acanthamoeba* share another characteristic which may be related to the intranuclear microfilaments. Both the slime mold and the soil amoeba differentiate to form large amounts of cellulose under the conditions of the Fukui and Tomlinson experiments. While cellulose is the chief structural component of the cyst wall in *Acanthamoeba*, it is also the chief structural component of the stalk which supports the fruiting bodies in *Dictyostelium*. In fact, it is tempting to regard *Acanthamoeba* phylogenetically as an "Unaggregated cellular slime mold". That is, *Acanthamoebae* exemplify many of the characteristics which might be expected of cellular slime molds such as *Dictyostelium* if such slime molds were to lose or never acquire the capacity to aggregate into a multicellular organism and form a slug.

What triggers cellulose synthesis in *Acanthamoeba*? In general, starvation or other unfavorable environmental conditions can clearly induce cyst wall formation. In these studies, Ca^{++} and Mg^{++} accumulation in cells may be especially significant as evidenced by intramitochondrial bodies (Fig. 3) which have been attributed to storage of excess cellular calcium in this organism (Sobota, et. al., 1981). Such mitrochondrial inclusions occur regularly in encysting *Acanthamoebae*. In view of the role of Ca^{++} in the sliding filament model of vertebrate muscular contraction, it may be that the aggregation of actin microfilaments in the nucleus of *Acanthamoeba* is triggered by changes in calcium concentration in the cell. Storage of excess calcium in mitochondria may alter respiratory mechanisms and induce encystment with concomitant cellulose synthesis.

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