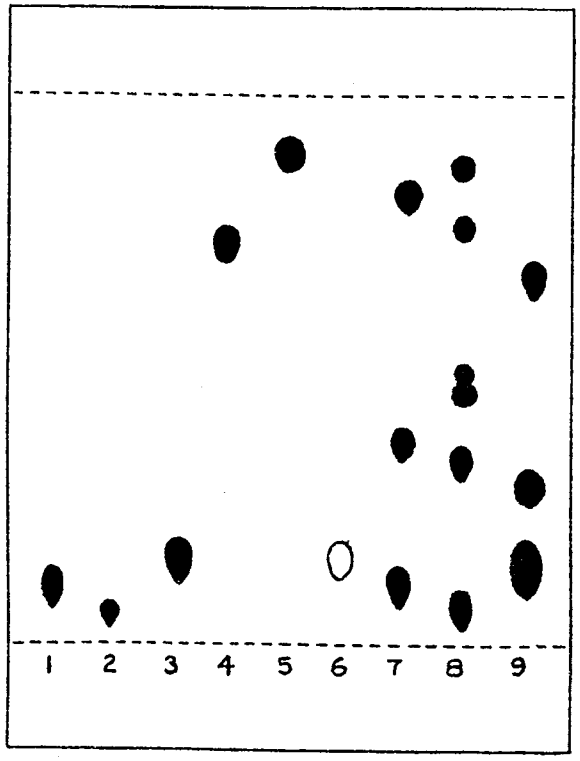

FIGURE 1. *TLC comparison of reaction products with citric acid cycle intermediates. (1) citric acid, (2) isocitric acid, (3) alphaketoglutaric acid, (4) succinic acid, (5) fumaric acid, (6) malic acid, (7) UV exposed citric acid products, (8) citric acid pyrolysis products, (9) alphaketoglutaric acid pyrolysis products.*

metabolism to emerge? In regard to the origin of the citric acid cycle, the results of this study suggest that complex catalysts such as proto-coenzymes (Lipmann, 1965) or protenoids (Fox et al., 1970) were needed and probably evolved first since non-catalyzed Krebs cycle reactions have not been observed. Hartman (1975) has speculated that the citric acid cycle evolved early *in a chemically simple environment* and formed the basis for the buildup of amino acids, fatty acids, and carbohydrates in the biosphere. In view of the results reported here and those of earlier workers (vide supra), the experimental evidence available seems to support the widely accepted notion that metabolism evolved within simple organisms in a complex environment.

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INDUCTION OF INTRANUCLEAR MICROFILAMENTS WITHOUT DMSO

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ABSTRACT

Large intranuclear bundles of microfilaments are visible by transmission electron microscopy in *Acanthamoeba castellanii* after they have been induced to form cyst walls. The bundles, which may traverse an enlarged nucleus and be as large as 10 μm long x 0.85 μm wide, are ultrastructurally similar to giant bundles of actin microfilaments in *Dictyostelium mucoroides* which are reported to be induced by dimethyl sulfoxide (DMSO). Since the intranuclear bundles are formed in *Acanthamoeba* without DMSO treatment, an alternate mechanism is proposed for their induction.

INTRODUCTION

Intranuclear microfilaments have been reported in dividing nuclei of humans (Fustwara, et al., 1976), other mammals (Cande, et al., 1977) and in interphase nuclei of birds (Masurovsky, et al, 1970), rabbits (Clattenberger, et al., 1972), amphibians (Lane, 1969) and slime molds, (Ryser, et al., 1970; Jockush, et al., 1973).

Giant bundles of intranuclear microfilaments 3 μm long and 0.85 μm wide are visible in nuclei of cellular slime molds, *Dictyostelium mucoroides* and *Dictyostelium discoideum* when cultures are harvested at aggregation, dissociated by treatment with cellulase-macerozyme

mixture and induced to form macrocysts synchronously in Bonner's salt solution (Fukui, 1978). 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, on the basis of experiments repeated 10 times, that such giant bundles of intranuclear microfilaments were specifically induced in *Dictyostelium* in response to dimethyl sulfoxide (DMSO) treatment which immediately preceded fixation for electron microscopy.

In this study, large intranuclear bundles of microfilaments, ultrastructurally similar to those which occur in *Dictyostelium,* are reported in a small soil amoeba, *Acanthamoeba castellanii* without DMSO treatment. Since DMSO is not required for induction of intranuclear bundle formation in *Acanthamoeba,* an alternate mechanism for their induction in this organism is proposed.

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

Acanthamoeba were fixed for electron microscopy in 8% glutaraldehyde 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.

Result

Encystment in *Acanthamoeba* is characterized by the transformation of an irregular, highly vacuolated amoeboid cell into a rounded, apparently quiescent cell enclosed by a cyst wall. Mature cysts range in size from 10 to 30 um in diameter. The cyst wall is double with the major structural component of the wall being cellulose (Tomlinson, 1962, 1981). When cells are induced to encyst, they typically contains one near-spherical nucleus with a single, centrally located nucleolus that is very conspicuous (FIG. 1). The nuclear membrane is porous but without any attached particles.

By the T_8 period of preencystment, 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. 2). The cystoplasm has become highly vacuolated and morphological changes in mitochondria and other cytoplasmic organelles also occur as has been previously reported (Bowers and Korn, 1968; Tomlinson, 1981).

By T_{12} of the preencystment period, *Acanthamoeba* have rounded up to a near-spherical shape, nucleolar mass remains dispersed throughout the nucleus, and large intranuclear bundles of microfilaments are visible by transmission electron microscopy (FIG. 3). The bundles, which may traverse an enlarged nucleus and be as large as 10 um long X 0.85 wide, are intrastructurally similar to giant bundles of actin microfilaments which have been reported in the slime mold, *Dictyostelium mucoroides* after specific induction by dimethyl sulfoxide (DMSO) treatment (Fukui, 1978). *Acanthamoeba* have no contact whatsoever with DMSO in this experimental system.

Under higher magnification (FIG 4), it can be seen that the mean diameter of individual microfilaments in *Acanthamoeba* is 6 nm. It has not been possible to determine whether individual microfilaments run the entire length of the giant intranuclear bundles, which may be 10 um in length, or if branching occurs as they traverse the bundle. The giant intranuclear bundles of microfilaments disperse or disappear as encystment proceeds and cyst wall material whose major structural component is cellulose accumulates at the periphery of the cell.

Discussion

The presence of actin in the cytoplasm of *Acanthamoeba castellanii* and the experimental interraction of this protein with rabbit skeletal muscle tropomyosin has already been demonstrated (Yang, Y. Z., *et. al.,* 1977). The presence of such large intranuclear actin bundles in *Acanthamoeba* and the correlation of their appearance in time with the onset of cyst wall formation is being reported for the first time in this communication.

Since giant bundles of intranuclear microfilaments have been demonstrated in the slime mold, *Dictyostelium,* and the soil amoeba, *Acanthamoeba,* one wonders about the similarities and differences of the two systems. When *Acanthamoeba* were induced in this study, they were placed under cultural conditions of carbon and nitrogen deprivation. Nevertheless, these soil amoeba produced large quantities of cellulose for their cyst walls from endogenous carbon sources. In similar manner, when *Dictyostelium* was induced by Fukui (1978), they also produced large quantities of cellulose from endogenous sources under starvation conditions. But while Fukui concluded that such giant

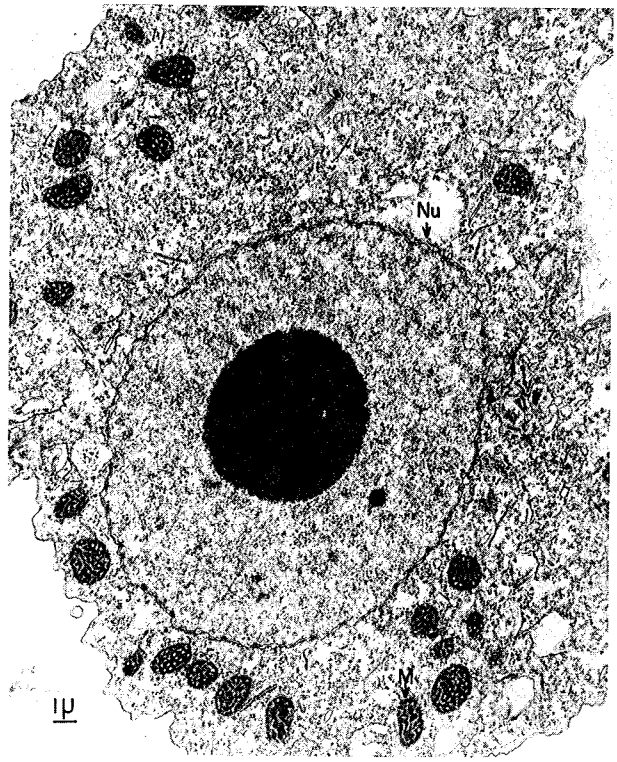

FIG. 1. *A section of* Acanthamoeba *trophozite showing a typical spherical nucleus, central nucleolus and mitochondria with tubular cristae. Nu, Nucleus: M, mitochondria X 26, 250.*

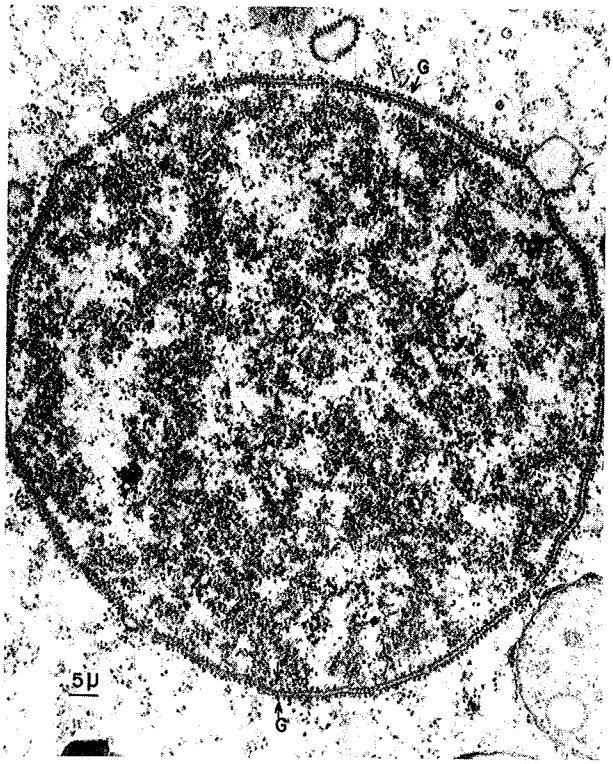

FIG. 2. *A section of* Acanthamoeba *8 hours after induction to encyst showing the nucleus with granules on both inner and outer nuclear membrane. G, granules. X 70000.*

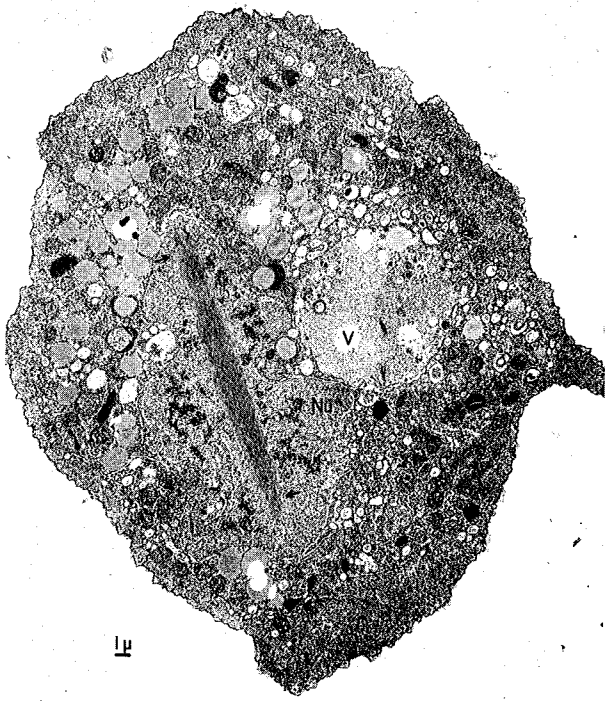

FIG. 3. *A section of* Acanthamoeba *at the 12 hour stage of preencystment. The arrows point to an intranuclear body surrounded by diffuse nucleolar granules. L, lipid; V, vacuole; Nu, nucleus. X 14000.*

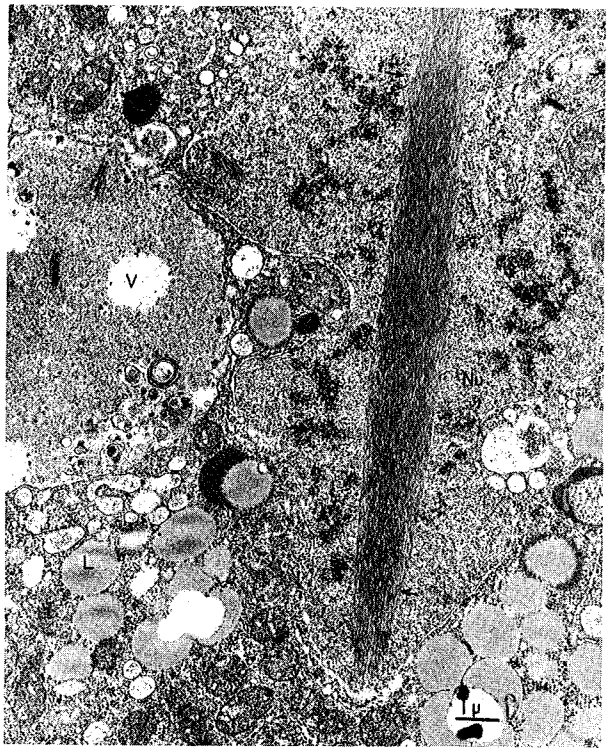

FIG. 4. *A section of* Acanthamoeba *during 12 hour stage of preencystment at higher magnification. The arrows point to the same intranuclear bundle as in Fig. 3. V, vacuole; L, lipid; Nu, nucleus. X 35000.*