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SEQUENTIAL INDUCTION OF MITOCHONDRIAL CHANGES DURING ENCYSTMENT IN *ACANTHAMOEBA CASTELLANII*

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ABSTRACT

Cup-shaped mitochondria are visible by transmission electron mocroscopy in *Acanthamoeba castellanii* during preencystment after this soil amoeba has been induced to form a cyst wall. Later in the encystment process, after cyst wall has become morphologically detectable, large electron dense intramitochondrial bodies reaching 0.5 μm diameter are formed and persist in the fully differentiated cyst. These sequential ultrastructural changes are induced under reproducible cultural conditions and correlate in time of appearance with large intranuclear microfilaments which may traverse the entire nucleus.

INTRODUCTION

The first report of "Intra-cristal vesicles" in mitochondria of *Acanthamoeba castellanii* was made by studying monoxenic cultures of Acanthamoeba grown with *Aerobacter aerogenes* (Vickerman, 1960). Later this same phenomenon was observed in axenic cultures of *Acanthamoeba* by Bowers and Korn (1968). In 1981, Tomlinson reported the appearance of large intranuclear bodies in *Acanthamoeba* after these soil amoebae were induced to undergo encystment. He observed that nuclear ultrastructural changes were also induced by the cyst induction procedure. Such nuclear changes included loss of nucleolar mass, irregular and convoluted nuclear membranes, and diffuse distribution of remaining nucleolar material. In 1984, intranuclear microfilaments and intramitochondrial bodies were observed in the same cells of *Acanthamoeba* cultures which had been induced to form cyst walls (Tomlinson, 1984). It was not clear from the foregoing studies however whether mitochondrial bodies could be specifically induced in *Acanthamoeba* by cyst induction techniques under reproducible cultural conditions.

This report documents the sequential induction of cup-shaped mitochondria and large electron dense intramitochondrial bodies in *Acanthamoeba* which have been induced to encyst synchronously in axenic culture under reproducible cultural conditions. Correlations of mitochondrial changes and nuclear changes are also considered.

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, 1981). 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 encystment 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 concentrated by centrifuging 3 minutes at 500 X g in an HR-1 centrifuge.

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

The mitochondria of trophozoite *Acanthamoeba* are usually oval or elongate in shape and measure 1.0 μm X 1.5 μm in their elongate form. They are surrounded by the classical membrane and possess tubular cristae which characterize other free-living Protozoa. Mitochondrial outer membranes rarely show invaginations in trophozoites and generally appear relatively smooth under low magnification. Occasionally, tubular cristae traverse an entire mitochondrion, and in rare cases, a single intracristal inclusion less than 0.1 μm may be observed (Fig. 1).

Acanthamoeba which have been induced to encyst synchronously, but have not yet produced detectable cyst wall, possess both normal shaped mitochondria as well as cup-shaped mitochondria formed via invagination of the outer mitochondrial membrane. The cups may appear as round vesicles when sectioned transversly (Fig. 2). The invaginated cups, which may become 0.5 μm in diameter and reach deeply into the interior of the mitochondrion, often appear "empty" but may show electron dense particles under staining procedures utilized in this study. It is often difficult to differentiate cups sectioned transversly, if they contain electron dense material, from intracristal bodies which generally appear later in encystment in *Acanthamoeba* after cyst wall synthesis has begun. In some cases, the same mitochondrion will show both the cup-shaped feature and a single, darkly staining, intra- or extracristal inclusion which may reach 0.5 μm diameter in a near-spherical shape (Fig. 3). These mitochondrial changes often occur subsequent to the appearance of large intranuclear microfilaments which also occur in *Acanthamoeba* during encystment (Tomlinson, 1982).

As encystment in *Acanthamoeba* proceeds and cyst wall accumulates, many mitochondria retain a single, darkly staining body which stops increasing in size at approximately 0.5 μm or about half the diameter of the mitochondrion. Such a body may be intracristal or extracristal with

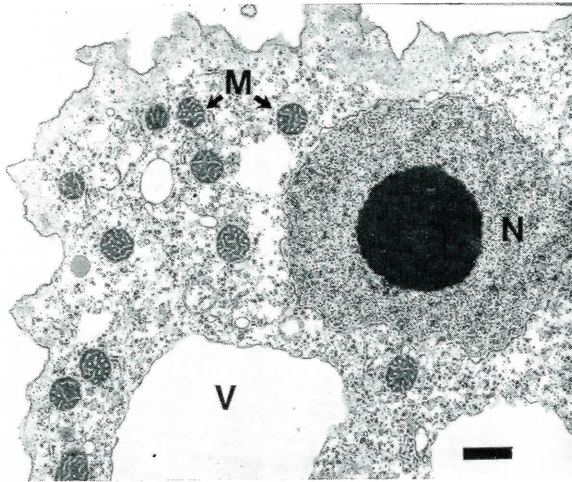

FIG. 1. *A section of* Acanthamoeba *in the trophozoite form prior to induction to encyst. The bar represents 1.0 μm. M, mitochondrion; N, nucleus; V, vacuole. X 26,250.*

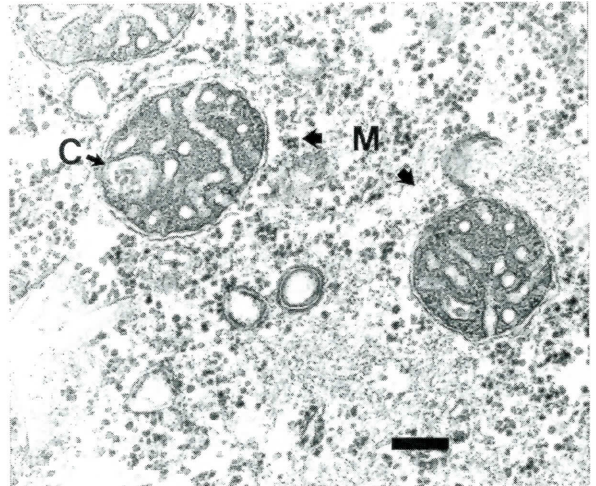

FIG. 2. *A section of mitochondria from* Acanthamoeba *during preencystment after induction to encyst. The bar represents 0.5 μm. M, mitochondrion; C, mitochondrial cup X 140,000.*

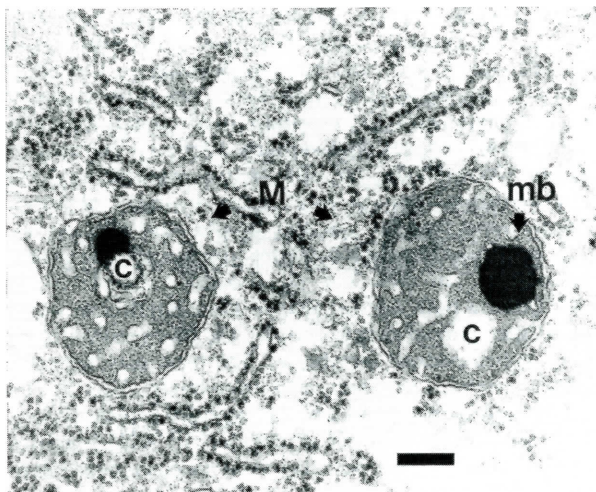

FIG. 3. *A section through mitochondria from* Acanthamoeba *during encystment showing mitochondrial cups and bodies. The bar represents 0.5 μm. M, mitochondrion; Mb, mitochondrial body; C, mitochondrial cup. X 140,000.*

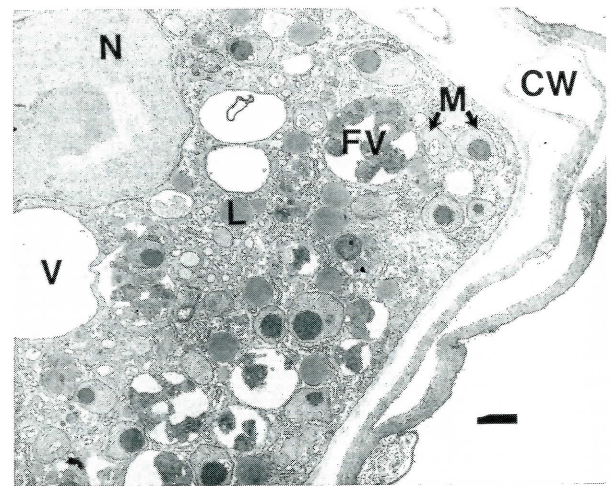

FIG. 4. *A section through an* Acanthamoeba *cyst showing many mitochondrial bodies and cups (double arrow). The bar represents 1.0 μm. M, mitochondrion; N, nucleus, V, vacuole, CW, cyst wall, FV, food vacuole; X 26,250.*

respect to its location in the mitochondrion. Super-dense granules (100-400 A°) which are irregular in shape are often included in the mitochondrial bodies. Endoplasmic reticulum in the cytoplasm is sometimes arranged so that it appears continuous with the outer mitochondrial membrane.

Discussion

The mechanism whereby *Acanthamoeba* mitochondria become cup-shaped or form large dense bodies in their interior during cyst induction is not clear. However, it is well known that aerobic respiration as measured by oxygen consumption decreases rapidly during encystment (Klein and Neff, 1960). In addition, Sobota *et. al.* (1981) reported Ca^{++} and Mg^{++} accumulation in *Acanthamoeba castellanii* under conditions of high cultural tonicity and attributed specific developmental changes in mitochondria and other cellular organelles to calcium binding sites where these ions accumulated. Since large intranuclear microfilaments are also induced in *Acanthamoeba* during preencystment (Tomlinson, 1984), it is tempting to postulate a "Ca^{++} trigger" for *Acanthamoeba* analogous to the role of this ion in vertebrate muscular contraction. Storage of excess calcium in mitochondria of *Acanthamoeba* may alter respiratory mechanisms, induce mitochondrial membrane invagination via local tonicity alteration, and serve in some manner as binding sites for electron dense stains such as those utilized in this study. And how are these changes "reversed" in *Acanthamoeba*? That, too, is not clear at this time. However, the fate of intramitochondrial cups and large electron dense bodies in *Acanthamoeba* are currently under investigation in this laboratory and will be the subject of a future communication.

Acknowledgments

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Literature Cited

Bowers, B, and Korn E. D. 1967. Fine structural changes during the encystment of an ameba. J. Cell Biol. *35*:15A.

Klein, R. L., and Neff, R. J. 1960. Homeostatic mechanisms for cation regulation in *Acanthamoeba*. J. Exptl. Cell Research *19*:133.

Sobota, A., and Przelecka, A. 1981. Developmental changes in the localization of calcium binding sites in *Acanthamoeba castellanii*. Histochemistry *71*:(1)135-144.

Tomlinson, G. 1981. Nuclear changes in *Acanthamoeba* during preencystment. J. Tenn. Acad. Sci. *56*:(3)107-108.

Tomlinson, G. 1982. Induction of intranuclear microfilaments without DMSO. J. Tenn. Acad. Sci. *57*:(2)50-53.

Tomlinson, G. 1984. Intranuclear Microfilaments and cellulose synthesis in *Acanthamoeba*. J. Tenn. Acad. Sci. *59*:(4)90-92.

Vickerman, K. 1960. Structural changes in mitochondria of *Acanthamoeba* at encystation. Nature *188*:248.

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EFFECTS OF VARIOUS PROSTAGLANDINS ON OVARIAN FUNCTION IN THE RAT

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Abstract

The hypothesis postulating that prostaglandin $F_2\alpha$ ($PGF_2\alpha$) induces luteolysis by restricting blood flow to the corpus luteum was tested. Immature female rats bearing functional corpora lutea were treated with various vasodilating or vasoconstricting prostaglandins, and levels of progesterone and 20α-dihydroprogesterone in serum and in medium of cultured luteal cells were used as the index of luteal cell function. Although luteal function generally follows the vascular action of prostaglandins (i.e., vasodilators are luteotrophic and vasoconstictors are luteolytic) this logic does not apply to all prostaglandins. These results indicate that a vasoconstricting property does not ensure luteal regression and $PGF_2\alpha$-induced luteolysis is caused by factors in addition to decreased ovarian blood supply.

Introduction

In the mammalian ovary, the corpus luteum (CL) is formed after ovulation from the follicle which releases the egg. The main function of the corpus luteum is to secrete progesterone which maintains pregnancy by inhibiting uterine contraction. In the absence of conception, however, the CL degenerates which is characterized by a sharp drop in serum progesterone levels and a subsequent rise in the level of its metabolite, 20α-dihydroprogesterone (20α-DHP). This process, known as luteolysis, is caused by one prostaglandin, $PGF_2\alpha$ which is a 20-carbon unsaturated carboxylic acid with a cyclopentane ring. Prostaglandin $F_2\alpha$ has been noted for its potent luteolytic effect in many species including humans (Vijayakuman and Walters, 1983). Evidence for the luteolytic action comes partly from the ability of exogenously administered $PGF_2\alpha$ in regressing the CL and the identification of $PGF_2\alpha$ as the naturally-occurring luteolysin in sheep by Goding et al. (1974). Other evidence includes: Increased length of luteal phase in the rat following injection of indomethacin, which suppresses production of all classes of prostaglandins (Patrono et al., 1976); prolonged estrus cycle in the cow and sheep when passively immunized with $PGF_2\alpha$ antibodies (Fairclough et al., 1981); and morphologic degeneration of the CL by $PGF_2\alpha$ in the guinea pig (Pavvola, 1979).

Other prostaglandins are effective in modifying luteal function. For example, PGE_1, although less potent than $PGF_2\alpha$, is luteolytic in some species, while $PGE_2\alpha$ appears to be luteotrophic in many species. Because of the major physiological effects of prostaglandins is their ability to constrict or dilate blood vessels, a hypothesis has been formulated to explain the underlying mechanism of $PGF_2\alpha$-induced luteolysis. The hypothesis is supported by fragmented evidence showing that some vasodilating prostaglandins are luteotrophic, while vasoconstricting prostaglandins are luteolytic (Milvae and Hansel, 1983). In