Division Stages of the Mitochondria in Normal and Actinomycin-Treated Plasmodia of *Physarum polycephalum*[1]

Multiplication of mitochondria by division has been postulated for many years[2] and has been experimentally demonstrated more recently[3,4]. As mitochondria of a large variety of organisms have been shown, by electron microscopy[5] and by autoradiography[6–8], to contain DNA, their nature as self-replicating units seems now established. Morphological variability of mitochondria, suggestive of division stages, has been reported for various organisms[9–15], and in some of these reports the occurrence of 'division' stages has been correlated to specific stages of cell growth[14] and multiplication[9,10,15]. The mitochondria of *Physarum polycephalum* contain a 'nucleoid'[12] and they are remarkably rich in DNA[16,17]. The following report provides additional evidence that the mitochondria of *P. polycephalum* have a regular division cycle in which division of the mitochondrion is preceded by that of the nucleoid.

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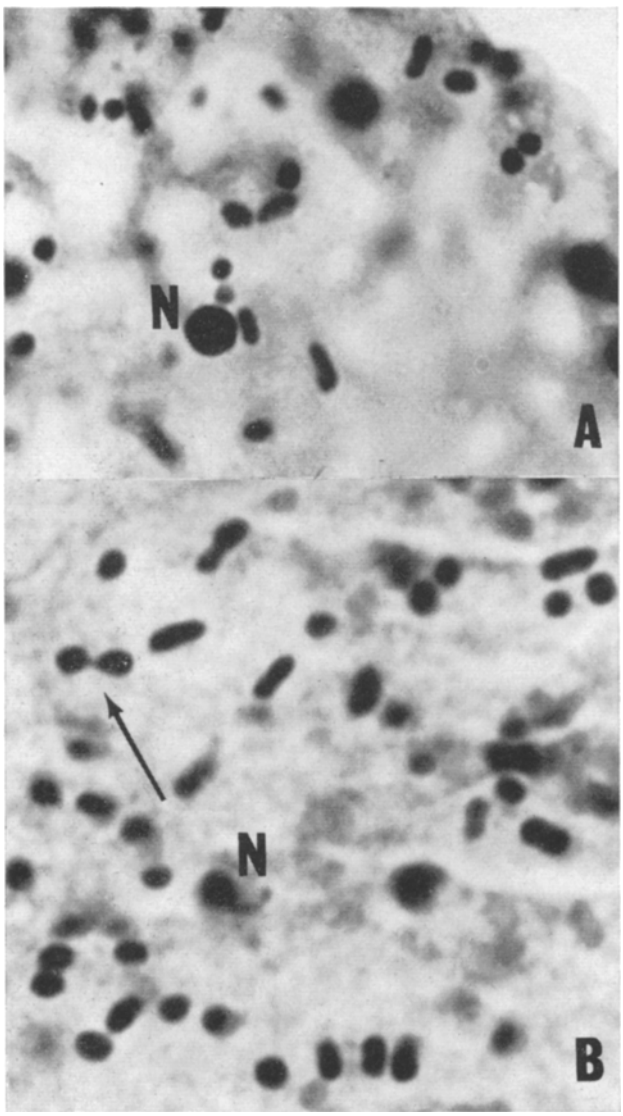

Fig. 1. Mitochondria, fixed with Champy's mixture and stained with acid fuchsin. × 1550. (A) Plasmodium untreated. N, nucleus. (B) Plasmodium treated with actinomycin. N, nucleus with abnormal spindle. Arrow: mitochondrion with narrow constriction.

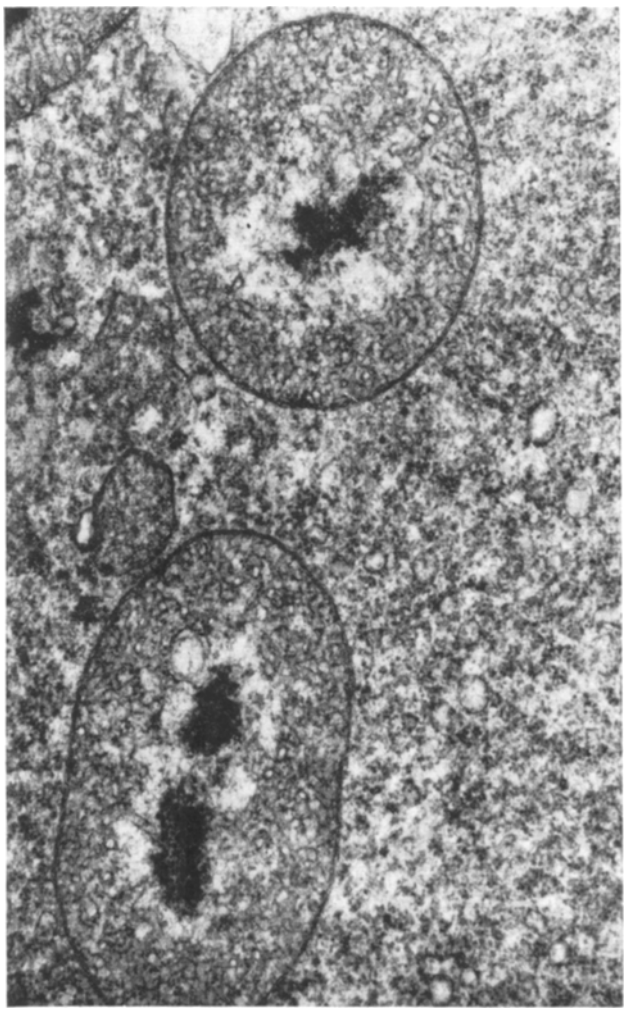

Fig. 2. Two mitochondria from untreated plasmodium. × 15,800.

The organism was grown according to DANIEL and BALDWIN[18]. Mitochondria of normal plasmodia were compared with those of plasmodia treated with actinomycin C[19]. For microscopic observation, plasmodial pieces were fixed with Champy's mixture, and sections (5 μ) were stained with acid fuchsin. For electron microscopy, pieces were fixed with glutaraldehyde followed by osmic acid and embedded in Epon 812 as described previously[20]. Sections were stained with lead citrate according to REYNOLDS[21].

Plasmodia from rapidly growing cultures (Figure 1A) contain a large variety of morphologically different mitochondria. About 32% of all mitochondria are rod-shaped, some of them slightly constricted in the middle. They are approximately 4.5 μ long. Less than 0.2% of the mitochondria are dumbbell-shaped with a narrow constriction in the middle as one would expect from a division stage just prior to the separation into 2 halves. The large majority of mitochondria are globular, with gradual transitions to an elliptic form which approaches that of the rod-shaped mitochondria. Since the length of the rod-shaped mitochondria does not exceed the thickness of the sections (5 μ) it is not likely that the presences of all, or even most, of the mitochondrial globules in sections is due to fragmentation by sectioning of rod-shaped mitochondria into smaller pieces. After treatment of the plasmodia for 18 h with actinomycin C at a concentration of 30 μg/ml, the number of mitochondria having a narrow constriction in the middle increased to approximately 1.4%. This increase might be due to an extension of the time required for mitochondrial division since treatment of *Physarum* with actinomycin is known to prolong nuclear division for several hours[22]. After treatment with actinomycin for 36 h almost all mitochondria were globular and larger than the globular mitochondria in untreated plasmodia.

The above interpretation of the different morphological types of mitochondria found both in normal and actinomycin-treated plasmodia as division stages is supported by electron micrographs (Figures 2–4). Figure 2 shows 2 mitochondria from an untreated plasmodium. One of them appears globular and has a large central nucleoid, while the other is ovoid and contains 2 nucleoids. The mitochondrion in Figure 3 is about to divide into 2 pieces of similar size and each half receives 1 nucleoid.

Figure 4 shows mitochondria after treatment with actinomycin C (30 μg/ml for 18 h). The mitochondria exhibit a coarser tubular structure than those in untreated control plasmodia. While many mitochondria are similar in shape to those from untreated plasmodia, there is a large number, whose appearance is different in a way which is best explained by the assumption that, in the presence of actinomycin, the division time of the mitochondria and that of the nucleoid is prolonged. One commonly found type (Figure 4A) is elongated, with a slight constriction in the middle and an incompletely divided nucleoid. Others (Figure 4B) have a much

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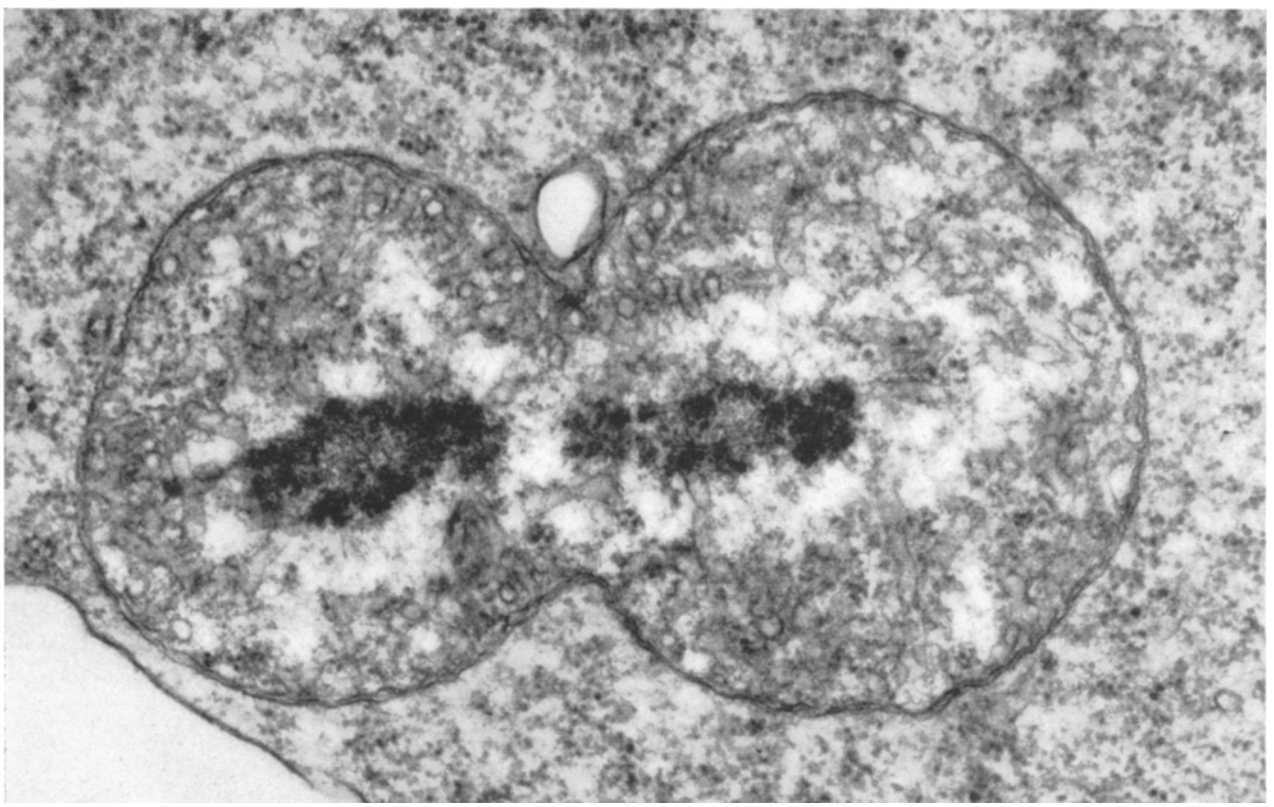

Fig. 3. Mitochondrion from untreated plasmodium. × 45,000.

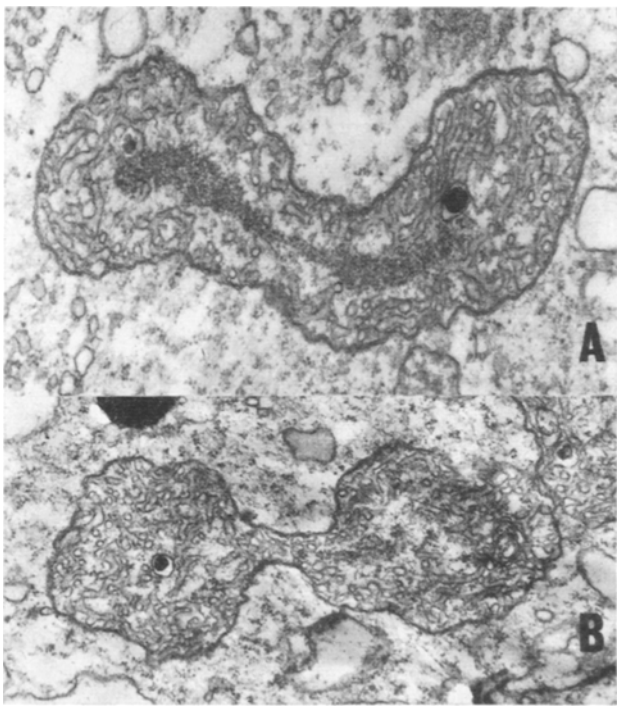

Fig. 4. Mitochondria from plasmodium treated with actinomycin. (A) Elongated mitochondrion showing delayed division of nucleoid. × 13,000. (B) Mitochondrion with narrow constriction, probably similar to that in Figure 1B. Nucleoid understained. × 17,400.

narrower constriction in the middle. They may be similar to the type shown in Figure 1B. In these mitochondria also, division of the nucleoid appears to be inhibited. Incomplete division of the nucleoid in mitochondria that had a narrow constriction in the middle was not found in untreated plasmodia.

The finding that the mitochondria of *P. polycephalum* are rich in DNA[16,17] and that they appear to have a regular division cycle involving division of a nucleoid would suggest the possibility that, during evolution, they might have retained a comparatively high level of autonomy.

The above-described division stages were not correlated with a specific stage of the mitotic cycle of the plasmodia.

Zusammenfassung. Die Form der Mitochondrien und das Verhalten des Mitochondrien-Nukleoids im Schleimpilz *Physarum polycephalum* werden durch Behandlung mit Actinomycin C in einer Weise modifiziert, die darauf schliessen lässt, dass die Mitochondrien einen regelmässigen Teilungszyklus durchlaufen, der mit der Teilung des Nukleoids korreliert ist.

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Inactivation of Tissue-Specific Inhibitors by a Carcinogen (Diethylnitrosamine)

The deletion of tissue-specific growth-regulating proteins by carcinogens has been postulated to be of primary importance during carcinogenesis[1]. Carcinogens become bound to specific proteins (h-proteins)[2–5], which are eliminated during carcinogenesis[3,5,6]. A direct inactivation of growth-regulating factors could indeed lead to neoplastic growth, provided that the deletion becomes hereditary; a model to explain such hereditary changes has been proposed[7]. Purified fractions of h-proteins were shown to inhibit reversibly cell growth in tissue-culture[8]; however it was found that liver arginase is responsible for most of the inhibition[9].

Starting from the study of wound healing in mouse skin, BULLOUGH developed the concept of tissue-specific mitosis inhibitors ('chalones') acting together with hormones, which are lacking or reduced in tumours[10].

In the following experiments we studied the effect of liver supernatants from adult rats (1:4 homogenate; S_3: 78,000 g; 2.5 h) containing carcinogen-binding h-proteins on liver explants. Despite some drawbacks (time consumption, non-uniform size, diffusion rate, internal necrosis) these explants offer a better test system for potential liver specific substances than HeLa or L-cells[8] or ascites cells[11]. Some tissue culture cells and kidney explants were used for comparison. Diethylnitrosamine (DENA) was used as a fast reacting carcinogen (10 mg/kg i.v.). Supernatants listed in experiments 1–3 in Table II and in Table I were partially purified as suggested by BULLOUGH using 50–80% ethanol precipitates[10].

Livers from new-born rats were cut into 1 mm pieces, incubated as described under Table I and processed as described elsewhere[12]. Kidney explants were similarly handled. Tissue culture cells were grown as monolayers in flasks in their proper media, distributed into roller tubes ($2.5–3.5 \times 10^5$ cells/ml and tube) and incubated overnight. The medium was removed and 0.9 ml TCM-199 and 0.1 ml supernatant were added. The monolayers were incubated for 3 h, then 1 μC ^{3}H-thymidine was added. The incorporation was stopped after 1 or 2 h by cooling and adding 0.1 ml 1.0*N* NaOH. Controls without cells were run alongside. The monolayers were broken by stirring and ultrasonicating. TCA-soluble material was

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