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INFLUENCE OF ZERO GRAVITY SIMULATION ON TIME COURSE OF MITOSIS IN MICROPLASMODIA OF *PHYSARUM POLYCEPHALUM*

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ABSTRACT

Detrimental effects of weightlessness are no longer expected to hinder successful mitosis. Experiments in space and on the fast clinostat give no hints of this. Nevertheless we are thinking of a g sensitivity during the process of chromosome condensation and distribution. The time course of nuclear division in microplasmodia of the slime mold Physarum polycephalum was investigated under 0 g simulation on the fast rotating clinostat in comparison to 1 g controls. The result of this experiment is: A significant shortening of mitosis under 0 g simulation compared to 1 g controls.

INTRODUCTION

In the last twenty years an increasing number of cell physiologists have been studying the acellular slime mold Physarum polycephalum. Two milestones of Physarum research are the observations of synchronous nuclear divisions in the multinucleate plasmodium by Howard [1] and Daniel and Rusch's [2] success in growing microplasmodia in shuttle cultures in an axenic medium under sterile conditions. Today Physarum is a classical subject for cell cycle studies. The time course of synchronous intranuclear mitoses has been defined by Guttes and Rusch [3] and by Sachsenmaier [4] by photographic records of mitotic stages in macroplasmodia of Physarum in a series of alcohol-fixed preparations. Later, Wolf et al. [5] observed mitosis in macroplasmodia of Physarum in vivo by time lapse microcinematography. Morphological and temporal sequences of the mitotic events were established.

This paper addresses the question of whether mitosis, as an endogenously regulated distinct part of the cell cycle, may be sensitive to gravity. One way to answer this question is to exclude the effect of gravity. We performed this by simulating weightlessness.

MATERIAL AND METHODS

For the simulation of weightlessness we used the fast rotating clinostat method (Briegleb [6], Briegleb et al. [7], Schatz et al. [8] and Silver [9]).

Microplasmodia, grown in a shuttle culture at 130 vibrations per minute and 23 ± 1°C, were harvested 48 hours after inoculation into fresh medium and were taken for the experiments. These microplasmodia exhibit different, mostly spherical structures, varying in size between 50 and 500 µm (for details see Gawlitta et al. [10]). For the in vivo observation of mitoses in microplasmodia a microchamber (MC) with a diameter of 24 mm and a depth of 3.5 mm was constructed (Fig. 1a). To handle it on the fast rotating clinostat the MC was mounted inside a special steel holder of 76 x 38 x 5 mm (Fig. 1b) which is part of the necessary mechanical linkage between the different parts of the clinostat microscope and at the same time avoids transmission of torque to the MC during clinostat rotation. Figure 2 shows a part of the clinostat microscope including the tightly fixed holder. The optical axis is identical with the horizontal axis of rotation. This guarantees complete functional 0 g simulation.

At the beginning of the experiment one drop of a freshly harvested microplasmodia suspension is inoculated onto a cylindrical agar block with a diameter of 12 mm and a depth of 2.5 mm containing 4% Difco Bacto-agar and 20% axenic medium after Daniel and Rusch [2] which is placed onto a cover glass inside the bottom part of the MC. Now the top part including a second cover glass and the bottom part are screwed carefully into one another (see Fig. 1) until the microplasmodia are flattened by contacting the upper cover glass. Flattening is necessary to hold the nuclei in place and in focus because of the vigorous protoplasmic streaming which persists during mitosis (but decreases during anaphase to early telophase). To increase the viability of the microplasmodia the MC is continously

floated on a moistened gas mixture of 40% oxygen and 60% nitrogen introduced into the brass chamber by means of a capillary. Before final mechanical adjustment of the preparation to the clinostat microscope a suitable area within a microplasmodium containing nuclei in late interphase has to be selected for further observation. If a satisfying field of observation is found, the preparation is mechanically fixed (see Fig. 2). After that, the clinostat is accelerated to 80 rpm (revolutions per minute) within 10 seconds.

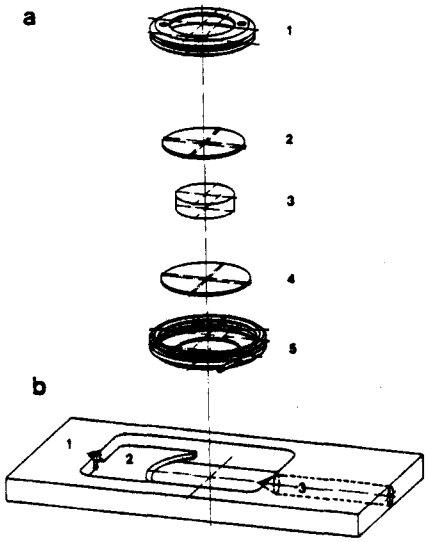

Fig. 1 Drawing of the microchamber (a) and holder (b). a. Microchamber: (1) Brass chamber top part with outer thread, (2) cover glass, (3) agar block, (4) cover glass, (5) brass chamber bottom part with inner thread and capillary for O_2 and N_2 supply. b. Steel holder: (1) Steel holder, (2) brass tongue and (3) screw for fixing the microchamber.

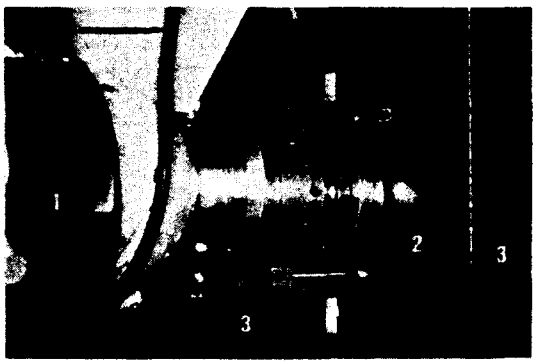

Fig. 2 View on a part of the clinostat microscope. Support and traction elements for the mechanical connection of (1) tube, (2) holder and (3) microscope table. Bar represents 2 cm.

Microscopy was performed with a Zeiss Neofluar Ph 63/1.25 oil immersion lens with phase contrast technique, using red light (630 nm) and a Zeiss heat protection filter. A television camera (Philips, Eindhoven, The Netherlands) is mounted within the clinostat. The camera signal is transmitted via a video timer (For-A Company Lim., Tokyo, Japan) to a time lapse video recorder (International Video Corporation, Sunnyvale, California) and a monitor. In our experiments we used a time compression factor of 30. When the nucleoli began to move out of their central position (see Fig. 4a) the time course of mitosis was recorded. When the divided daughter nuclei became visible the experiment was terminated. Evaluation of the recorder tapes was carried out frame by frame. The measured mitotic stages were classified according to the norm established by Wolf et al. [5] .

RESULTS

A 0 g simulation of high quality is only achieved with small subjects (Briegleb [11] , Sobick and Sievers [12] and Cogoli et al. [13] . Microplasmodia (Fig. 3), a special growing type of Physarum, fulfill this precondition. As a spontaneous reaction to the above mentioned flattening process the microplasmodia stop protoplasmic streaming. After an adaptation time of 30 to 60 minutes they get back their streaming activity which is accompanied by amoeboid movement. This movement, however, is nearly supressed by flattening. In contrast to Wolf et al. [5] we use stage 3 as a distinct starting point of mitosis which is characterized by the beginning dissolution of the peripherally located nucleoli. This is necessary, because the duration of stage 1 to 2 could vary between 30 minutes and 4 hours under

1 g as well as under 0 g simulation. The results are based on the evaluation of six sequences of mitoses under 1 g (control on the standing clinostat microscope) and eight sequences of mitoses on the rotating clinostat microscope comprising 45 dividing nuclei. The mitotic stages which were selected for the evaluation are shown in Figure 4a. This selection was done, because these stages represent distinct points of mitosis. Others were also registered but not evaluated. The selected stages were taken for the calculation of the interval lengtn between single stages and the onset of anaphase (stage 13) as a normpoint (Fig. 4a)

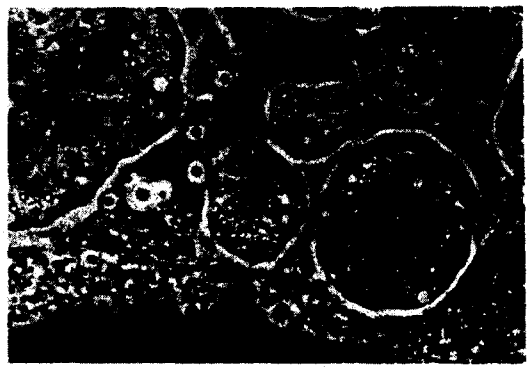

Fig. 3 Flattened microplasmodia within the microchamber after an adaptation time of 45 min show late interphase nuclei (►) with centrally located nucleoli. Bar represents 10 µm.

and for the calculation of the interval length between successive stages as shown in Figure 4b.

The main result of this experiment is a significant shortening of mitosis under 0 g simulation compared to the 1 g control. Figure 4a shows time reductions in interval length of 18.7% (stage 3-13); 27.9% (stage 6-13); 44.9% (stage 10-13); 54% (stage 12-13); 28.6%

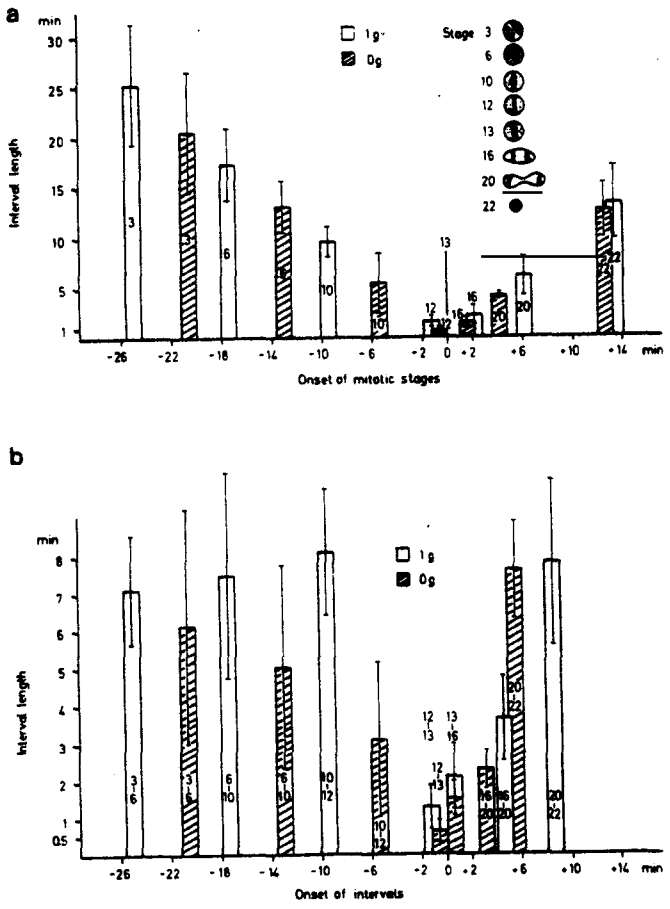

Fig. 4 Time course of selected mitotic stages in microplasmodia of Physarum polycephalum under 1 g and on the fast rotating (80 rpm) clinostat at 23°C. The hatched columns always represent the time schedule of mitotic events under 0 g simulation, the white columns are centered at the beginning of each interval. a. Length and onset of intervals between single mitotic stages and the normpoint anaphase (stage 13). b. Length and onset of intervals between successive mitotic stages relative to the normpoint anaphase. Vertical bars represent twice mean error.

(stage 13-16) and 32.8% (stage 13-20). The maximum shortening is observed in interval stage 12-13. With the beginning of the reconstruction phase of the daughter nuclei the difference in interval length (stage 13-22) between 0 g simulation and 1 g control is still 6% which is not significant at the 95% level. A confirmation of this result is given in Figure 4b. Here the interval length between successive mitotic stages were compared to the 1 g control. Under 0 g simulation we observed time reductions in interval length of 13.3% (stage 3-6), 27.9% (stage 6-10), 61.6% (stage 10-12), 52.3% (stage 12-13), 27.9% (stage 13-16) and 37% (stage 16-20). With the beginning of the reconstruction phase (stage 20-22) of the daughter nuclei the time difference still amounts 2.8% which is not significant at the 95% level in comparison to the 1 g control.

CONCLUSIONS

Results of American and Russian space experiments concerning the influence of weightlessness on cell division and on cell cycle of various biological systems have shown only small effects. The constitution and distribution of the genetic material as well as morphological differentiation is not disturbed under 0 g. Some of those findings, mostly based on chemically fixed material, are the following: Cells of wheat roots divided more slowly [14] . In Crepis capillaris seedlings the mitotic index was slightly higher [15] . A trend towards a higher division rate was found in amoebae [16] . A stimulating effect on cell proliferation was observed in paramecia cultures [17] .

This paper contributes to the question of a gravitational sensitivity during the dynamic course of mitosis which need not necessarily lead to an influence on further differentiation steps. Our findings prove the presence of a gravitational reaction during the process of chromosome condensation (supercoiling and weight doubling of chromatin fibers during prophase) and distribution (movements of condensed chromosomes from prophase to telophase) represented by an overall shortening of mitosis under 0 g simulation (Fig. 4). The shortening of the chromosome condensation period (stage 3 to 10, Fig. 4a) is difficult to explain. One may suspect that the interaction between DNA and the core particles (histones) which is responsible for the final folding of the DNA will be slightly retarded by the weight of the chromosome under 1 g [18] . More plausible is the faster movement of the condensed chromosomes (stage 10 to 16, Fig. 4a) under 0 g simulation, because the size and weight of the condensed chromosomes should be great enough to influence the motive forces of the spindle apparatus under 1 g.

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