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Cell proliferation of *Paramecium tetraurelia* on a slow rotating clinostat

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Abstract

Paramecium is known to proliferate faster under microgravity conditions, and slower under hypergravity. Experiments using axenic culture medium have demonstrated that hypergravity affected directly on the proliferation of *Paramecium* itself. In order to assess the mechanisms underlying the physiological effects of gravity on cell proliferation, *Paramecium tetraurelia* was grown under clinorotation (2.5 rpm) and the time course of the proliferation was investigated in detail on the basis of the logistic analysis. On the basis of the mechanical properties of *Paramecium*, this slow rate of the rotation appears to be enough to simulate microgravity in terms of the randomization of the cell orientation with respect to gravity. *P. tetraurelia* was cultivated in a closed chamber in which cells were confined without air bubbles, reducing the shear forces and turbulences under clinorotation. The chamber is made of quartz and silicone rubber film; the former is for the optically-flat walls for the measurement of cell density by means of a non-invasive laser optical-slice method, and the latter for gas exchange. Because of the small dimension for culture space, *Paramecium* does not accumulate at the top of the chamber in spite of its known negative gravitactic behavior. We measured the cell density at regular time intervals without breaking the configuration of the chamber, and analyzed the proliferation parameters by fitting the data to a logistic equation. As a result, *P. tetraurelia* showed reduced proliferation under slow clinorotation. The saturation of the cell density as well as the maximum proliferation rate decreased, although we found no significant changes on the half maximal time for proliferation. We also found that the mean swimming velocity decreased under slow clinorotation. These results were not consistent with those under microgravity and fast rotating clinostat. This may suggest that randomization of the cell orientation performed by slow rotating clinostat has not the same effect on *Paramecium* as that under microgravity that may affect the proliferation as the result of the reduced cost of propulsion.

Keywords: *Paramecium*; Clinorotation; Simulated microgravity; Proliferation rate; Swimming velocity

1. Introduction

Previous space experiments aboard the Soviet orbital station Salyut 6 (CYTOS experiment) and the Space Shuttle (D1 mission) reported that the proliferation of *Paramecium* became faster (about 160%) under microgravity in space (Planel et al., 1981; Richoilley et al., 1986). The same authors reported that proliferation became slower (about 70%) under hypergravity (20*g*) provided by centrifugation (Tixador et al., 1984; Planel et al., 1990; Richoilley et al., 1993). Experiments using axenic culture medium have demonstrated the direct effect of hypergravity on the proliferation of *Paramecium* itself, other than the indirect effect on the proliferation of nutrient bacteria grown in non-axenic culture (Richoilley et al., 1993; Kato et al., 2003).

Mechanisms underlying the gravity dependent changes in proliferation could be explained in terms of the energetics of the proliferation as well as of the motile activities of the cell. Because *Paramecium* modulate their propulsive thrust depending on the orientation of the cell body with respect to the gravity vector, i.e. increasing propulsive thrust in upward swimming and decreasing it in downward (Machemer et al., 1991; Ooya et al., 1992). This gravity-induced change in propulsion, gravikinesis, is explained

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on the basis of cellular mechanosensitivity in combination with close coupling between the membrane potential and ciliary locomotors activity (Machemer, 1990). It seems likely that *Paramecium* consumes much energy when swimming upwards than that when swimming downwards. Mogami et al. (2001) demonstrated that the gravitactic orientation of *Paramecium* is mechanically biased by the torque mainly due to the fore-aft asymmetry of the cell body (gravitaxis). The upward-orienting torque increases with increase in gravity acceleration, so that the fraction of the upward-orienting cell would decrease under reduced gravity, and increase under hypergravity. This would result in a change in energy expenditure for propulsion under different gravity accelerations. Thrust force would be increased under hypergravity and hence reduced under microgravity. If the energy available for physiological events of the cell is limited, changes in the energy for propulsion may lead to the changes in the energy stock for the proliferation. The larger stock of energy under microgravity as the result of the reduced propulsive work might enhance the proliferation activity and smaller stock would reduce this activity under hypergravity.

In order to assess the feasibility of the assumption above, *Paramecium tetraurelia* was grown under simulated microgravity performed by clinorotation. If it is the case, we may expect the cell proliferation of *Paramecium* to be enhanced by randomization of the cell orientation by means of clinorotation.

2. Materials and methods

P. tetraurelia was cultivated axenically as described previously (Kato et al., 2003) in the medium of Soldo et al. (1966) with the modifications by Fok and Allen (1979). We used a closed chamber for the cell culture under clinorotation in which cells were confined without air bubbles for reducing the shear forces and turbulences under rotation (Fig. 1). The chamber had a culture space with an inner dimension of $3 \times 3 \times 60$ mm, which had an inlet and outlet for the medium. Three walls of the culture space were made of quartz and the rest of silicone rubber film. The quartz walls provided the optically-flat windows for the measurement of cell density by means of a non-invasive laser optical-slice method (Mogami et al., 2000; Kato et al., 2003). Gas exchange for respiration was carried out through the silicone rubber film between the medium and the moist air in the space placed next to the culture space. This prevented the formation of air bubbles in the culture medium caused by the evaporation of water in association with gas exchange. The depth of the culture volume was restricted to 3 mm when the chamber was placed horizontally. In this shallow space, *Paramecium* showed roughly even vertical distribution rather than the apparent accumulation at the top of the culture volume as observed in a deep water column.

The culture chamber was placed on a rotator (RT-5 TAITEC, Tokyo) with the long axis of the chamber kept horizontal and rotated around the long axis (clinorotation) or around the short axis perpendicular to the long axis (control rotation) (Fig. 2). Both the direction and the speed of rotation (2.5 rpm) were kept constant throughout an experiment. For the horizontal rotation, the chamber was placed with the silicone rubber as the side wall of the culture space.

Cell density was measured without breaking the configuration of the chamber by optical-slice method (Kato et al., 2003) with removing the chamber from the rotators. The time course of the cell proliferation under clinorotation was analyzed on the basis of the logistic growth equation. According to the three parameters determined by means of the least squares fitting of the equation to the experimental data as described in Kato et al. (2003), the kinetic parameter of proliferation (saturation cell density, maximum pro-

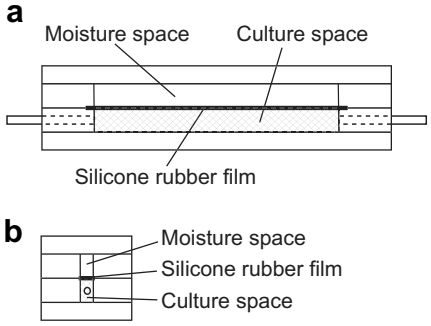

Fig. 1. Schematic drawings of the closed chamber for the culture of *Paramecium* under clinorotation. Side view (a) and front view (b) of the chamber showing the culture space and moist air space for gas exchange.

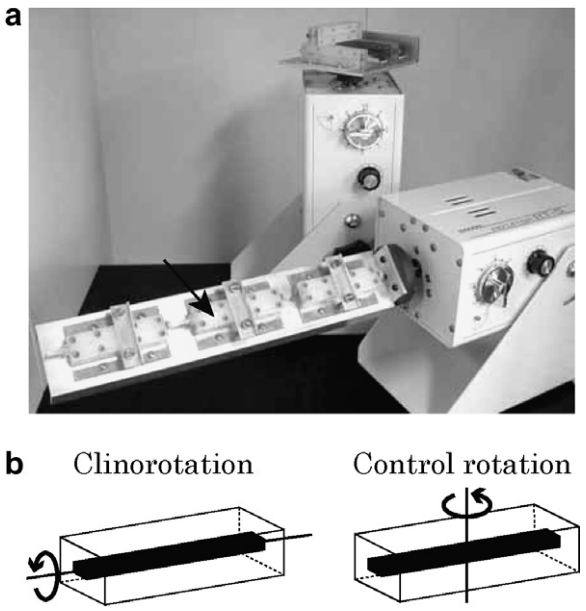

Fig. 2. (a) An overview of the rotator developed for clinorotation. Arrow indicates one of the closed chambers. (b) Schematic drawing of the rotation of the culture space in the clinorotation and the control horizontal rotation.