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# Responses to Hypergravity in Proliferation of *Paramecium tetraurelia*

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**ABSTRACT**—It has been reported that *Paramecium* proliferates faster when cultured under microgravity in orbit, and slower when cultured under hypergravity. This shows that the proliferation rate of *Paramecium* is affected by gravity. The effect of gravity on *Paramecium* proliferation has been argued to be direct in a paper with an axenic culture under hypergravity. To clear up uncertainties with regard to the effect of gravity, *Paramecium tetraurelia* was cultured axenically under hypergravity ( $20 \times g$ ) and the time course of the proliferation was investigated quantitatively by a new non-invasive method, laser-beam optical slice, for measuring the cell density. This method includes optical slicing a part of the culture and computer-aided counting of cells in the sliced volume. The effects of hypergravity were assessed by comparing the kinetic parameters of proliferation that were obtained through a numerical analysis based on the logistic growth equation. Cells grown under  $20 \times g$  conditions had a significantly lower proliferation rate, and had a lower population density at the stationary phase. The lowered proliferation rate continued as long as cells were exposed to hypergravity ( $>$  one month). Hypergravity reduced the cell size of *Paramecium*. The long and short axes of the cell became shorter at  $20 \times g$  than those of control cells, which indicates a decrease in volume of the cell grown under hypergravity and is consistent with the reported increase in cell volume under microgravity. The reduced proliferation rate implies changes in biological time defined by fission age. In fact the length of autogamy immaturity decreased by measure of clock time, whereas it remained unchanged by measure of fission age.

**Key words:** *Paramecium*, hypergravity, axenic culture, proliferation rate, autogamy immaturity

## INTRODUCTION

The increased opportunity of microgravity experiments in space provides a challenge to investigate the effect of gravity on biological activities. In addition to the fact that gravity affects the large-sized phenomena such as growth and morphogenesis of individual organisms, several lines of evidence indicate that it acts on the activities of organisms of cellular dimensions. Since an experiment conducted in 1983 in Spacelab-1 revealed amazing effects of microgravity on human lymphocytes (Cogoli *et al.*, 1984), a wide range of investigation was triggered on the sensitivity to gravity of the cell of various sources (Moore and Cogoli, 1996; Cogoli and Cogoli-Greuter, 1997; Lewis and Hugens-Fulford, 1997). Among them *Paramecium* has been subjected to the research of gravitational biology, because of its

pronounced behavioral response to gravity, gravitaxis (Machemer *et al.*, 1991; Ooya *et al.*, 1992; Braecker *et al.*, 1994; Hemmersbach *et al.*, 1996). A series of experiments, Cytos in Salyut 6, first demonstrated that the proliferation rate of *Paramecium tetraurelia* was stimulated during the space flight (Planel *et al.*, 1981). This stimulatory effect was confirmed by the experiment carried out during the Space Shuttle D1 mission, which offered the first opportunity to use an onboard centrifuge to provide an inflight  $1 \times g$  control for direct comparison to microgravity (Richoille *et al.*, 1986). On the other hand, the inhibitory effect was found on the proliferation activity when *Paramecium* was grown under increased gravity (hypergravity) provided by the centrifugal rotation (Tixador *et al.*, 1984; Planel *et al.*, 1990; Richoille *et al.*, 1993). These results indicate that *Paramecium* might be sensitive to a gravity environment and could manage the proliferation activity according to changes in the magnitude of gravitational force.

Richoille *et al.* (1993) compared the effect of hyper-

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gravity on the proliferation of *P. tetraurelia* grown under axenic conditions to that under non-axenic, and revealed a reduced proliferation under both culture conditions. This may exclude the possibility that gravity did not affect directly on *Paramecium* but on bacteria which are known to respond to gravity conditions and also to alter the proliferation rate as in the case of *Paramecium*: microgravity in space has stimulatory effects on the proliferation, and hypergravity has inhibitory ones (Brown *et al.*, 2002).

In order to gain an insight into the mechanisms underlying the sensitivity to gravity, *P. tetraurelia* was grown axenically using an improved culture medium and the effect of hypergravity was investigated quantitatively on the proliferation and morphology of the cell. The time course of proliferation was investigated on the basis of a new non-invasive method for density measurement, optical slice method. By this method, cells in a given volume of the culture were illuminated by a slit laser beam and the cells recorded as bright spots were counted by a computer-assisted image analysis. This method enables us to measure the density without breaking the confinement of the culture, which is much advantageous for us to keep the cell culture axenic and also free from disturbances due to sampling procedures. The effects of hypergravity were assessed by comparing the kinetic parameters of proliferation, which were obtained through a numerical analysis based on the logistic growth equation.

We also assessed the effects of hypergravity on autogamy immaturity. Autogamy is a form of sexual reproduction which occurs in a single cell. It consists of meiosis and self-fertilization. In *P. tetraurelia* autogamy is induced by starvation after a certain period, which follows the previous autogamy or conjugation. The period of autogamy immaturity includes the period during which autogamy can not be induced by natural starvation and the transitory period during which cells gradually come to respond to starvation by autogamy (Ishikawa *et al.*, 1998). This process of maturation leading to autogamy maturity is known to occur in close

relation to the number of fission (fission age) of the cell from the former events of sexual reproduction. Does altered gravity affect the maturation process of *P. tetraurelia*? If hypergravity reduces the proliferation rate, one question should be examined whether the length of autogamy immaturity period is extended by measure of clock time, while it is maintained by measure of fission age. In the present study the maturation process into a 100% autogamous culture, i.e. autogamy can be induced in all the cells in that culture by starving them at an appropriate fission age, was compared under normal and hypergravity conditions according to the measure of universal clock time or fission age.

## MATERIALS AND METHODS

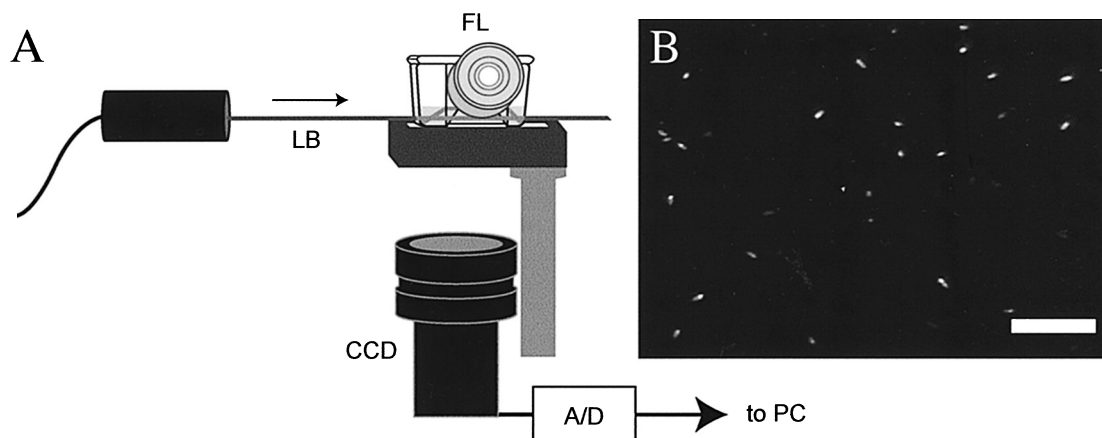
### Cells and culture medium

*Paramecium tetraurelia* (stock 29) was a gift from Dr. Birgit Satir of Albert Einstein College of Medicine, New York. Cells were cultivated axenically in the medium of Soldo *et al.* (1966) with the modifications by Fok and Allen (1979). The culture medium consists of 0.02 mM calcium pantothenate, 0.04 mM nicotinamide, 0.02 mM pyridoxal HCl, 0.01 mM pyridoxamine HCl, 0.01 mM riboflavin, 0.06 mM folic acid, 0.04 mM thiamine, 2.56 nM biotin, 0.24  $\mu$ M dl-thictic acid, 2.03 mM MgSO<sub>4</sub>, 6.06  $\mu$ M stigmasterol, 10.0 g/l proteose peptone, 5.0 g/l polypepton, 1.0 g/l yeast RNA, 0.2 g/l phosphatidylethanolamine (pH7.0). The temperature during culture was 24°C unless otherwise specified.

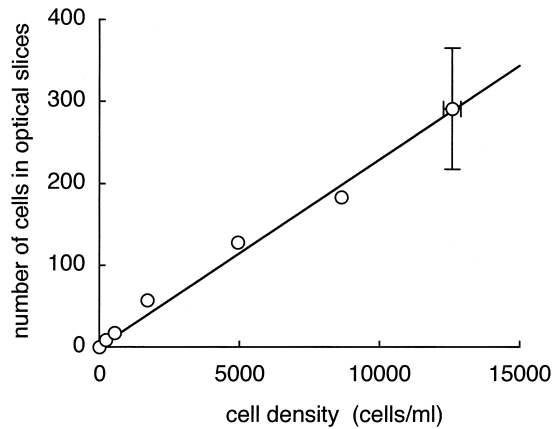
### Optical slice method

Cells were cultivated using a commercial Falcon cultivation flask (#353107, Becton Dickinson, NJ, U.S.A.). A small quantity (usually 5 ml) of culture medium was placed into the flask, which made the depth of the medium 5 mm or less. Since *Paramecium* has the property of negative gravitaxis, cells usually accumulate at the top of the water column. In the shallow medium, however, cells were observed to almost evenly distribute vertically as well as horizontally.

We introduced a new optical slice method for measurement of the cell density. As shown in Fig. 1A, a cell suspension in the shallow medium in the flask was illuminated through a sidewall with a horizontal slit-laser of a known beam thickness (half maximum width of 0.2 mm; LD-30J, Sakai Glass Engineering, Tokyo, Japan).



**Fig. 1.** Optical slice method for measurement of the cell density. A: a rough sketch of the equipment for the optical slice method, FL; culture flask, LB; slit laser beam of a known thickness, CCD; CCD camera, A/D; image board, PC; personal computer. B: an example of a recorded dark field image. Cells are shown by bright spots. Scale bar, 1 mm.

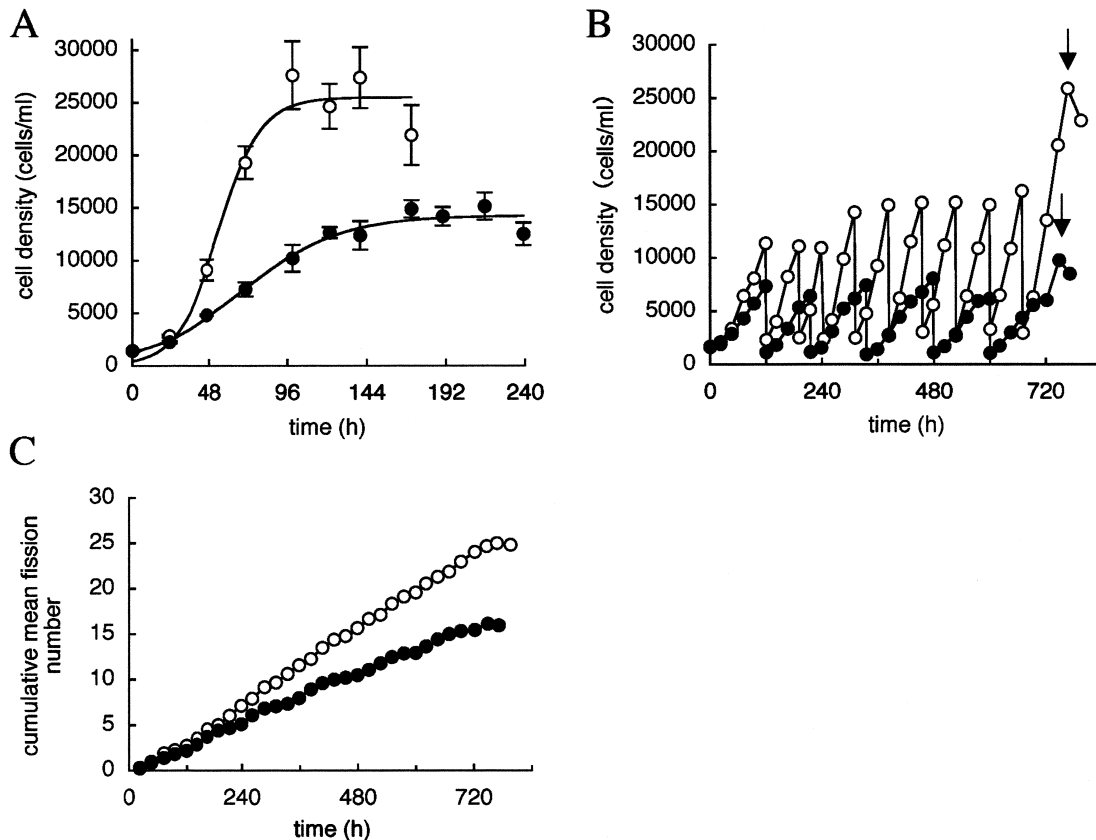


**Fig. 2.** Number of cells counted in optical slices as a function of the cell density. Cell cultures in the stationary phase were used for the measurement of cell density either by the optical slice method or a conventional counting method. The mean and S.D. ( $n=5$ ) for measurement of the undiluted are shown at the upper-right. The optical slice method was then applied to cell suspensions prepared by diluting with a known volume of a fresh culture medium. The solid line shows the result of the linear regression (slope=0.023 ml, correlation coefficient=0.98).

The light scattered by cells within a laser beam was monitored from the direction perpendicular to the beam plane with a CCD camera (DC-77RR, SONY, Tokyo, Japan) and the resultant dark field images were recorded into a computer through an image board (LG-3, Scion Corp. ML, USA) (Fig. 1B). The number of the optically sampled cells in the recorded images was counted with an assistance of a public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). Fig. 2 shows a representative calibration for the method. The plot of the optical count against cell density, which was determined more directly in a conventional way, shows a linear relation, which has been confirmed in the density range up to several ten thousands  $\text{cells}\cdot\text{ml}^{-1}$  (Mogami *et al.*, 1996). These indicate that the optical slice method is a reliable tool for measurement of the cell density in a practical range. The mean fission number at time  $t$  was calculated as  $\log_2\{D(t)/D(0)\}$ , where  $D(t)$  and  $D(0)$  are the densities measured at time  $t$  and the beginning of culture, respectively.

### Hypergravity experiment

For the cell culture under hypergravity conditions, cells in the culture flask were spun by a centrifuge, which was customized on the basis of inverter-microcomputer-controlled machine (LX-130, Tomy Seiko, Tokyo) to attain prolonged operation with high stability. Culture flasks were placed on the swing buckets of the centrifuge so that the direction of artificial hypergravity, as a vector sum of the earth's gravity and centrifugal acceleration, was always perpendic-



**Fig. 3.** Proliferation of *Paramecium tetraurelia* under different gravity conditions. A: Time course of the cell proliferation at  $1 \times g$  (open circles) and  $20 \times g$  (closed circles). Curves are the results of the least squares fitting of the logistic growth equation;  $K=2.55 \times 10^4$ ,  $\alpha=59.4$  and  $\beta=0.076$  for  $1 \times g$ , and  $K=1.43 \times 10^4$ ,  $\alpha=10.28$  and  $\beta=0.034$  for  $20 \times g$ . B: Time course of the cell proliferation following dilutions repeated to maintain the logarithmic growth phase. Open circles:  $1 \times g$  and closed circles:  $20 \times g$ . Arrows indicate the stationary phase. C: Cumulative mean fission number as a function of time.

ular to the 'bottom' of the flask, on which a shallow culture medium had been layered. These culture flasks were picked out from the centrifuge for measurement of the cell density by the optical slice method.

The temperature during centrifugal rotation was regulated at 24°C. Since it took a long time to complete the culture experiment, the temperature on the bucket of rotating centrifuge as well as that in the incubator for 1 × g control was measured and logged by thermo recorders (TR-71S (ONDOTORI), T and D Co. Ltd., Matsuyama, Japan) throughout the culture. Data obtained from the culture experiment, in which the temperature was kept well within a technical limit of the control devices (i.e., ±0.5°C), were only used for the further analysis.

#### Measurement of the length of autogamy immaturity

In order to investigate the effect of hypergravity on the length of autogamy immaturity, a 100% autogamous culture was prepared. Cells in the stationary phase were diluted with a fresh culture medium and put in a culture flask at the density of  $1 \times 10^3$  cells·ml<sup>-1</sup>. After a three-day incubation the cells at the logarithmic growing phase ( $5$  to  $8 \times 10^3$  cells·ml<sup>-1</sup>) were diluted to  $5 \times 10^2$  cells·ml<sup>-1</sup>. This dilution process was repeated several times until all the cells experienced to proliferate more than twenty times, and the cells were grown to the stationary phase and starved for a couple of days to induce autogamy. Cells were sampled and stained by 0.7%(v/v) methyl green in 1%(v/v) acetate to survey fragmentation of the macronucleus, which is indicative of autogamy (Wichterman, 1986; Ishikawa *et al.*, 1998). Cultures in which all of the sampled cells had a fragmented macronucleus were only used for the following measurement.

At the beginning of measurement of the length of autogamy immaturity, 625 µl of a 100% autogamous culture was added to 4375 µl of a fresh culture medium and incubated with the cell density being monitored by the optical slice method. At the mid to late logarithmic phase, 625 µl of the culture was again transferred to 4375 µl of a culture medium. This procedure was repeated to continue logarithmic proliferation more than twenty times (cf. thick solid lines in Fig. 5A, B). The remaining cells were cultivated further on undiluted and then kept starved for several days to induce autogamy (cf. dotted lines in Fig. 5A, B). During starvation cells were sampled from the culture and the cells were stained to determine the fraction of autogamous cells. The clonal age at which the fraction reached a maximum was determined (cf. filled symbols in Fig. 5A, B) in terms of the clock time as well as the mean fission number from the beginning of the experiment. The maximum values were plotted against the clonal age. The length of autogamy immaturity was defined as the length of the clonal age before the percent autogamy got to 50%, and was determined by the least squares fitting of a sigmoid function to the plot of the percent autogamy either against clock time or fission number. More than 100 cells each time were used to determine the percent autogamy. Six continuous cultures were run in parallel, with three cultures under hypergravity (20 × g) and others under normal (1 × g) conditions, and the mean values from the parallel measurements were used for the analysis.

#### Morphometry of the cell

For measurement of the morphological parameters of *Paramecium*, an aliquot of the culture was mixed with the same volume of a solution of 0.02% Brilliant Green in 5.0% Formaldehyde. The fixed and stained samples were covered by a cover slip with a silicone rubber spacer of 0.5 mm thick. On the microscopic images of the cell recorded by a digital camera (HC300Zi, Nikon, Tokyo), parameters characteristic of the cell morphology of *Paramecium* (such as the lengths of short and long axes of the cell) were measured using a laboratory made software for image analysis (Bohboh, Shiba *et al.*, 2002).

#### Numerical analysis

The time course of the cell proliferation under different gravitational conditions was analyzed on the basis of the logistic growth equation:  $D(t)=K/(1 + \alpha \exp(-\beta t))$ , where  $D$  is the cell density as a function of time,  $t$ , and  $K$ ,  $\alpha$  and  $\beta$  are the parameters characteristic of the individual culture. According to the three parameters determined by means of the least squares fitting of the equation to the experimental data, the kinetic parameters of proliferation (saturation cell density and maximum proliferation rate) were calculated. Autogamy immaturity was analyzed by fitting a sigmoid function:  $R(x)=A(x/\tau)^\lambda/(1 + (x/\tau)^\lambda)$ , where  $R$  is the fraction of autogamous cell as a function of variable,  $x$  (clock time or fission age), and  $A$ ,  $\tau$  and  $\lambda$  are the maximum (saturation) amplitude of the ratio, the half maximum variable  $x$  corresponding to  $R=A/2$  and the exponent representing the sharpness of the sigmoid curve, respectively. The length of autogamy immaturity was obtained as a value of variable  $x$  at which  $R=0.5$ . The curve fitting was done by the least squares method using KaleidaGraph, a commercial package for scientific data analysis (version 3.51, Synergy Software, Reading, PA, USA).

## RESULTS

### Reduced activity of proliferation of *P. tetraurelia* under hypergravity

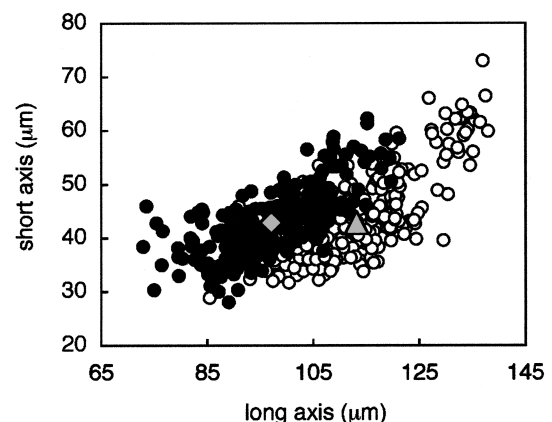
Fig. 3A shows the time course of proliferation under hypergravity (20 × g) as well as control normal gravity con-

**Table 1.** Kinetic parameters of the proliferation of *Paramecium tetraurelia* under normal (1 × g) and hypergravity (20 × g) conditions.

	Saturation density (× 10 <sup>4</sup> cells·ml <sup>-1</sup> )	Maximum proliferation rate <sup>1</sup> (× 10 <sup>2</sup> cells·ml <sup>-1</sup> ·hr <sup>-1</sup> )	n
1 × g	2.72±0.15	4.39±0.51	5
20 × g	1.49±0.17**	1.34±0.17**	5

<sup>1</sup>: maximum slope of a tangent to the regression curves.

\*\* :  $P < 0.01$ .

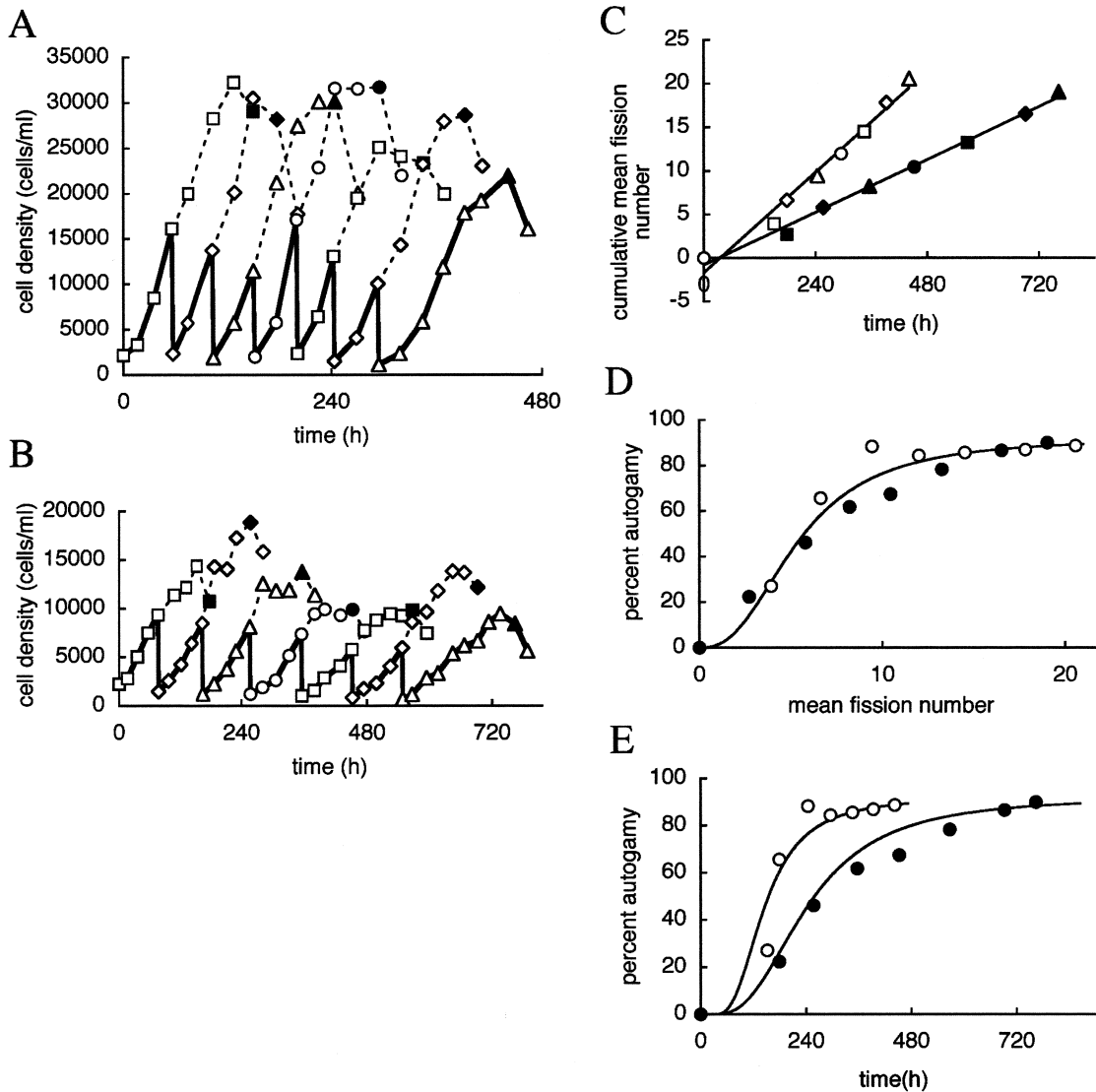


**Fig. 4.** Changes in the cell morphology of *Paramecium tetraurelia* under different gravity conditions. The lengths of short axis of the cells at the stationary phase are plotted against those of long axis. Open circles and closed circles represent data obtained at 1 × g and 20 × g, respectively. Data from a single experiment are shown. A triangle indicates the mean values for 1 × g; 113.2±9.8 and 42.9±7.8 µm (n=286) for the long and short axes, respectively. A diamond indicates the mean values for 20 × g; 97.1±9.7 and 42.8±6.5 µm (n=225) for the long and short axes, respectively.

ditions. As clarified by the regression curves of the logistic growth equation, at  $20 \times g$  *P. tetraurelia* proliferated to a lower saturation density with a reduced growth rate. Significant inhibitory effects of hypergravity were not found in the culture at  $10 \times g$  (data not shown), where the saturation density appeared to be slightly lower but still not significant, whereas Planel *et al.* (1990) reported a significant inhibition at similar values of hypergravity. Thus effects of hypergravity were evaluated here mostly for the culture under  $20 \times g$

conditions.

Table 1 shows kinetic parameters obtained from the numerical analysis on the basis of the logistic growth equation. It shows that hypergravity greatly affected the saturation density and the maximum growth rate. These inhibitory effects lasted as long as cells were cultivated under hypergravity. Fig. 3B shows the time courses of the proliferation following repeated dilutions to maintain a logarithmic growth under hypergravity and normal gravity conditions. In the



**Fig. 5.** Proliferation and autogamy maturity of *Paramecium tetraurelia* under different gravity conditions. A: Time course of the cell proliferation following repeated dilutions at  $1 \times g$ . B: Time course of the cell proliferation following repeated dilutions at  $20 \times g$ . Thick solid lines in A and B show the time course of the cell proliferation to maintain the logarithmic growth phase. Dotted lines show the time course of proliferation to the stationary phase, in which autogamy inducibility was tested by starvation. Filled symbols indicate the time when the maximum percent autogamy was reached. C: Cumulative mean fission number as a function of time. Open symbols and closed symbols represent data obtained from  $1 \times g$  and  $20 \times g$ , respectively. The symbols of respective shapes correspond to the filled ones of the same shape in A and B. Solid lines show the results of the least squares fitting of a linear function. D: Time course of the percent autogamy as a function of mean fission number for  $1 \times g$  (open circles) and  $20 \times g$  (closed circles). The mean fission numbers at 50% autogamy are 5.2 for  $1 \times g$  and 6.4 for  $20 \times g$ , respectively. The solid line is the result of the least squares fitting of the sigmoid function (see DISCUSSION). E: Time course of the percent autogamy as a function of time for  $1 \times g$  (open circles) and  $20 \times g$  (closed circles). The lengths of autogamy immaturity are 166.1 and 295.9 hours at  $1 \times g$  and at  $20 \times g$ , respectively. Solid lines are the transformation of the curves in D by translating the time axis from fission number to clock time (also see DISCUSSION).

growth phase extended by a repeated dilution, the growth rate was always lower under hypergravity conditions, which resulted in a slower increase in the cumulative mean fission number as a function of time (Fig. 3C).

Planel *et al.* (1990) reported an increase in the cell volume of *P. tetraurelia* grown under microgravity. The effect of hypergravity on the morphology of the cell was examined at the stationary phase. As shown in Fig. 4, cells appeared to become small when they were grown under hypergravity. On average, the long axis decreased to  $0.88 \pm 0.03$  ( $n=5$ ,  $P < 0.01$ , Student's *t*-test) relative to the control and the short axis to  $0.95 \pm 0.05$  ( $n=5$ ,  $P=0.06$ ). Assuming the cell body to be a prolate spheroid, these values indicate the volume decreased to 78% of the cell grown under  $1 \times g$ .

### Effects of hypergravity on autogamy immaturity

Fig. 5A, B show the time courses of the proliferation at  $1 \times g$  and  $20 \times g$ , respectively. In these figures thick solid lines indicate the time course of continuous logarithmic growth maintained by repeated dilutions and dotted lines show those of the remaining cultures in which measurements giving the maximum fraction of autogamous cells were shown by filled symbols. Fig. 5C shows the cumulative mean fission number corresponding to the measurements (filled symbols in Fig. 5A, B) as a function of the clock time, and also demonstrates that the reduced growth under hypergravity lasted to the end of the experiment. In Fig. 5D, E, the percent autogamy induced under altered gravity conditions is plotted as a function of the mean fission number and the clock time, respectively. These figures imply that data of the percent autogamy are plotted closely along the same curve when plotted against the mean fission number, but along separate curves when plotted against the clock time. As a result, the length of autogamy immaturity measured at  $20 \times g$  was  $1.1 \pm 0.3$  times of that at  $1 \times g$  ( $n=3$ ,  $P > 0.5$ ) by measure of mean fission number, and  $1.8 \pm 0.6$  times ( $n=3$ ,  $P=0.15$ ) by measure of clock time. These findings suggest that the length of autogamy immaturity is equal under altered gravity conditions when measured by the fission number, whereas it tends to be extended under hypergravity when measured by the clock time.

## DISCUSSION

We confirmed the finding of Richoille *et al.* (1993) that *P. tetraurelia* in axenic culture reduces the proliferation activity under hypergravity. The fact that the inhibitory effect was revealed under axenic conditions may exclude the possibility that gravity does not affect directly on *Paramecium* but on the nutrient bacteria in non-axenic culture.

In their short paper, Richoille *et al.* (1993) reported the inhibitory effect of hypergravity on the proliferation of *P. tetraurelia* grown under axenic conditions. The medium they used, however, contained skim milk powder as the major carbon and nitrogen sources, which was reported to be insoluble and gave a turbid 'milky' appearance to the

medium at natural pH (Schoenefeld *et al.*, 1986). Therefore, it might be inferred that the insoluble powder particles are the actual target of gravitational force in the medium used in Richoille *et al.* (1993). Hypergravity could accumulate the powder particles at the bottom and, as a result, might separate paramecia from the nutrient resources otherwise available for them.

The medium used in the present study, to the contrary, appears clear and transparent to the laser illumination giving a very low background to the dark field images obtained. It is therefore highly plausible that the effects of hypergravity revealed in the present study are the results of the direct action of gravity on the proliferation activity of *P. tetraurelia*.

In the present paper, the kinetics of proliferation of *P. tetraurelia* was analyzed on the basis of the logistic growth equation. The equation is derived from a differential equation of

$$dD/dt = \gamma D(K-D), \quad (1)$$

where the growth rate is defined by the product of a proportional constant ( $\gamma$ ) and the terms related to the cell density in culture ( $D$ ) and to the density dependent inhibitory effect, i.e., reduction in available resources ( $K-D$ ). Although the logistic equation is useful to describe the proliferation of bacteria (Yano *et al.*, 1998; Kacena *et al.*, 1999), a conventional non-axenic growth of *Paramecium* does not necessarily conform to the equation 1 because the nutrient bacteria grow in the culture concomitantly with *Paramecium*. The fact that the logistic growth curve fitted well to the data (Fig. 3A) may indicate the availability of the logistic analysis to the time course of proliferation of *P. tetraurelia* especially under axenic conditions. This may also indicate that the parameters shown in Table 1 accurately characterize the kinetics of proliferation under altered gravity conditions: hypergravity affects proliferation to reduce the saturation density and to lower the proliferation rate. Here it is interesting to point out that the proliferation under normal as well as hypergravity conditions has not significantly different values of  $\gamma$  in the equation 1 above:  $2.39 \pm 0.37$  ( $\times 10^{-6}$  ml $\cdot$ cell $^{-1}\cdot$ hr $^{-1}$ , mean  $\pm$ S.D.,  $n=5$ ) for control and  $2.44 \pm 0.41$  for hypergravity, respectively ( $P=0.86$ ). Since  $\gamma$  is the essential parameter in the differential equation representing the magnitude of feedback from an accelerating as well as deceleration term (Peitgen *et al.*, 1992),  $\gamma$  of similar values suggests that proliferation occurred on the basis of common regulations under altered gravity conditions.

Cytos experiments showed that the cell volume of *P. tetraurelia* increased when cultivated under microgravity. Thus the decrease in cell volume under hypergravity demonstrated in the present paper suggests that hypergravity affects *Paramecium* oppositely to microgravity in the cell morphology as well as proliferation kinetics. Since the cell dry weight and the total protein content reduced in the culture under microgravity, Planel *et al.* (1990) related the increase in cell volume to an increase in cell water content. This osmosis-dependent change in cell volume could not

solely explain the reduction of cell volume in the hypergravity culture, where the decreased ratio of long/short-axis lengths indicates a tendency for *Paramecium* to become spherical.

The length of autogamy immaturity may be defined in several ways, e.g., as the period during which autogamy cannot be induced at all by ordinary starvation. In the present paper it was defined as the time up to which autogamy can be induced in 50% of cells, and obtained on the basis of the least squares fitting of a sigmoid function. This method of measurement is more suitable for a continuous mass culture of *Paramecium* than for a single-cell culture with daily isolation. The results presented in the present paper indicate that the length of autogamy immaturity is defined uniquely by fission number in spite of the reduced proliferation rate under hypergravity. This is in line with the results of Ishikawa *et al.* (1998) where the length of autogamy immaturity is conserved by measure of fission number when cells are grown at higher temperatures. In Fig. 5D, a single sigmoid function is fitted to the data on the assumption of the fission number dependent control of autogamy immaturity. This curve was transformed to a function for each gravity condition against clock time using a linear correlation of cumulative mean fission number and time (Fig. 5C). The curves appear to fit to each individual data set for control and hypergravity (Fig. 5E). This would support the notion that the fission number dependent control of autogamy immaturity works irrespective of how the proliferation rate alters in different environments. It is therefore expected that autogamy immaturity would be shortened by measure of clock time under microgravity, which is known to raise the proliferation rate. Since the length of clonal life span of *Paramecium* may be related to the length of autogamy immaturity (Smith-Sonneborn, 1981; Ishikawa *et al.*, 1998), proliferation at an increased rate under microgravity would result in a decrease in clonal life span.

As a plausible explanation for changes in the cell-proliferation rate, Planel *et al.* (1990) raised the possibility of changes induced by altered gravity in energy metabolism linked to cell motility. *Paramecium* requires some energy for propulsion by ciliary beating, which is supplied by cellular metabolism. Under normal  $1 \times g$  conditions, energy released from the metabolic pathway is used to perform physiological activities including cell division and motility. Under microgravity in space, cells would require less energy for swimming because of reduction in the mechanical load due to gravity. Hypergravity, on the other hand, would increase the load, and, hence the energy for swimming. As a result, these situations would change the amount of energy stock for cell division. The large stock of energy under microgravity would induce an increase in the proliferation rate and the small stock under hypergravity a decrease.

This explanation on the basis of energy budget should be considered in terms of the energetics of swimming behavior of *Paramecium*. As demonstrated previously, paramecia are able to modulate their propulsive thrust depend-

ing on the orientation of the cell body with respect to the gravity vector (Machemer *et al.*, 1991; Ooya *et al.*, 1992). As a result of 'gravikinesis' of *Paramecium*, the magnitude of the propulsive velocity ( $p$ ) increases with an increase of its angular direction with respect to gravity ( $\phi$ ):  $\Delta p/\Delta \phi > 0$  (Ooya *et al.*, 1992). In swimming at low Reynolds numbers, as in the case of *Paramecium* ( $Re \approx 0.01$ ), the propulsive force is directly proportional to the velocity, so that the power ( $P$ ) of a single *Paramecium* cell swimming with a propulsive velocity  $p(\phi)$  is given by

$$P(\phi) = cp(\phi)^2, \quad (2)$$

where  $c$  is a drag coefficient, such as  $c = 6\pi a\eta$  if *Paramecium* is assumed to be a sphere with a Stokes radius ( $a$ ) moving in a fluid of viscosity ( $\eta$ ) (Vogel, 1994). The average power of the individual cells in a culture is obtained by

$$P_{av} = \Sigma P(\phi)F(\phi), \quad (3)$$

where  $F(\phi)$  is a fraction of cells swimming with the propulsive velocity  $p(\phi)$ .  $P_{av}$  would increase when there is an increase in the fraction of cells orienting upwards. Mogami *et al.* (2001) demonstrated that the gravitactic orientation of *Paramecium* is mechanically biased by the torque generated mainly due to the fore-aft asymmetry of the cell body. The upward-orienting torque increases in proportion to gravity, so that the fraction of cells orienting upwards, i.e.,  $F(\phi)$  for a large  $\phi$ , increases under hypergravity, and decreases under microgravity. This would result in a decrease and increase in energy expenditure for propulsion under microgravity and hypergravity, respectively.

Klaus *et al.* (1997) proposed a hypothesis for a stimulated proliferation of bacteria under microgravity that gravity may have a significant impact on the suspended cells via hydrodynamic properties of a fluid environment. In their hypothesis, the zonal accumulation of metabolic byproducts surrounding a cell would affect the cell metabolism only when the velocity of bacterial swimming is comparable to or less than that of diffusive motion. This may not be the case of *Paramecium* because its swimming velocity is far beyond the velocity of diffusive motion of the cell.

The results presented in the present paper indicate that gravity affects *Paramecium* directly to modulate its proliferation activity. Further analysis of the response to hypergravity in proliferation may open the way to gain an insight into the mechanisms of gravity responses, which will be a help for analyzing the phenomena found in future space experiments (Mogami *et al.*, 1999).

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