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Growth of the Cellular Slime Mold, *Dictyostelium discoideum*, Is Gravity Dependent

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ABSTRACT

The effect of artificial gravity on the growth of a microorganism, *Dictyostelium discoideum*, was studied and the following results were obtained: (a) Germination efficiency increased as gravity increased up to 3 gravities. (b) Cell differentiation was influenced by gravity. Retardation of spore formation or reduction in the spore fraction was observed at hypergravity. (c) Fruiting bodies were taller at hypergravity and smaller at simulated microgravity when compared at 1 gravity. It is suggested that modulation of gravity provides useful information on the mechanisms of life.

Gravity is a unique parameter in the sense that organisms on the earth, in natural condition, have never experienced its chronic deviation from 1*g*. Based on this fact, following questions can be posed: (a) Will environmental adaptation be established without any exposure to chronic fluctuations of environmental factors? (b) Is 1*g* optimum for growth, and if not, which is optimum? (c) Is 1*g* essential for growth? (d) Does comparative study under 1*g* and other gravity conditions give useful information on the mechanisms of life? Answers to these questions could contribute to a fundamental understanding of the mechanisms of life.

For these reasons, we undertook the present study. The initial step is to find a suitable biological system. In order to study whether the mechanisms of gravity perception are universal or specific to individual species, it is necessary to study a wide variety of organisms. There have been many studies on the gravi-response of higher green plants (6, 9, 12, 14) but not so much on the gravi-response of primitive organisms (2, 4, 8, 11, 13). The cellular slime mold, *Dictyostelium discoideum*, a familiar microorganism in some moist environments, is one of the most promising for this purpose. The advantages of using *D. discoideum* are: It has several phases including unicellular and multicellular phases and animal-like and plant-like phases; it exhibits a characteristic morphogenesis; it consists of only two kinds of cells; and one generation is complete in only 5 d under optimal experimental conditions (3). For these reasons, we investigated the growth of *D. discoideum* under artificial gravity. Here we report that *D. discoideum* is an excellent object for the study of the effect of gravity on the growth of organisms.

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MATERIALS AND METHODS

Cell Culture and Cell Counting

Strain NC-4 of *Dictyostelium discoideum* was obtained from Dr. G. Higuchi of Higuchi Biological Laboratory in Tokyo, and strain ATCC was obtained from Dr. M. A. Benjaminson of New York College of Osteopathic Medicine, New York Institute of Technology in New York. For cell culture, the following procedure was employed: Spore cells (1×10^4 cells/cm^2) harvested from fruiting bodies were plated on agar plates in 35 mm Petri dishes containing salts and nutrients (glucose 5 g, polypepton 5 g, yeast extract 0.5 g, KH_2PO_4 2.25 g, K_2HPO_4 0.67 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, bacto-agar 15 g in 1000 mL of distilled water). Bacteria (*Klebsiella aerogenes* or *Escherichia coli*) were plated at the same time as one of the essential nutrients. The Petri dishes were tightly covered with aluminum foil and placed on a centrifuge (for artificial hypergravity), on a clinostat (for simulated microgravity) or left standing (for 1*g* control). Some of the dishes were inverted. The cells were cultured at 22°C in the dark.

For the experiments on germination efficiency, the following procedure was employed: Spore cells were cultured on agar plates without nutrients as explained above (2×10^3 cells/cm^2). Heat-treated (120°C 30 min) bacteria were plated at the same time. After culturing 14 h at 20°C under artificial gravity, cells were scraped off the agar plates and washed three times with Sussmann's saline. Then, the cells were fixed with 2% glutaraldehyde for 18 h at room temperature. The ratio of the number of amoeboid cells to total cells (spore cells and amoeboid cells) was defined as germination efficiency.

For the experiments on cell proliferation, cultured cells were scraped off at the indicated time, washed three to five times with Sussmann's saline (KCl 1.5 g, $MgCl_3$ 0.5 g, in 1000 mL distilled water), and the number of amoeboid cells was counted.

For the experiments on the heights of fruiting bodies, cells were cultured 7 d under artificial gravity. Then, 30 μL of 25% glutaraldehyde was dropped onto the edge of the dishes to fix fruiting bodies. The dishes were left standing more than 2 h at room temperature.

For the experiments on cell differentiation, the following procedure was employed. After preculturing spore cells in liquid medium (the same medium used for the agar plate but without agar) with bacteria at 22°C for 3 d, amoeboid cells were washed three times with Sussmann's saline. Cells were plated on agar plates without nutrients. After culturing on the plates at 19°C for 24 h under artificial gravity (at an early

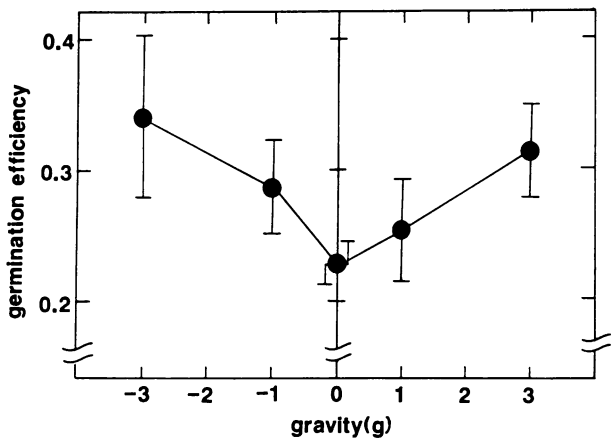

Figure 1. Dependence of germination efficiency of strain NC-4 on artificial gravity. Germination efficiency is defined as the number of amoeboid cells divided by total cells. Negative values of gravity mean that culture dishes were inverted. 0*g* is simulated by a clinostat. Vertical bars represent standard errors of the measurements using three dishes. $P < 0.01$ (for the difference between 0*g* and 3*g*).

stage of culmination), fruiting bodies and slugs were scraped off the agar plates and were suspended in dissociation solution (KCl 20 mM, Na_2HPO_4-KH_2PO_4 buffer 40 mM at pH 7.4, ethylenediaminetetraacetic acid 2 mM). The cells were dissociated by vigorous agitation in pronase solution (0.1 wt% pronase in 25 mM 2,3 dimercapto-1-propyl alcohol and 2 mM ethylenediaminetetraacetic acid) for 40 s at room temperature. After washing two times with dissociation solution, cells were fixed and the numbers of spore and amoeboid cells were counted.

For the experiments on the effects of gravity on morphogenesis, the following procedure was employed. Preculture in liquid solution was the same as for the cell differentiation experiments. Collected amoeboid cells were trapped on filters (Millipore, HABP02500) at a cell density of $1.3 \times 10^7/cm^2$ and the filters were placed on agar plates without nutrients. The cells were cultured for 24 h at 22°C under artificial gravity. To fix fruiting bodies, 30 μL of 25% glutaraldehyde was dropped onto the edge of the dishes, and the dishes were left standing more than 2 h at room temperature. Then the heights of mature fruiting bodies were measured.

Generation of Artificial Gravity

Hypergravity was obtained by a centrifuge with a diameter of 23 cm. Under this condition, Coriolis' force acting on moving slugs is less than 10^{-5} of the centrifugal force, and the difference of the centrifugal force within a single slug is less than 3% of the average value. Therefore, in this article, acceleration was regarded as artificial gravity. Microgravity was simulated with a clinostat. A clinostat is an apparatus that rotates specimens around the axis perpendicular to the direction of gravity. During one rotation, the relative direction of gravity to the specimens changes through 360°, so gravity averaged over time becomes zero. The clinostat was rotated at 4 s/rotation. The resultant acceleration caused by this rotation was $10^{-3}g$, which is a microgravity condition (not zero gravity).

Measurements of the Heights of Fruiting Bodies

Petri dishes with agar were placed on the stage of a microscope. The focus was first set at the base of the fruiting bodies, and was set at the widest part of the sorus, and the distance between these two settings was read. Thirty to 50 fruiting bodies were counted in each Petri dish, and three to six Petri dishes were measured.

RESULTS

The life cycle of *D. discoideum* is composed of several stages. We studied the effects of artificial gravity on these stages one by one and finally on the whole life circle.

Germination

Figure 1 shows the effect of artificial gravity on the germination efficiency of strain NC-4. Under simulated microgravity, germination efficiency was lowest. The efficiency increased as the increase in gravity. The similar results were

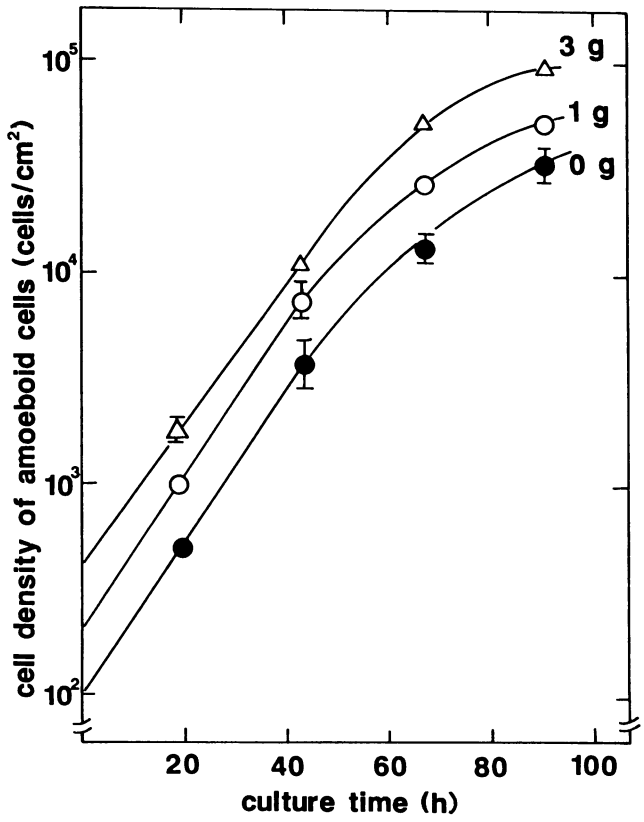

Figure 2. Dependence of proliferation of strain NC-4 on artificial gravity. Vertical bars represent standard errors of the measurements using three dishes.

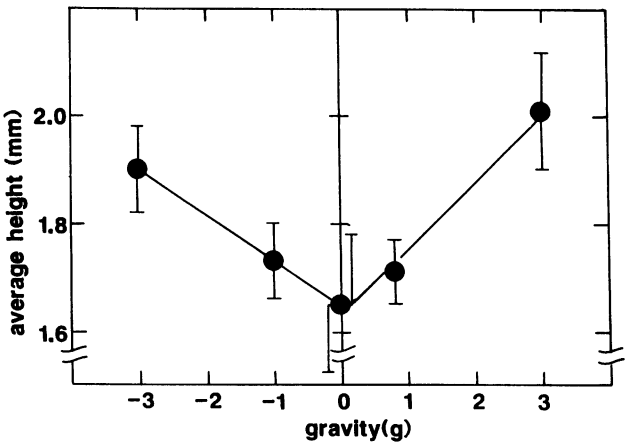

Figure 3. Effect of artificial gravity on the stage of morphogenesis of strain NC-4. Cells in the postproliferation stage were subjected to the levels of gravity indicated, and the heights of fruiting bodies were measured. Vertical bars represent standard errors of the measurements using three dishes. $P < 0.05$ (for the difference between $1g$ and $3g$). $P < 0.05$ (for the difference between $0g$ and $3g$).

obtained in inverted orientation. Even at $10g$, the promotion of germination efficiency was observed.

Cell Proliferation

Figure 2 shows the proliferation curves of germinated cells. The slopes of the curves are identical. Therefore, it can be concluded that the proliferation rate of germinated cells is not influenced by artificial gravity from simulated microgravity to $3g$. The difference in the extrapolated values of the growth curves to time zero is a reflection of the dependence of germination efficiency on gravity.

Morphogenesis

The effect of artificial gravity on the morphogenesis of NC-4 was examined. In this case, only the postproliferation stage was subjected to artificial gravity, and the heights of fruiting bodies was measured. As shown in Figure 3, morphogenesis of NC-4 was also influenced by gravity. The heights of fruiting bodies increased as the increase in gravity.

Of the postproliferation stages, which step is responsible? As shown in Figure 4, the fraction of spore cells in early culmination stage decreased as gravity increased. In this case, the total number of cells per single organism remained constant. Therefore, the increase in the fraction of stalk cells under hypergravity may be responsible for the taller fruiting bodies.

Whole Life Cycle

We have shown that some stages in the life cycle of *D. discoideum* were influenced by artificial gravity. Then, we examined whether the growth of *D. discoideum* is influenced when its whole life cycle is subjected to artificial gravity. As shown in Figure 5, the heights of fruiting bodies were influenced by gravity. Fruiting bodies were taller in hypergravity and smaller in simulated microgravity. Again, inverted orientation of culture dishes gave the same result. Another strain, ATCC, also showed a similar tendency as shown in Figure 6.

DISCUSSION

In this article we reported the phenomenological results of the growth of *D. discoideum* under artificially altered gravity (simulated microgravity and accelerated force). The significance of the present results depends on whether they can be ascribed only to gravity, because rotation induces many undesirable effects. The primary artifacts possible are as follows.

(a) Polarized distribution of water and/or nutrients in the agar plates.

(b) Mechanical pressing of cells against substrate.

(c) Local changes in temperature and humidity.

(d) Micro-vibration accompanying rotation.

(e) Electromagnetic effects by the motors

We would like to show that these are not so serious.

As for (a) and (b), both normal and inverted orientation of culture dishes gave the same results (see Figs. 1, 3, 5, and 6). If (a) and/or (b) are the main reasons for the present results, the inverted orientation should show an opposite tendency. This was not the case.

As for (c), the entire system was contained in a doubly temperature controlled chamber, and culture dishes were located as close together as possible. In some cases, relative

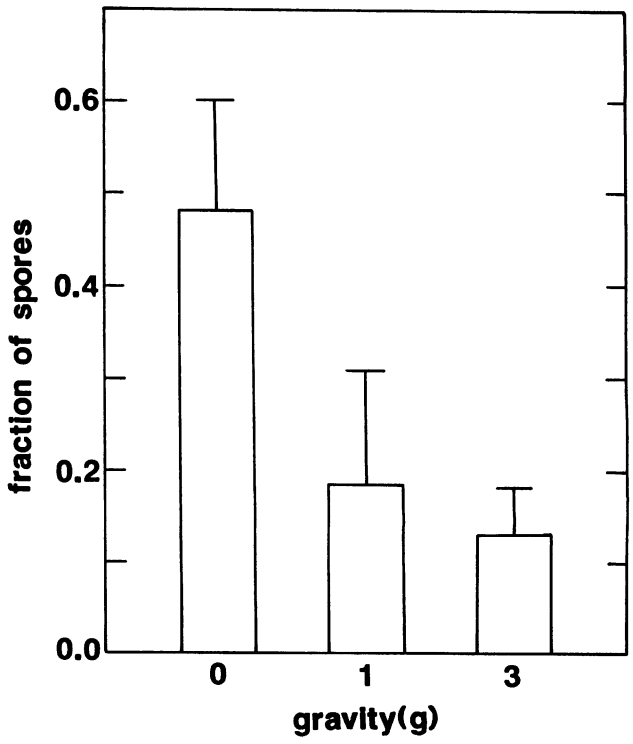

Figure 4. Fraction of spores of strain NC-4 in the early culmination stage. Vertical bars represent standard errors of the measurements using four dishes. $P < 0.05$ (for the difference between $0g$ and $1g$). $P < 0.01$ (for the difference between $0g$ and $3g$).

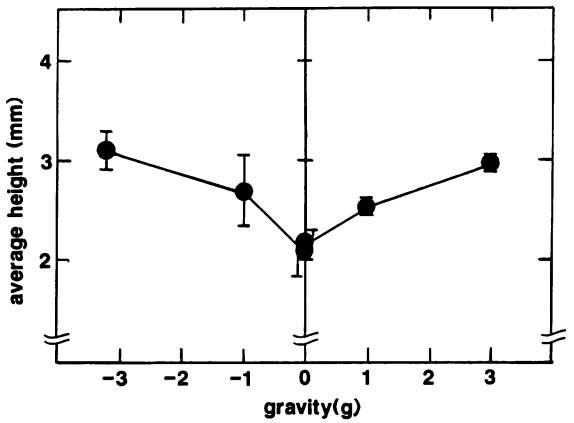

Figure 5. Height of fruiting bodies of strain NC-4 as a function of artificial gravity. Vertical bars represent standard errors of the measurements using three dishes. $P < 0.05$ (for the difference between 0*g* and 1*g*). $P < 0.001$ (for the difference between 1*g* and 3*g*).

locations of the equipment for 1*g*, simulated microgravity, and hypergravity were changed. Yet the same results were obtained. Based on these reasons, we think (c) is negligible.

As for (d), we cannot measure the power spectrum of the possible vibration of our apparatus. Microvibration exists both in the centrifuge and the clinostat. If vibration itself exerts some effect on *D. discoideum*, 3*g* and simulated microgravity should give similar results. Actually, they gave opposite results. Moreover, when we used a centrifuge that had much larger vibrational motion (with at least a 10 times larger amplitude), we obtained similar results. For these reasons, we think that (d) did not exert serious effects on the present experiments.

As for (e), the relative positions of the culture dishes were changed. Even under this condition, similar results were obtained.

Combining these reasons, it might be concluded that the growth of *D. discoideum* is directly influenced by artificial gravity.

Of the stages of *D. discoideum*, growth, germination, and morphogenesis (cell differentiation) were influenced by artificial gravity, while cell proliferation was not influenced. Reduced cell proliferation under microgravity has been reported in human lymphocytes (1), while *Paramecia* and *HeLa* cells showed increased cell proliferation under microgravity and hypergravity, respectively (7, 11). Therefore, response to gravity may be different among cell types.

As shown in Figure 4, the fraction of spore cells was reduced as artificial gravity increased. Since the number of cells per single fruiting body was independent of gravity, this result indicates the increase in stalk cell fraction. Another possible interpretation is that spore formation is retarded under hypergravity. Benjaminson (2) reported that cell differentiation of *D. discoideum* is accelerated under hypergravity. His result is not compatible with the latter interpretation. Therefore, the reduction in the fraction of spore cells may be plausible. Gravity may modulate the mechanisms of cell differentiation of *D. discoideum*. It is reported that cell differentiation in *D. discoideum* is influenced by intracellular pH (5) and diffusible molecules (10). It should prove interesting to examine the interrelationship between the effects of gravity and these factors.

When whole life cycle was subjected to artificial gravity, matured fruiting bodies became taller as gravity increased (Figs. 5 and 6). This result is out of our expectation, since hypergravity is thought to retard the upward growth in the normal orientation of the culture dishes. Considering the result shown in Figure 4, the increase in the fraction of stalk cells may be responsible for this phenomenon. Because higher green plants do not show such a phenomenon, only primitive organisms can exhibit this property significantly.

Fruiting bodies grew upward when agar plates were placed to normal orientation and they grew downward when the plates were inverted. This means that the culmination of *D. discoideum* is not influenced by gravity. Indeed, when agar plates were oriented vertically, no curvature of matured fruiting bodies was observed in NC-4.

What mechanisms can be considered to explain our results? We can give no decisive answer to this question at present. In the root cap cells of higher green plants, there exist amyloplasts which have a large specific weight and sediment rapidly with respect to the direction of gravity within the cell (9). These heavy amyloplasts are thought to be the gravisensors. In *D. discoideum*, there have been no reports concerning amyloplast-like organelles. However, some organelles that have relatively large specific weights and large volumes could be sensors for gravity. Supporting systems for these organelles, such as cytoskeleton, can also be considered as sensors. The cytoskeleton may influence such cell activities as germination and cell differentiation. It is known that germination efficiency is enhanced by perturbing spore cells with detergent and heat treatments. Hypergravity may perturb spore cells by pressing organelles to cytoskeleton or to cell membranes, and successively, to cell walls leading to germination.

We would like to emphasize again that *D. discoideum* is an

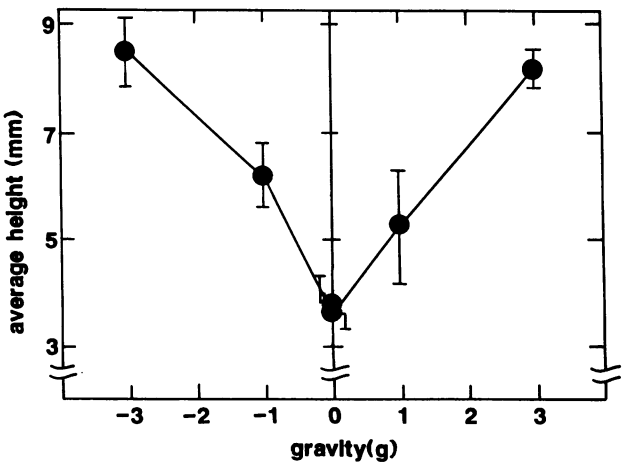

Figure 6. Height of fruiting bodies of strain ATCC as a function of gravity. Vertical bars represent standard errors of the measurements using four dishes. $P < 0.001$ (for the difference between 0*g* and 1*g*). $P < 0.001$ (for the difference between 1*g* and 3*g*).

excellent object for the study of the effect of gravity on the growth of organisms.

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