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# Cell growth and morphology of *Dictyostelium discoideum* in space environment

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***Abstract*** Two strains of cellular slime mold *Dictyostelium discoideum*, a radiation-sensitive mutant and the parental wild-type strain, were used to investigate the effects of microgravity and/or cosmic radiation on their morphology through the whole life span from spores to fruiting bodies for about 7 days in space shuttle of NASA. We found almost no effect of space environment on amoeba cell growth in both strains. It was also observed that almost the same number and shape of fruiting bodies in space compared to the control experiments on earth. These results suggest that there is little effect of microgravity and space radiation on germination, cell aggregation, cell differentiation and cell morphology in the cellular slime mold.

*Key words;* morphology, fruiting body, *Dictyostelium discoideum*, space radiation, radiation sensitive mutant.

## *Introduction*

A simple eukaryote of *Dictyostelium discoideum*, lives among decaying forest leaves and in topsoil, is a member of a complex ecosystem of the soil. The amoeboid cells, which emerge from spores through swollen spores, can grow by feeding on bacterial cells. After cell multiplication, the vegetative cells aggregate to form slug-shaped cell masses and synchronously start to differentiate (Bonner, 1967). The slug undergoes a series of morphological movements and eventually constructs a fruiting body consisting of three parts: a spore mass (sorus), a stalk and a basal disc (Murata and Ohnishi, 1980). During the formation of the fruiting body (culmination), the anterior cells differentiate into stalk and basal disc cells, while the posterior cells differentiate into spores. During the fruiting body construction, cells in the two regions trigger high cAMP production to accumulate and release cellulose fibrils which form the stalk sheath and the thickened cell wall which are characteristics of both the stalk and basal disc regions (Bonner, 1947). Then the spore cells move upward in response to cAMP produced uniquely in the apical tip. Finally, the spore coats are formed as the last act in spore differentiation. After aggregation, most strains can complete the differentiation in about 24 hours at 23 °C. It is very interesting to study the effect of microgravity on this morphotic differentiation.

Space environment mainly consists of microgravity and space radiations. Microgravity may affect movement and spread of chemicals. Therefore, many kinds of biological experiments about cell differentiation, cell development from eggs (Heinrich et al., 1989; Souza and Black,

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1985; Snetkova et al., 1995; Ijiri, 1995) or seeds (Kordyum, 1997; Parfenov, 1988; Tripathy et al., 1996) to adults have been done in space. However, there are no reports about cell aggregation from differently spread cells, because almost all experiments were started from single cell like egg or seed in which developing cells gather together in contact with each other and progress toward cell development.

Nevertheless, it is interesting to study the biological effect of space radiations on organisms, because space radiations consist of more ionizing radiations and high linear energy transfer (LET) beams than the surface of earth. In the present paper, we used two kinds of *D. discoideum* strains which are the most radiation sensitive mutant (γs13) and the parental wild-type strain (NC4). Many radiation sensitive strains of *D. discoideum* have been established. The radiation sensitive mutant (γs13) is sensitive to most DNA damaging agents, for example, about 100 times sensitive to γ–ray and UV, because this mutant lacks DNA repair capacity (Ohnishi and Nozu, 1979, Deering et al., 1972). On the other hand, the wild-type strain, NC4, is relatively resistant to most DNA-damaging agents, exhibiting, when graphed, extensive shoulders followed by approximately exponential decreases in survival with high levels of damage, since NC4 has DNA repair mechanisms. Treatment with X-ray, UV or DNA damaging agents induces inhibition of cell growth and abnormal morphology (Ohnishi and Nozu, 1979; Ohnishi, 1988). Hence, this led us to investigate whether space conditions such as microgravity and space radiation induce biological toxicity on cell growth and morphological abnormality in *D. discoideum* in comparison with γs13 and NC4.

## *Materials and methods*

*Organisms.* A strain of *Escherichia coli* Bs-1 was used as food to culture slime mold amoebae. A radiation sensitive mutant of *D. discoideum* was isolated from the parental wild-type strain by Dr. R. A. Deering (The Pennsylvania State University, USA).

*Preparation of bacterial cells*. After the bacterial cells were cultured overnight in Bouillon broth, the cells were washed twice with phosphate (Pi)-buffer. The bacterial cells used as food to amoebae *D. discoideum* and were used within 1 month. For the amoebae culture on the membrane, the bacterial cells of $5x10^{10}$ cells were filtered in the range of 60 mm in diameter on a membrane (70 mm in diameter, cellulose nitrate membrane filter, 0.45 μm pore size, black, ADVANTEC, Toyo Roshi Kaisha, Ltd, Tokyo, Japan) and dried in air for about 30 min at room temperature, and then kept in the cold at about 5 °C.

*Preparation of spores.* Amoebae were cultured in Pi-buffer containing bacterial cells with vigorous aeration. The doubling time of NC4 and γs13 is about 4 and 6 hours in this liquid condition, respectively. When cell growth reached to almost static phase, the amoebae were washed twice with Pi-buffer and suspended to concentration of $2x10^7$ cells/ml. The cell suspensions were spread on a membrane filter without bacterial cells and incubated for 2 days at 22-23 °C. Fruiting bodies appear after many stages of morphology. The initial amoeboid cells differentiate to stem, spore bag and spores. The complete morphology requires about 24 and 36 hours in NC4 and γs13, respectively. We collected the spore samples by absorption with dried pads to one side. We used it for experimental samples after drying with silica gel powder at about 5 °C. The viability of these spores were about 80 % survival for 1 month, when kept at about 5 °C in the dark. Other details were described previously (Nozu et al., 1982).

*Cell culture kit*. This kit is a set of experiment apparatuses to cultivate cells in microgravity environment. The Pi-buffer can be easily added by using syringe-type container with high accuracy. Sterility can be maintained through the crew operation without clean bench. Oxygen and carbon dioxide gas were exchanged through a special gas permeable membrane. This kit is normally used with a Thermo-Electric Incubator. The chamber is composed of aluminum outer chamber (126 diameter × 31 (H) mm) and polyethylene carbonate inner chamber (90 diameter × 17.7 (H) mm). The inner chamber has a connection with syringe (22.5 diameter × 76.2 (W) mm) to inject Pi-buffer solution in it and a window for observation and microscope.

The double chambers are located for each strain. The cell culture kit has two holes to inject Pi-buffer and exhaust air. This kit was made by Mitsubishi Heavy Ind., Ltd. (Kobe, Japan). Four pads absorbed with NC4 and γs13 spores were set at one side of the chambers. The spores were attached on the surface of the membrane with bacterial cells. The picture of the cell culture kit are shown in Fig. 1. The syringe contains 3 ml of Pi-buffer. The cell culture kit and syringe were kept at about 5 °C before injection of Pi-buffer.

*Experimental procedures.* One cell culture kit was taken into space on a Space Shuttle (STS-84, May 1997, 9 days flight in total) and three cell culture kits were left on earth as controls. Three ml of Pi-buffer were injected into the first cell culture kit by an astronaut (Dr. C. I.Noriega) on the first day after launch. On the third day after launch, the wet condition of the cell culture kit was checked and estimated as fully wet. Therefore, we continued the incubation for 7 days in total at 22-23 °C.

*Observation of fruiting bodies.* For a permanent record of the experimental results, we used 8 mm video camera (Cannon L1, Cannon, Tokyo, Japan) to record the observations. After fixing the cell culture kit, he focused the view for measurement of the growing range of amoebae, number of fruiting bodies and the shape of fruiting body (by Olympus camera system; SZH10 and PM20; Olympus, Tokyo, Japan). The data were down-linked to Marshal Space Center (Houston, TX, USA).

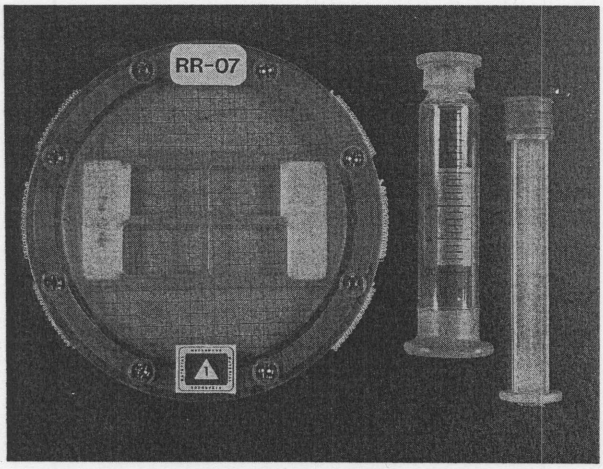


Fig. 1 Cell culture kit.

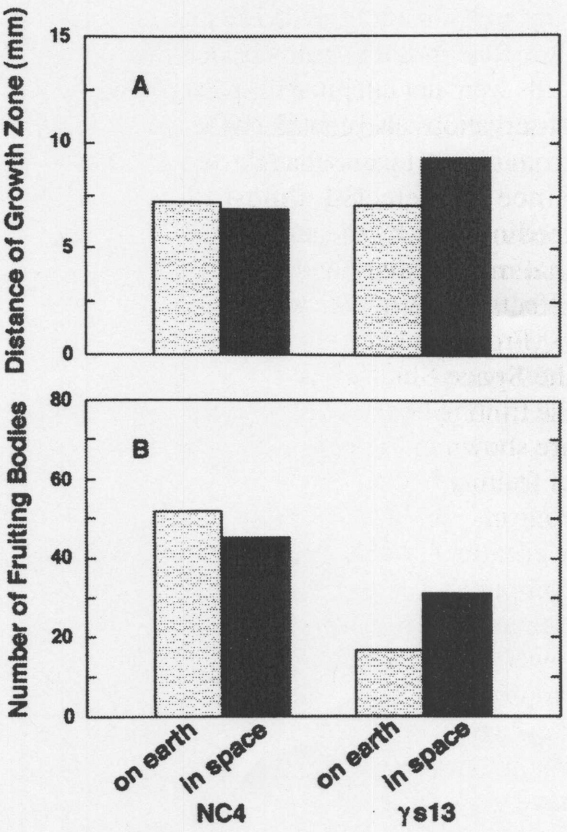


Fig. 2. A, Cell growth of amoebae emerged from spores. B, Number of fruiting bodies. The numbers represent complete shape of fruiting bodies, namely cell aggregation with incomplete shape of slug and Sombrero in the middle stage of morphology were not counted. The distance of growth zone and numbers of fruiting bodies represent the average of individual samples in the both strains.

## *Results and discussion*

After injection of Pi-buffer, the two strains of *D. discoideum* emerged from spores to amoeba cells, this stage is called as germination. Since the amoebae fed on the bacterial cells, the bacterial cells disappeared from the membrane. It is easy to measure the distance of fed area. We could get two data from each two chambers of NC4 and γs13. The experimental data are shown in Fig. 2A. There was no difference in the growth rate of amoebae between space and ground samples in NC4. The amoebae from γs13 spores of ground control grew almost the same level as NC4 amoebae, on the other hand, the growth zone of γs13 in space was a little greater than the control on earth at Kennedy Space Center and Nara Medical University. These results suggest

that cell dividing activities of *D. discoideum* amoebae in both strains by feeding on bacterial cells were not inhibited in space condition. These observations also contained the germination stage from spores to amoebae through swollen spores. Since we detected almost the same width of feeding area by the amoebae, it can be deduced that microgravity and space radiation have few effects on germination in space.

From the analysis of down-linked tape from the Space Shuttle, we measured the number of the fruiting bodies in the both strains. The results are shown in Fig. 2B. We found similar number of fruiting bodies formed in space and in the NC4 control. In the case of γs13, we observed rather a greater number of fruiting bodies in space compared to the ground controls, although the efficiency of fruiting body formation of γs13 was lower than NC4. These findings show that space environment hardly inhibits, rather it enhances the formation of fruiting bodies a little. These results suggest that the grown amoeboid cells have cell aggregation activity at almost the same level. It further suggests that for cell aggregation, amoebae could move in the direction of high concentration of cAMP.

From the recorded video tape, we also detected very clear shape of fruiting bodies in NC4 and γs13. Typical photographs are shown in Fig. 3. In NC4, there were no abnormal fruiting bodies in space and in ground samples. Even in the case of γs13, abnormal shape of fruiting bodies was hardly detected. In addition, no difference in size of the fruiting bodies was observed between the space and the earth samples. We also found several numbers of slug-shape cell masses and Sombrero (data not shown). Therefore, we postulate that the whole process of cell aggregation, cell differentiation and morphology might have progressed like the controls observed on earth.

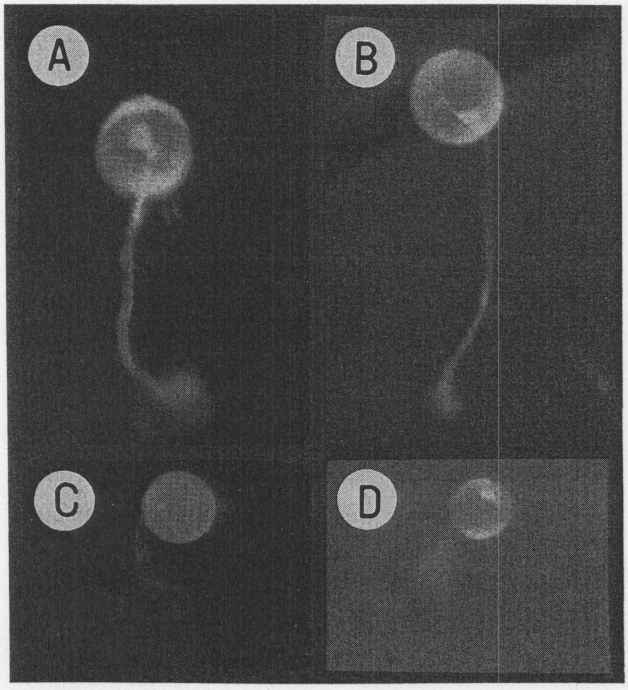


Fig. 3. Typical photographs. A and B, NC4; C and D, γs13; A and C, on earth; B and D, in space.

In space condition, the most typical difference from earth condition is microgravity. Microgravity affects the relative weight and circulation of heat transmission. If such physical factors interact with biological process of cell aggregation, cell differentiation, morphological change and cell development, we might find abnormal or suppressive effect. It is well known that the diffusion of cAMP is a very important factor for morphological process. In the present experiments, we detected normal morphology in space. Therefore, we suggest that the cAMP diffusion is normal, the amoeboid cells can aggregate to each other, and cell differentiation proceeds to completion. We detected normal shape of fruiting bodies , which have spore bag, in NC4 and γs13, nevertheless there is no direction of gravity. From these results, we suggest that spore bag might be oriented in opposite direction to membrane through the conformational structure such as a stalk and a basal disc of fruiting body which may work to separate spores from water.

Many kinds of DNA damaging treatments with X-rays, UV and chemicals inhibit cell division and cell differentiation, especially in γs13 as compared to NC4. The radiation sensitivity of γs13 to ionizing radiation and UV is about 100 times as compared to NC4. The abnormal differentiation is easy to be induced in γs13. The typical abnormal shape of fruiting bodies is a double fruiting which consists of linearly connected two spore bags on one stalk. In the present experiments, we hardly detected the depression of cell growth and such abnormal shape of fruiting body in space environment.

Therefore, we postulate that space radiations may not inhibit to cell growth and formation of abnormal fruiting body. It is suggested that space radiations might be negligible to cell growth and thus abnormal fruiting body formation, even though space radiations contain high LET radiations. In this flight, Dr. Doke measured 0.8-0.9 mSv/day of high LET radiation by a Real-time Radiation Monitoring Detector (Waseda University, Japan; personal communication). Therefore, we could calculate the total doses of high LET radiations were less than 4.5 mSv for formation of fruiting body from spores, because normal cell growth and formation of fruiting body were observed at least on the 5th day after launch by the astronaut . It has been reported that double fruiting body was detected with X-ray (Deering et al., 1972) or UV(Ohnishi and Nozu, 1979) at 50 Gy or 25 J/m$^2$, respectively. Furthermore, it has been reported that microgravity enhances the radiation effects on abnormal development in *Carausius morosus* (Bucker et al., 1986) and induced mutation frequency in melanogaster (Ikenaga et al, 1997). Even taking this into considerations, we speculate that space environment containing microgravity and space radiations may not affect cell growth and the morphology in *D. discoideum*. Further analysis is necessary to measure the viability and the mutation frequency of spores formed in space environment.

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