Centrifugal displacement of nuclei in epidermal cells of azuki bean epicotyls

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Abstract
We examined the effects of hypergravity on nuclear positioning in epidermal cells of azuki bean (Vigna angularis) epicotyls. The nucleus was positioned almost in the center of the cell under 1 G conditions. When the epicotyls were exposed to basipetal hypergravity-by centrifugation, the nucleus was displaced toward the centrifugal side of the cell in a linear dose-response manner. The nuclear displacement started within 20 min by exposure to basipetal hypergravity at 300 G, and reached ca. 35% from the centrifugal edge of the cell after 2 h. The displaced nucleus recentered by removal of hypergravity stimuli. The nucleus was also displaced by acropetal hypergravity at the similar degree as basipetal hypergravity. The displacement by hypergravity of the nucleus was stimulated, when actin filaments were disrupted by cytochalasin D treatment. These results suggest that the positioning of the nucleus in epidermal cells is maintained by actin filaments, which is affected by gravity in azuki bean epicotyls. ©2019 Jpn. Soc. Biol. Sci. Space; doi: 10.2187/bss.33.1

Introduction
Centrifugal hypergravity, the gravitational acceleration of more than 1 G, has been utilized for gravitational biology experiments. Various plants have been grown under hypergravity conditions, and shown to develop a short and thick body (Hoson and Soga, 2003; Wakabayashi et al., 2005; Nakano et al., 2007; Murakami et al., 2016). Based on the results of hypergravity experiments, it was hypothesized that plant body would become longer and thinner under microgravity conditions in space. This hypothesis was proven by space experiments such as STS-95 Rice and Aniso Tubule (Hoson et al., 2002, 2014; Soga et al., 2002, 2014, 2018a). The development of a short and thick body is considered as a key part of the response that enables plants to grow against the gravitational acceleration.

The changes in the dynamics of the cytoskeleton such as microtubules and actin filaments are involved in the modification by gravity of plant body shape (Tanabe et al., 2018; Soga et al., 2018b). The shape of the plant body depends generally on the shape of its individual cells, which is primarily controlled by the orientation of cortical microtubules (Shibaoka, 1994). Hypergravity, which stimulated the development of a short and thick body, induced reorientation of cortical microtubules from transverse to longitudinal directions in epidermal cells of azuki bean epicotyls (Soga et al., 2006) and Arabidopsis hypocotyls (Matsumoto et al., 2010; Murakami et al., 2016). On the other hand, microgravity, which stimulated the development of a longer and thinner body, induced reorientation of cortical microtubules from longitudinal to transverse directions in epidermal cells of Arabidopsis hypocotyls (Soga et al., 2018a). The changes in the dynamics of actin filaments are also involved in the regulation of cell growth of plants (Thimann et al., 1992). Recently, we have shown that the modification of the dynamics of actin filaments is involved in reorientation of cortical microtubules, which leads to develop a short and thick body in azuki bean epicotyls under hypergravity conditions (Tanabe et al., 2018). However, it has not been clarified whether hypergravity affects the dynamics of other organelles.

The nucleus is the dense and largest organelle. Actin filaments, whose dynamics was modified by hypergravity (Tanabe et al., 2018), are present near the nucleus in various plant species (Clayton and Lyoyd, 1985; Parthasarathy et al., 1985; Seagull et al., 1987). These facts suggest that the dynamics of the nucleus in the cell is affected by gravity. The effects of hypergravity on nuclear position have been examined in various cells including animals (Beams and King, 1939). The nucleus was displaced toward the centrifugal side of the cell with increasing the gravity doses. For example, Luyet and Ernst (1934) showed that the nucleus did not change the position at 500 G, but 2000 G caused it to move in the centrifugal direction and it was reached at the bottom of the cell at 5000 to 10000 G in onion roots. On the other hand, Sievers and Heyder-Caspers (1983) reported that the nucleus was displaced even at 50 G in cress roots. The effects of hypergravity on nuclear position have mainly been investigated in roots. Also, the relationships between the nuclear displacement and the modification by gravity of body shape have not been clarified yet. In the present study, we analyzed the effects of hypergravity on nuclear positioning in epidermal cells of azuki bean epicotyls, where the mechanisms of gravity-induced modification of body shape have been well studied (Soga, 2010, 2013). We also examined the effects of disruption of actin filaments by cytochalasin D on the hypergravity induced nuclear displacement.

Materials and Methods
Plant materials and hypergravity experiments
Seeds of azuki bean (Vigna angularis (Willd.) Ohwi et Ohashi ‘Erimowase’) were soaked in running tap water for 1.5 days at 30°C and they were allowed to germinate.
Centrifugal Displacement of Nuclei in Azuki Bean

Results and Discussion

Figure 1 shows representative fluorescence micrographs of DAPI stained nuclei in epidermal cells of epicotyls grown at 1 G and 300 G. Under 1 G conditions, the nucleus was almost centrally positioned in epidermal cells of apical region of epicotyls. The nucleus was also positioned almost in the center of the cell in the middle or basal region of epicotyls under 1 G conditions; the nucleus was positioned at 48.9±0.5% in apical region, 50.1±0.4% in middle region and 50.9±0.9% in basal region, when the relative position from the basal end of the cell was calculated. These data indicate that the nucleus is centrally positioned irrespective of the epicotyl region, namely cell age, in the epidermal cells of azuki bean under 1 G conditions.

The nuclear position in epidermal cells of Arabidopsis hypocotyls, and got a similar result that the nucleus was almost centrally positioned. On the other hand, it has been shown that in the columnella cells, statocytes of roots, the nucleus is positioned at the upper side of the cell in various plants such as Arabidopsis (Sievers et al., 1991; Hashiguchi et al., 2013). Thus, the position of the nucleus in the cell may be determined and maintained depending on the cell type.

Under basipetal hypergravity conditions at 300 G, the

Nuclear Position

For the observation of the nuclei, the segments excised from azuki bean epicotyls were immediately fixed with 4% (w/v) paraformaldehyde in PMEG buffer (50 mM PIPES, 1 mM MgSO₄, 5 mM EGTA, 1% (v/v) glycerol, pH 6.8) at 25°C for 1 h after infiltration with a vacuum pump for 10 min. The air pressure was restored, and the samples were washed four times (15 min each) with phosphate-buffered saline (PBS) at 25°C. Epidermal strips were peeled from the segments. The specimens were treated with DAPI solution (Cellstain; Dojindo, Japan) for 20 min, and then washed with PBS. Fluorescence images were collected with a fluorescence microscope (Axio Imager. A1; Carl Zeiss, Germany) equipped with a cooled CCD camera (VB-7000; Keyence, Japan).

The nuclear position of each epidermal cells was analyzed by using ImageJ software (http://rsbweb.nih.gov/ij/, NIH). The length of long axis of cell and the distance from the basal end of cell to the center of the nucleus were measured. Then, the relative position of nucleus from the basal end of cell was calculated. The data of the nuclear position were analyzed using the Student’s t-test (Figs. 2 and 3) and Tukey HSD test (Figs. 4 and 6), respectively.

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**Fig. 1.** Fluorescence micrographs of nuclei in epidermal cells of azuki bean epicotyls grown under 1 G or basipetal hypergravity conditions at 300 G. Seedlings with an epicotyl about 30-35 mm long were selected and grown under 1 G or basipetal hypergravity conditions at 300 G for 2 h in the dark. Epidermal strips were prepared from subapical region of epicotyls, and the nuclei were stained with DAPI. Bar=20 μm.
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In hypergravity conditions at 300 G, the nucleus was also displaced toward the centrifugal side of the cell (Fig. 4). The value of the nuclear positioning from the basal end of the cell was significantly larger than that of acropetal 1 G at 5% level. The degree of displacement caused by acropetal hypergravity was almost the same as that by basipetal hypergravity. These results indicate that the nucleus was displaced regardless of the direction of hypergravity.

In order to clarify the contribution of actin filaments to the nuclear positioning, we examined the effects of cytochalasin D, an actin-disrupting reagent, on the nuclear position of epidermal cells. Figure 5 shows that the nucleus tended to move to the centrifugal direction (Fig. 1). Therefore, we calculated the relative nuclear positioning from the basal end of the cell. Figure 2 shows the dose-response relationship between the nuclear position and the magnitude of basipetal hypergravity in 2 h-treatment. At 30 G, the nuclear position was almost the same as that at 1 G. On the other hand, at 100 G and above, the value of nuclear position was significantly smaller than that of 1 G at 5% level. Namely, the nucleus was displaced toward the centrifugal side of the cell by increasing the magnitude of gravity. Nuclear displacement was also caused by hypergravity in epidermal cells of the middle or basal region of epicotyls as well as in the apical region; the nucleus was positioned at 35.8±0.6% in apical region, 36.1±0.6% in middle region and 37.2±0.7% in basal region under 300 G conditions. The magnitude of gravity that displaces the nucleus is different depending on the report. Luyet and Ernst (1934) showed that 2000 G was required for nuclear displacement in onion roots. In cress roots, 50 G displaced the nucleus (Sievers and Heyder-Caspers, 1983). Katsuta and Shibaoka (1988) reported that 100 G displaced the nucleus in tobacco BY-2 cells. Taken together, hypergravity generally displaces the nucleus, but the magnitude required may differ by the plant material, age, or the type of organs.

The relationship between the magnitude of gravity and the nuclear position was regressed linearly (Fig. 2 upper panel) or logarithmically (Fig. 2 lower panel). The data on the nuclear position fitted better to the straight line on a linear scale of the magnitude of gravity than on a logarithmic scale. We also examined the dose-response relationship in the epidermal cells of Arabidopsis hypocotyls and found the linear relation on a linear scale \( R=−0.99 \) than on a logarithmic scale \( R=−0.85 \). Thus, the nuclear displacement may be caused in a linear dose-response manner. We showed previously that the body shape of azuki bean epicotyls varied in proportion to the logarithm of the magnitude of gravity (Soga et al., 2006). Also, in azuki bean epicotyls, the orientation of cortical microtubules and the transcript levels of a 65 kDa microtubule-associated protein varied in proportion to the logarithm of the magnitude of gravity (Soga et al., 2006, 2012). These results indicate that the nuclear displacement and the modification of body shape show different dose-response relationships. Therefore, these two events may be independent responses.

The nuclear positioning of epicotyls grown under 1 G or basipetal 300 G conditions was examined at several time points. A significant difference in nuclear positioning was detected at 20 min after the start of hypergravity treatment at 300 G (Fig. 3). The nucleus reached ca. 35% from the centrifugal edge of the cell after 2 h, and was not displaced further. On the other hand, the nucleus recentered by removal of hypergravity stimuli. These results indicate that the position of the nucleus changes reversibly in response to the magnitude of gravity. Does hypergravity affect the nuclear positioning independently of the direction of the stimulus? To clarify this question, we examined the effects of acropetal hypergravity on the nuclear positioning. Under acropetal hypergravity conditions at 300 G, the nucleus was also displaced toward the centrifugal side of the cell (Fig. 4). The value of the nuclear positioning from the basal end of the cell was significantly larger than that of acropetal 1 G at 5% level. The degree of displacement caused by acropetal hypergravity was almost the same as that by basipetal hypergravity. These results indicate that the nucleus was displaced regardless of the direction of hypergravity.

In order to clarify the contribution of actin filaments to the nuclear positioning, we examined the effects of cytochalasin D, an actin-disrupting reagent, on the nuclear position of epidermal cells. Figure 5 shows...
representative fluorescence micrographs of nuclei in epidermal cells of azuki bean cuttings kept at 1 G or basipetal hypergravity conditions in the presence or absence of cytochalasin D. Cuttings were kept at 1 G or hypergravity conditions for 2 h in the presence or absence of 10 μM cytochalasin D. Epidermal strips were prepared from cuttings, and the nuclei were stained with DAPI. Bar=20 μm. 

Fig. 3. Changes in the nuclear position in epidermal cells of azuki bean epicotyls under hypergravity conditions. Seedlings were grown under basipetal hypergravity at 300 G for 2 h, and then a half of the hypergravity-treated seedlings were transferred to 1 G conditions for an additional 2 h. The relative nuclear positioning from the basal end of the cell was calculated. Values are means±SE (n=20).

Fig. 4. Effects of basipetal and acropetal hypergravity on the nuclear positioning in epidermal cells of azuki bean epicotyls. Seedlings were grown under 1 G or 300 G conditions applied in basipetal or acropetal direction for 2 h. The relative nuclear positioning from the basal end of the cell was calculated. Values are means±SE (n=20). Different letters above bars represent significant differences (Tukey HSD test: P<0.05).

Fig. 5. Fluorescence micrographs of nuclei in epidermal cells of azuki bean cuttings kept at 1 G or basipetal hypergravity conditions in the presence or absence of cytochalasin D. Cuttings were kept at 1 G or hypergravity conditions for 2 h in the presence or absence of 10 μM cytochalasin D. Epidermal strips were prepared from cuttings, and the nuclei were stained with DAPI. Bar=20 μm.

Fig. 6. Effects of cytochalasin D on the nuclear positioning in epidermal cells of azuki bean cuttings kept at 1 G or basipetal hypergravity conditions. Cuttings were treated as described in the legend of Fig. 5. The relative nuclear positioning from the basal end of the cell was calculated. Values are means±SE (n=20). Different letters above bars represent significant differences (Tukey HSD test: P<0.05).
At 300 G for 2 h, the nucleus was significantly displaced toward the centrifugal side of the cell in the actin-disrupting cells, but it was not displaced in the control cells. At 300 G, the nucleus was significantly displaced toward the centrifugal side of the cell in both control and the actin-disrupting cells, although the degree of displacement in the actin-disrupting cells was significantly larger than the control cells at 5% level. These results indicate that the nuclear displacement by hypergravity was stimulated, when actin filaments were disrupted in azuki bean epicotyls. Similarly, the nuclear displacement by hypergravity was stimulated in tobacco BY-2 cells, when actin filaments were disrupted (Katsuta and Shibaoka, 1988). Recently, we reported that the filamentous actin was observed even in the hyergravity conditions at 300 G in epidermal cells of azuki bean epicotyls (Tanabe et al., 2018). We also showed that the density of actin filaments in the apical region was decreased by hypergravity. Therefore, the hypergravity-induced decrease in the density of actin filaments may be involved in the displacement of nuclei under hypergravity conditions. Taken together, these facts suggest that the positioning of the nucleus is maintained by actin filaments, which is affected by gravity.

In gravitropism of plants, the sedimentation of amyloplasts in the direction of gravity trigger graviperception (Sievers, 1991; Hashiguchi et al., 2013). Analogously, the displacement of the nucleus may also be involved in the graviperception of some response. The nucleus was displaced by hypergravity at 100 G and above in azuki bean epicotyls (Fig. 2). Also, 2000 G was required for nuclear displacement in onion roots (Luyet and Ernst, 1934). Therefore, the nuclear displacement might be involved only at the higher dose, even if it is involved in graviperception. We have previously reported that mechanoreceptors on the plasma membrane is involved in the graviperception of the modification by gravity of body shape (Soga et al., 2004). As described above, the nuclear displacement and the modification by gravity of body shape show different dose-response relationships. Therefore, the nuclear displacement may not be directly involved in the gravity sensing in the modification by gravity of body shape, although the reversibility and the independence to gravity direction are common between two events. In conclusion, the present results suggest that the positioning of the nucleus is maintained by actin filaments, which is affected by gravity. Further studies are needed to clarify the physiological functions of nuclear displacement by gravity.

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