Summary report of the ISS-Kibo utilization mission,
"Dynamism of auxin efflux facilitators, CsPINs, responsible for gravity-regulated growth and development in cucumber (CsPINs)"

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Plants sense gravity as an environmental signal and use it for governing their morphology and growth orientation. We have found that dynamism of auxin efflux facilitators, CsPINs, plays an important role in the regulation of gravity-dependent redistribution of auxin and thereby controls gravimorphogenesis (peg formation) in cucumber (*Cucumis sativus* L.) seedlings. Moreover, we have found that gravitropism interferes with hydrotropism in cucumber roots, in which the dynamism of auxin efflux facilitators may also play a role. In this space experiment, we used cucumber seedlings to analyze the effect of gravity on the expressions of CsPIN1 and unravel their contributions to peg formation. At the same time, we attempted to differentiate hydrotropism from gravitropism in cucumber roots and compare the expression of CsPIN5 between the two tropisms.

The gravity-regulated localization of CsPIN1 and negative control of gravimorphogenesis in cucumber seedlings

The formation of a specialized protuberance, the peg, in cucurbitaceous seedlings is a unique gravimorphogenesis. When cucumber seeds (*Cucumis sativus* L.) are placed in a horizontal position and allowed to germinate, a peg forms on the lower side of the transition zone between the hypocotyl and the root. The peg anchors the lower seed coat in soil so that the elongation of the hypocotyl pulls the cotyledons out of the seed coat. Cucumber seedlings have the potential to develop a peg on each side of the transition zone as, when seeds are placed prior to germination in a vertical position with the radicles pointing down or under microgravity conditions, a peg develops on each side. However, peg formation on the upper side of the transition zone is suppressed in response to gravity when the seedlings are grown in a horizontal position on the ground. A phytohormone, auxin, plays an important role in the lateral placement of peg formation in the transition zone. We hypothesized that gravity-modified transport of auxin is required for the differential decrease in auxin level on the upper side of the transition zone in cucumber seedlings grown in a horizontal position. We suggested that an auxin efflux carrier CsPIN1 was involved in the gravity-modified auxin transport and redistribution. Here, we examined whether CsPIN1 changes its localization in response to gravity. Immunohistochemical analysis indicated that, when cucumber seedlings were grown entirely under microgravity conditions in space, CsPIN1 in endodermal cells was mainly localized to the cell side parallel to the minor axis of the elliptic cross section of the transition zone. However, when cucumber seeds were germinated in microgravity for 24 h and then exposed to 1G-centrifugation in a direction crosswise to the seedling axis for 2 h in space, CsPIN1 was re-localized to the bottom of endodermal cells of the transition zone. The results showed that endodermal cells re-localize CsPIN1 due to gravistimualtion and laterally transport auxin from the upper to the lower flank, which explains the redistribution of auxin responsible for the lateral placement of peg formation in cucumber seedlings (Fig. 1).

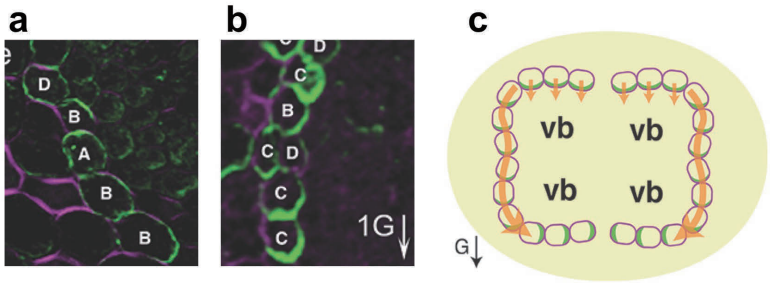

Fig. 1. Auxin efflux carrier CsPIN1 re-localizes in endodermal cells following gravistimulation in space (a, microgravity; b, 1G), which could cause an auxin transport from the upper to the lower side through endodermal layer and thereby an auxin decrease on the upper side (c). (Yamazaki *et al*., npj Microgravity 2016)

The CsPIN5-regulated auxin distribution in counteractive relationship between gravitropism and hydrotropism in cucumber roots

Land plants orient root growth along moisture gradients and gravity via hydrotropism and gravitropism, respectively. Gravitropism interferes with hydrotropism, although the mechanistic aspects are poorly understood. Here, we differentiate hydrotropism from gravitropism in cucumber roots by conducting clinorotation and spaceflight experiments. We also analyzed and compared mechanisms regulating hydrotropism and auxin-regulated gravitropism. Clinorotated or microgravity-grown cucumber seedling roots hydrotropically bent toward wet substrate in the presence of moisture gradients, but they grew straight along the direction of stationary 1G on the ground or centrifuge-generated 1G in space. The roots appeared to become hydrotropically more sensitive to moisture gradients in microgravity. Auxin transport inhibitors significantly reduced the hydrotropic response of clinorotated seedling roots. The auxin efflux protein CsPIN5 differentially expressed in hydrotropically and gravitropically responding roots, with higher expression in the high-humidity (concave) side versus higher expression in the lower (concave) side, respectively. These results suggest that roots become hydrotropically sensitive in microgravity, by inducing CsPIN5-mediated auxin re-distribution in cucumber roots. (Morohashi *et al*., New Phytologist 2017)

Thus, CsPINs spaceflight experiments revealed the gravity-regulated CsPIN1 re-localization in gravisensing cells, polar auxin transport, and thereby a reduction of auxin level and a suppression of peg formation on the upper side of gravistimulated transition zone of cucumber seedlings. Furthermore, CsPINs experiments suggested a possible role of CsPIN5 of which differential expression is induced by both hydro- and gravi-stimulation and causes a counteractive relationship between hydrotropism and gravitropism in cucumber roots.