Summary of Research:

Seedlings of most cucubitaceous plants develop a protuberance, peg, on the concave (lower/gravistimulated) side of the bending transition zone (TR zone) between hypocotyl and root when seeds are germinated in a horizontal position. The peg plays an important role in pulling the seed coat out from the seedling by holding the coat during upward elongation of the hypocotyl. In spaceflight experiment (STS-95, 1998), we found that cucumber seedlings develop a peg on each side of the TR zone in microgravity. This finding verifies that gravity is not required for peg formation, but on Earth seedlings suppress peg formation on the convex (upper/non-stimulated) side of the TR zone in response to gravity, resulting in one peg on the concave side. Thus, gravity negatively controls morphogenesis of cucumber seedlings. By analyzing expression of an auxin-inducible gene, we have shown that in response to gravity the upper side of the gravistimulated TR zone reduces auxin content to below the threshold required for peg formation. We have also shown that hydrotropism interferes with gravitropism on Earth. Our spaceflight experiment suggested that positive hydrotropism could occur in response to a moisture gradient that might exist in microgravity. The gravity’s negative control of morphogenesis could provide a clue to understand the molecular mechanisms of auxin dynamics that play an essential role in plant graviresponse. Hydrotropism in seedling roots could be a tool for separating growth response from graviresponse and for comparing mechanisms underlying each of those responses and their integration in controlling growth orientation.

In the present study, therefore, we attempted to; 1) analyze the role of auxin dynamics in gravimorphogenesis, gravitropism and hydrotropism, 2) establish models of auxin dynamics that are induced by gravi- and hydro-stimuli and clarify their mechanisms, 3) develop new experimental systems for achieving the above-mentioned objects and establish systems that allow us to maintain and monitor moisture gradient in space, and 4) ultimately establish a model to be verified by spaceflight experiment for understanding the gravity’s negative control of morphogenesis and its interaction with other growth responses at molecular level.

By measuring distribution of endogenous auxin and analyzing expression patterns of auxin-inducible genes in cucumber seedlings stimulated by gravity or treated with auxin transport inhibitors, we verified that auxin level substantially decreases on the non-peg (upper) side of the TR zone in a horizontal position and found that the TR zone of cucumber seedlings serves as a pool and source of auxin. Also, our studies demonstrate that auxin transporters play important roles in auxin distribution induced by graviresponse. To study the molecular mechanism of auxin movement and dynamics in graviresponse, we then isolated cDNAs encoding auxin transporters, CS-AUX1 and CS-PIN1, and analyzed their expression and localization at both transcriptional and post-transcriptional levels. The results of those analyses revealed that balance of the influx and efflux carriers regulates cytoplasmic auxin concentration and auxin distribution. In gravistimulated TR zone, CS-AUX1 was abundant on the peg-formed (lower) side compared with that of the non-peg (upper) side. In contrast, CS-PIN1 was more abundant on the non-peg side than the peg side. These auxin transporters were inducible by auxin at both transcriptional and post-transcriptional levels. CS-AUX1 in particular increased in response to auxin, suggesting its feedback regulation due to differential distribution of auxin. Namely, higher level of auxin causes an increase in CS-AUX1 in the lower side of gravistimulated TR zone. Thus, this study did reveal not only the mechanism for the gravity’s negative control of morphogenesis in cucumber but also the roles of auxin transporters in regulating cytoplasmic auxin concentration and auxin distribution.

Auxin that is re-distributed via graviresponse may promote or suppress the transcription of auxin-regulated genes for peg formation. We next examined the possible mechanism of transcription of auxin-inducible genes, which might involve with the gravity’ negative control of morphogenesis in cucumber seedlings. For this purpose, in addition to CS-IAA1 and CS-IAA2, we cloned five cDNAs of
auxin response factors (CS-ARFs) from cucumber and analyzed the expression patterns by in situ hybridization. The results suggested that CS-ARF2 is involved in activation of CS-IAA1 transcription and repressed at post-transcriptional level by the reduction of auxin concentration in the non-peg (upper) side of the gravistimulated TR zone. Other experiments suggested that auxin-induced ethylene facilitates the development of peg. This was consistent with facts that mRNA of CS-ACS1 (ACC synthase) accumulated on the peg side of TR zone and that the expression of CS-ACS1 was induced by auxin.

To obtain new type of molecules that could involve with the gravity’s negative control of morphogenesis, we compared accumulation of mRNAs between the peg (lower) side and the non-peg (upper) side of the gravistimulated TR zone by using Arabidopsis DNA arrays and fluorescence differential display. Among the cDNAs isolated, CS-GRP1 (cucumber glycine-rich protein) was expressed more abundantly on the non-peg (upper) side of the gravistimulated TR zone just before and after peg initiation on the lower side. Interestingly, auxin starvation caused an increase in mRNA accumulation of CS-GRP1. Thus, CS-GRP1 could involve with the gravity’s negative control of morphogenesis or interact with auxin signaling pathway via its anti-auxin action.

Furthermore, we have established experimental systems for the study of hydrotropism in Arabidopsis and cucumber roots, which could be used for spaceflight experiments in future. These studies led us find a unique mechanism that accounts for the interaction between hydrotropism and gravitropism and unique hydrotropic mutants that allow us to dissect molecular mechanism of hydrotropism and its interaction with other growth responses such as gravitropism and waving response. It was also shown that auxin re-distribute in hydrostimulated roots of cucumber, which allows us to examine and compare the roles of auxin transporters in hydrotropism and gravitropism. In addition, we found that shoots of agravitropic growth of weeping morning glory, Shidare-asagao, lack gravisensing endodermal cells, which might be caused by the mutation of SCARECROW-related gene. Analyzing circumnutation of such agravitropic shoots of morning glory and Arabidopsis, we found that gravitropic response is required for circumnutation. Molecules and experimental systems newly found or established in this study contribute to our understandings of the gravity’s negative control of morphogenesis, interactions between graviresponse and other growth responses, and their verification by spaceflight experiments.

Publication Lists: