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植物ホルモン・ブラシノステロイドによる遺伝子発現調節機構に関する生化学的解析

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Summary

Biochemical analysis of the mechanism for regulation of brassinosteroid-responsive gene expression in *Arabidopsis* cultured cells

Brassinosteroids (BRs) are unique polyhydroxylated steroidal hormones that affect a wide range of plant growth and development at an extremely low concentration. They also control several important agronomic traits, such as flowering time, number of branches, plant architecture, biomass production and seed yield, and tolerance to biotic and abiotic stresses. Over the past decades, molecular genetic studies using *Arabidopsis* plants have made a great progress in our understanding of BR signal transduction pathway. Briefly, upon the binding of BR to a cell-surface receptor BRI1, its signaling cascade progresses with a series of phosphorylation-dephosphorylation reactions in both cytoplasmic and nuclear spaces and finally activates two closely related transcription factors, BES1 and BZR1 that together regulate thousands of BR responsive genes, thus leading to the numerous physiological processes mentioned above. However, other aspects of BR signaling-mediated gene expression, such as the involvement of other nuclear proteins and if so, their functions in it are largely unknown.

Therefore, for elucidation of the whole mechanisms underlying BR signaling, biochemical characterization was firstly performed to reveal the nature of nuclear proteins in *Arabidopsis* cultured cells grown under different BR levels. As a result, it was observed that sixteen spots increased in their abundance in response to high BR contents among 551 protein spots detected on the 2D-PAGE gels, while fifty-five of spots decreased. The result indicates

that 2D-PAGE combined with pre-fractionation of nuclei is an effective approach for investigating the BR-induced changes in nuclear protein abundance. Then, LC-MS/MS analysis was carried out to characterize proteins derived from the 71 spots fluctuated BR-dependently in their amounts, and identification of 35 proteins was succeeded, among which 11 proteins were reportedly nuclear-localized. Among them, NAP1;2, SAM syn.2, and HD2B were recognized to be BR-induced while NAP1;1 was BR-repressed. All four proteins have been reported to be involved in chromatin remodeling. Thus, this finding strongly suggests that changes in chromatin structure are crucial for BR signaling-mediated gene expression.

Next, affinity-purification procedure was employed to characterize a protein complex containing BES1 and successfully identified a molecular chaperone heat shock protein 90 (HSP90) as a novel partner. High BR levels promoted not only BES1 interaction with HSP90, but also the formation of HSP90-containing macromolecular complexes with more than 480 kDa of molecular weight. Geldanamycin, a specific inhibitor of HSP90's ATPase activity caused prevention of BES1 binding to HSP90 and also disturbance of BR-dependent expression of two BR biosynthesis genes, *CPD* and *DWF4*. These observations suggest that BES1/HSP90 interaction plays a crucial role in BR signaling-mediated feedback control of endogenous BR contents through regulation of the corresponding genes.

Taken together, the current biochemical study firstly demonstrates the involvement of both the chromatin remodeling process and the chaperone activity of HSP90 targeted BES1 in BR signaling-mediated gene regulation.