別紙４

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主　論　文　の　要　旨

論文題目 Functional analysis of Haspin kinase role in plant cells

（植物細胞におけるハスピンキナーゼの機能解析）

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論　文　内　容　の　要　旨

Progression of the cell division is controlled by phosphorylation of various proteins, which triggers transitions from one stage to another. There are several kinase families known, such as CDKs, Plks, and Aurora kinases, involved into regulating cell division. Collaboration between kinases and other factors forms a highly complex, but remarkably accurate system, that orchestrates the events of cell division in time and space.

Proteins from Haspin family have been found in various eukaryotes; however, Haspin kinase role in regulating cell division was mainly investigated in animal cells. The most well-known function of Haspin kinase is phosphorylation of histone H3 at Thr3, which promotes centromeric Aurora B localization to regulate chromosome segregation. Nevertheless, recent studies strongly suggest that Haspin role in regulating cell division is not restricted to the histone phosphorylation, but can include other substrates and factors, possibly related to cytokinesis (Panigada et al. 2013; Maiolica et al. 2014).

Although AtHaspin was identified as histone H3Thr3 kinase in *Arabidopsis thaliana* (Kurihara et al. 2011; Ashtiyani et al. 2011), little is known about Haspin role in plant regulatory network. Current research purpose was further investigation of Haspin kinase in plant cells to shed new light on the plant regulatory network. I combined live cell imaging and the functional analysis, to learn more about Haspin functions, substrates and their down-stream mechanisms.

In this study, NtHaspin from BY-2 tobacco cells was identified and characterized as histone H3Thr3 and Thr11 kinase. A small molecule 5-Iodotubercidin (5-ITu), which was reported as an ATP-competitive inhibitor of human Haspin kinase (Wang et al. 2012), was used to investigate NtHaspin kinase function. 5-ITu significantly reduced phosphorylation levels both *in vitro* and *in vivo.* Inhibitor treatment in BY-2 plant cell culture led to decreased mitotic index and prolonged mitosis. It was demonstrated that inhibition of NtHaspin kinase in BY-2 cells delayed chromosome alignment during prometa- and metaphase stages of mitosis. NtHaspin inhibition also prevented the centromeric localization of NtAurora3 kinase (NtAUR3) kinase and disrupted its function. This suggested that NtHaspin kinase plays a role in the specific positioning of NtAUR3 on chromosomes in plant cells, a function conserved in animals. The results also indicated that NtHaspin and NtAUR3 kinases are involved in the same pathway, which regulates chromosome alignment during prometa-/metaphase. Remarkably, NtHaspin inhibition by 5-ITu also led to severe cytokinesis defect, resulting in binuclear cells with a partially formed cell plate. While many components are involved into cell plate formation and expansion, 5-ITu treatment did not affect microtubules, AUR1/2, or the NACK-PQR pathway. However, it did alter the distribution of actin filaments on the cell plate. Although actin filaments are prominent component of the phragmoplast, their function in cytokinesis remains unclear. These results indicate that NtHaspin kinase can be a part of a novel cytokinesis-related pathway, which also includes actin filaments.

To summarize, this study showed that NtHaspin kinase is an important factor in regulating cell division in plant cells. Presumably, NtHaspin kinase has several functions in mitosis: initially, during prometa/metaphase it promotes centromeric localization of NtAUR3 kinase and contributes to chromosome alignment, further NtHaspin is involved into regulating late cell plate expansion during cytokinesis.