DOI: 10.16262/j.cnki.1005-0841.2007.02.002

Dynamics and Regulation of Actin Cytoskeleton in Plant Cells*

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The actin cytoskeleton constituted of globular actin (G-actin) is a ubiquitous component of eukaryotic cells and plays crucial roles in diverse physiological processes in plant cells, such as cytoplasmic streaming, organelle and nucleus positioning, cell morphogenesis, cell division, tip growth, etc. In response to various extracellular and intracellular stimuli, the actin cytoskeleton is in a state of rapid dynamics. It is well-known that actin-binding proteins, whose spatiotemporal activities are under the control of a variety of parameters, such as, pH, Ca2 +, phosphoinositides, and reversible protein phosphorylation, directly regulate the dynamics of the actin cytoskeleton in living cells. The research group leaded by Dr. Ren Haiyun, Professor of Beijing Normal University, has conducted several studies on the function of several actin-binding proteins in the process of cell cycle and the tip growth of plant cells using tobacco Bright Yellow-2 suspension cell and lily pollen as models. The following are the results obtained.

Different actin arrays represent the typical feature of respective cell phase. Reorganization of the actin cytoskeleton occurs during transition from one cell phase to another. To visualize the reorganization of actin cytoskeleton and the regulation of ABPs in cell cycle, a living probe for actin cytoskeleton, GFP-m Talin, was introduced into suspension cultured tobacco BY-2 cells to visualize series of typical actin configuration during the process of cell cycle. In late metaphase, spindle actin filaments gradually shrank to the equatorial plane along both long and short axes. Soon after the separation of sister chromosomes, actin filaments aligned at the cell division plane parallel to each other to form a cylinder-like structure. During cytokinesis, one cylinder-like structure changes into two cylinder-like structures associated with the growing edge of cell plate. However, the two actin arrays stay overlapping at the margin of the centrifugally growing cell plate, forming an actin wreath. This GFP-mTalin cell line enables them to study how ABPs affect cell cycle through reorganizing actin cytoskeleton. This work is published in Biology of the Cell (Yu et al., 2006).

The pollen grain is the male gametophyte of seed plants, and it transports the male gametes towards the egg cell by pollen tube tip growth, a process called pollen germination. The dynamic organization of the actin cytoskeleton plays a fundamental role in pollen germination and pollen tube growth processes. Villin/gelsolin/fragmin superfamily was a multifunctional protein group that may play an important role in the processes. By using DNase I chromatography, a novel Ca2 +-dependent actin binding protein with a molecular mass of 41 kD is isolated from lily pollen (LdABP41). N-terminal sequencing and mass-spectrometry analysis show that it shares substantial similarity with trumpet lily villin and other members of the gelsolin superfamily. Biochemical experiments indicate that LdABP41 severs polymerized lily pollen F-actin in a Ca2 + sensitive manner. They also find that it localizes in the tip region of pollen tube and may be required in controlling actin organization in the pollen tube tip by responding to the oscillatory, tip-focused Ca2 + gadient. This work is published in Plant Physiology (Fan et al., 2004).

Furthermore, they cloned a 1006 bp full-length cDNA from Lilium longiflorum that encodes a 263-amino acid predicted protein sharing 100% identity with the N-terminus of 135-ABP (Lilium villin) except for 6 C-terminal amino acids. The deduced 29-kD protein, Lilium ACTIN BINDING ROTEIN29 (ABP29), contains only the G1 and G2 domains and

* NSFC Grant No.: 30325005, 30630005, 30470176.

it is the smallest identified member of the villin/gelsolin/fragmin superfamily. The purified recombinant ABP29 accelerates actin nucleation, blocks barbed ends, and severs actin filaments in a Ca2 + and/or phosphatidylinositol 4, 5-bisphosphate regulated manner in vitro. In vivo experiments also demonstrate the severing activity of ABP29. These results suggest that ABP29 is a splicing variant of Lilium villin as well as a member of the villin/gelsolin/fragmin superfamily, which plays important roles in rearrangement of the actin cytoskeleton during pollen germination and tube growth. The work is published in the Plant Cell (Xiang et al., 2007).

In addition, they used Phenylarsine oxide (PAO) and genistein, two well-known specific inhibitors of tyrosine phosphatases and kinases, to conduct the functional analysis of protein phosphotyrosine in pollen tube. The experiments show that both PAO and genistein arrest pollen germination and pollen tube growth and lead to the malformation of the pollen tubes, although genistein has a lesser effect. The malformations of the pollen tubes caused by PAO and genistein are quite different. It is found that the rate of pollen germination and tube growth recover to a certain extent when phalloidin, an actin filament-stabilizing reagent, is present during PAO treatment, but does not when it is present during genistein treatment. Furthermore, PAO treatment also has a great effect on the dynamic organization of filamentous actin in the pollen grain and pollen tube, while genistein only causes reorganization of actin at the turning point of the pollen tube. They conclude that reversible protein tyrosine phosphorylation is a crucial step in pollen germination and pollen tube growth, but that tyrosine kinases and phosphatases may have different effects. This work is published in Protoplasma (Zi et al., 2007).

Nucleation is the rate-limiting step for actin assembly, thus the study on nucleating protein is of extremely significance. Using RT-PCR strategy, they cloned a cDNA encoding a formin-like protein (AtFH8) from Arabidopsis. AtFH8 belongs to type-I Arabidopsis formin homologue characterized in the N-terminal transmembrane domain. The purified recombinant AtFH8(FH1FH2) has the ability of nucleating actin filament assembly and partial capping the barbed end of actin filaments. In addition, it binds actin filaments and severs them into short fragments at relatively lower concentration. Overexpression of the full-length AtFH8 in Arabidopsis causes prominent changes in root hair cell development and its actin organization, suggesting it is involved in the tip growth of root hairs through actin cytoskeleton remodeling. This work is published in Plant Physiology (Yi et al., 2005).

(Quoted from NSFC Web.)