(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Yanshu ZHANG
Thesis Title	Analysis of cortical actin dynamics and its regulatory proteins in living cells		

(Thesis Summary)

Intracellular actin-based architectures play critical roles in various cellular functions and events, such as cell migration, cell division, mechanical response and tissue organization. Cell cortex is composed of a random meshwork of actin filaments and other actin-related proteins, and lies beneath the plasma membrane. Due to technical limitations, the molecular mechanism maintaining and regulating the cortical actin dynamics has been less understood than other actin-based architectures such as stress fibers. In this study, the author tried to establish a microscope technique to visualize and analyze the dynamics of individual actin filaments in a living cell cortex by utilizing high-speed atomic force microscope (HS-AFM), and applied it for functional analyses of actin-regulating proteins.

The author used a tip-scan type HS-AFM to visualize actin filaments in the cell cortex by applying 5-10 % deeper indentation of the probe into the cell to obtain higher contrast of actin filament, which is stiffer than the plasma membrane. This allowed visualization of individual actin filaments in various cell types such as COS7, NIH-3T3, C2C12, ST2 and XTC. The detailed analyses of obtained AFM images elucidated several important kinetic parameters in a living COS-7 cell such as elongation rate and the frequency of new filament assembly. By using inhibitors of actin and its regulator proteins (cytochalasin D, SMIFH2, CK666) and overexpression of actin-binding proteins (profiling and thymosin β4), a dynamic equilibrium between G-actin and F-actin in the cortex has been elucidated.

Combining this system with the other microscopic techniques, the author tried to examine how transcription co-factor YAP, a well-known mechano-responsive regulator, controls cortical actin structures. HS-AFM analyses and other mechanical characterizations of YAP-knock out cells revealed that, the depletion of YAP increased the density of actin filaments, the stiffness of the cell cortex, and resistance against external damages. Additional biochemical and microscopic analyses suggested the involvement of E-cadherin, whose expression is regulated by YAP, in recruiting one of the Rho GTPase-activating enzymes, ARHGAP18, to the cell cortex, which inactivates RhoA. All of these results demonstrated a linkage between transcriptional control by YAP and structural changes of cortical actin via Rho GTPases, and demonstrated that HS-AFM-based visualization approach is a useful tool to analyze the dynamics of cortical actin network and the function of regulatory proteins.

(Form 2)

（Thesis Evaluation Summary）

Actin forms a variety of architectures within a cell. The cell cortex is composed of a random network of actin filaments, and regulates cellular morphology and mechanics in many cellular processes. Despite such important roles, the mechanism of how the cortical actin is regulated by other cellular proteins remains to be elucidated due to a lack of the technique to characterize individual actin filaments. In this study, the author aimed to establish a new microscopic approach to visualize and analyze individual actin filaments in the cell cortex of a living cell, and then applied it for characterizing its regulatory mechanisms.

In the first part (Chapter 2), the author utilized a tip-scan type of high-speed atomic force microscopy, to achieve high-contrast imaging of the cortical actin. This advancement greatly improved the spatial resolution of individual filaments, and successfully obtained important structural and kinetic parameters, such as the elongation rate and the initiation rate. This method has the advantage because it does not require any staining, labeling or expression of fluorescently labeled proteins in the target cell, but still resolves the dynamics of a single actin filament in a living cell.

In the following part (Chapter 3), the author applied this imaging technique to examine the mechanism of how cortical actin is regulated by YAP, which is a transcription co-factor responding to various mechanical stimuli and regulating a number of actin-related genes. The author analyzed the structure of the cell cortex in YAP-knockout cells, and demonstrated that YAP regulates the expression of a membrane-bound protein, which results in the activation of a RhoA in the cell cortex. These results clarified the linkage between YAP-dependent gene regulation and the structure of the cell cortex via small GTPase activity, and also demonstrated that the new imaging technique that the author established here can work as a new tool to analyze the dynamics of cortical actin.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of cell biology, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On January 28th, 2021, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

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Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2)　　:　mm　dd　, yyyy