(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	MAAROF Nur Diyana Binti
Thesis Title	Multisite phosphorylation regulates actin-binding and -bundling activities of MISP/Caprice		

(Thesis Summary)

An intracellular network of cytoskeletal filaments contributes to the morphological framework of a cell, and participates in the dynamic regulation of cellular activities. Precise regulation of the intracellular actin architectures by numerous actin-binding proteins is crucial for the maintenance of cellular activities, and its abnormalities are directly linked to pathological disorders. Mitotic interactor and substrate of Plk1 (MISP) is an actin-bundling protein, and plays critical roles in the proper orientation of mitotic spindles and the organization of actin-based architectures in interphase cells. MISP is also known as a disordered protein that contains multiple phosphorylation sites. Although it was previously demonstrated that the function of MISP is regulated by cell cycle-dependent phosphorylation, much less is known about how the effects of individual phosphorylation are integrated to control the actin-bundling activity. In this study, phosphorylation-dependent regulation of actin-bundling activity of MISP was investigated.

The co-immunoprecipitation assay demonstrated that MISP contained at least two independent actin-binding sites at the C-terminal region (amino acid residues 352-524 and 525-680). The analysis using Phos-tag gel demonstrated that the C-terminal region, but not the N-terminal region, was phosphorylated in interphase cells. This region contains 24 potential phosphorylation sites registered in PhosphoSitePlus™. Based on the probability score, fifteen residues were chosen and subjected to further analyses. Simultaneous substitutions of all 15 residues almost completely abolished phosphorylation, which is judged by Phos-tag gel, indicating that at least some of them are phosphorylated in interphase cells. Next, the effect of the mutations on the actin-based architectures was examined in HeLa cells. The overexpression of full-length MISP induced stress fiber formation, whereas the mutant which carries 15 mutations did not. A series of single point-mutants of individual phosphorylation sites was constructed, expressed in HeLa cells, and examined for the ability of stress-fiber induction. Out of 15 mutants, S394A, S395A, and S400A failed to induce the stress fiber in interphase cells, indicating that phosphorylation of all the three residues is required for the stress fiber formation. To elucidate this mechanism, actin-bundling activity of the wild-type and the mutants were examined by pull-down assay. Phosphomimetic mutation of S394, S395 and S400 promoted the actin-bundling activity. Simultaneous mutations of all the three residues further promoted the bundling activity, suggesting that the effect of phosphorylation is additive. Finally, the effect of possible mitotic phosphorylation sites were examined. Out of 12 residues tested, several phosphomimetic mutants including S376D and S471D exhibited lower actin-binding activity, indicating that mitotic phosphorylation reduced the affinity of MISP to actin. All of these results demonstrated that the actin-bundling activity of MISP is positively and negatively regulated by different sets of phosphorylation sites during the cell cycle.

(Form 2)

（Thesis Evaluation Summary）

The candidate studied the molecular mechanism of how actin-binding and -bundling activities of MISP is regulated by multiple phosphorylation during the cell cycle. MISP is one of the actin-binding proteins that contains a large portion of disordered region and undergoes hyperphosphorylation at more than 50 residues. MISP plays an important role in the regulation of intracellular actin-based architectures, including stress fibers in interphase cells and the cell cortex in mitotic cells. Therefore, the molecular mechanism of the functional regulation of MISP by multiple phosphorylation is of particular interest not only in the research field of cell biology, but also in structural biology.

The candidate constructed a series of point-mutants (non-phosphorylatable and phosphomimetic), and investigated their actin-binding and -bundling activities both *in vivo* and *in vitro*. Based on these analyses, she identified three residues (S394, S395 and S400) which up-regulate the actin-bundling activity and the stress-fiber formation upon phosphorylation, as well as the residues (S376, S471, and others) which down-regulate the actin-binding activity upon mitotic phosphorylation. These results clearly demonstrated that the function of MISP is regulated by different sets of phosphorylation during the cell cycle. By combining some bioinformatics analyses, she proposed that a negative charge cluster introduced by multiple phosphorylation enhanced the inter-molecular interaction of MISP, which resulted in the promotion of the actin-bundling and the stress fiber formation. This achievement contributed to the understanding of the regulatory mechanism of actin-based architectures, and the mechanism of how multiple phosphorylation regulates the function of a disordered protein.

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 July 12th, 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