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学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	Analysis of the Physiological Function of Scd6 through its Interaction and Methylation by Hmt1（Hmt1との相互作用とメチル化によるScd6の生理学的機能の解析）

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論文の内容の要旨
Abstract of thesis

【背景　Background】

Scd6, yeast homologue of hRAP55, was previously shown to act as a translational repressor and a decapping activator. In this report, arginine residues within its RGG motif in Scd6 were found to be methylated in a manner dependent on Hmt1, an arginine methyltransferase. Scd6 localization to the Processing bodies (P-bodies) was impaired by either deletion of *hmt1* or mutation that was introduced in the RGG motif of Scd6. Scd6 showed synthetic effects with Dhh1 on P-body formation and cell growth. The arginine methylation might negatively regulate Scd6 function toward cell growth at elevated temperature. The applicant elucidated that Hmt1-based arginine methylation is required for the physiological function of Scd6.

【目的　Purpose】

The major pathway of mRNA degradation in eukaryotes is initiated by deadenylation, followed by decapping, then 5′ to 3′ degradation. The decapping machinery can be concentrated in cytoplasmic processing bodies (P-bodies), where translationally repressed mRNPs and untranslated mRNAs accumulate. One of decapping activators, Scd6, was previously shown to repress translation, activate decapping *in vitro* and localizes to P-bodies. However, it remains unknown how Scd6 function to activate decapping and in the P-bodies. To resolve this issue, the applicant aimed to identify interacting factors of Scd6 and investigated for further analyses. Moreover, the applicant examined the

genetic and functional interactions of Scd6 with other decapping activators.

【対象と方法　Materials and methods】

Escherichia coli DH5α was used for DNA manipulations. *Saccharomyces cerevisiae* strains used in this study were derived from W303. Gene disruption and insertion were performed using PCR-based gene replacement. Yeast two-hybrid assay, immunoprecipitation (IP), and *in vitro* binding assay were used to detect interaction between Scd6 and its binding partners. For arginine methylation mapping of Scd6, Immunopurified Scd6Flag was analyzed by tandem mass spectrometry. For localization experiments, mCherry- , GFP- or mRFP-fused proteins were detected by fluorescent microscopy.

【結果　Results】

The applicant performed yeast two-hybrid screening of yeast cDNA library using Scd6 as bait and *in vitro* binding assays indicated that Scd6 interacts with Hmt1, an arginine methyltransferase. The *scd6* deletion impaired the formation of P-bodies marked by Dcp2-GFP under glucose starvation, and Scd6 overexpression induced P-bodies formation without stress induction. Neither methylation-deficient nor -mimic substitutions of Scd6 affected Scd6 overexpression-induced P-body formation, suggesting that Hmt1-dependent arginine methylation of Scd6 is important for its P-body targeting, but not required for its overexpression-driven induction of P-body formation. Overall, the applicant suggested that arginine methylation may regulate Scd6 function on cell growth upon environmental stimuli such as heat shock.

【考察　Discussion】

In the present study, the applicant demonstrated that Hmt1 associates with and methylates Scd6. Arginine methylation led to efficient targeting of Scd6 to P-bodies, and negatively regulates Scd6 function toward cell growth at elevated temperature. The applicant suggests that Scd6 was methylated not only by Hmt1 but also by another methyltransferase. Taken together, the applicant suggests that post-translational modification by arginine methyltransferases would be the key factor in understand functional and dynamic link among mRNP components.

【結果　Conclusion】

The applicant shows that physiological function of Scd6 is regulated by arginine methylation. The modification is important for cellular localization, interaction with decapping activators, and cell growth. The applicant's study provides a new insight into the regulation of mRNP components mediated by post-translational modification.

審査の結果の要旨
Abstract of assessment result

【批評　General Comments】

In the doctoral thesis, the applicant showed that Hmt1 associates with and methylates Scd6. However, molecular details of these arginine methylation-based regulations remain unknown. Hmt1 may affect either specificity or the binding affinity of Scd6 to mRNAs targets. Future studies are also needed to determine if Scd6 methylation is a reversible, especially under environmental stimuli.

【最終試験の結果　Assessment】

The final examination committee conducted a meeting as a final examination on June 13, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the

committee members reached a final decision that the applicant has passed the final examination.

【結果　Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medial Sciences.