氏　　名	NGUYEN THI MINH VIET
学位の種類	博士（医学）
学位記番号	博甲第　９２９６　号
学位授与年月	令和元年９月２５日
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	Post-transcriptional regulation of gene expression by the RNA-binding protein Puf5 and the Ccr4-Not complex in yeast （酵母における RNA 結合タンパク質 Puf5 と Ccr4-Not 複合体による遺伝子発現の転写後制御）
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Nguyen Thi Minh Viet describes the physiological roles of Puf5 and Pop2 as well as how they are regulated in response to glucose availability.

The summary is as follows:

（目的　Purpose）

The Ccr4-Not complex is an essential multi-subunit complex that is conserved from yeast to human and plays an important role in regulation of mRNA decay. Puf5 is a broad mRNA regulator that interacts with more than 1,000 mRNAs and is involved in multiple cellular processes. Puf5 binds to the recognition sequences in the 3'UTR of their target transcripts and recruits the Ccr4-Not complex through the interaction with Pop2 subunit. However, the physiological roles of Puf5 and Pop2 as well as how these two proteins are regulated remain unknown. Therefore, the author aimed to analyze the regulation of Puf5, in concert with Ccr4 and Pop2 deadenylases, on *LRG1* expression in CWI pathway and their regulation by glucose availability, focusing on glucose-regulated phosphorylation and its functional consequences.

（対象と方法　Materials and Methods）

The author used *Escherichia coli DH5α* for DNA manipulations and *Saccharomyces cerevisiae* strains were used for gene disruption and insertion based upon the PCR-based gene replacement method. To perform western blots, extracts were subjected to SDS-PAGE on acrylamide gels followed by electroblotting onto an Immobilon membrane. Phos-tag was added to a mix of SDS-PAGE gel when required. The membrane was incubated with antibody to detect protein. For RT-qPCR, first strands of cDNA were generated from isolated RNA. The cDNAs of *LRG1*, *HSP12*,

HSP26, and *PIR3* were quantitated by a quantitative real-time PCR method. The microarray analysis was performed by the KURABO Bio-Medical Department using the Affymetrix GeneChip Yeast Genome 2.0 Array. Finally, the poly(A) tail length of *LRG1* mRNA was measured by PCR-based poly (A) tail length assay and sequencing of its product..

（結果　Results）

The author analyzed the effect of Puf5 on *LRG1* expression, together with Ccr4 and Pop2 deadenylases, Dhh1 decapping activator, and other PUF proteins. She found that the growth defect of *puf5*Δ mutant was enhanced by *ccr4*Δ mutation, which was partially suppressed by *LRG1* deletion. Consistently, Lrg1 protein level was much more up-regulated in *ccr4*Δ *puf5*Δ double mutant than in each single mutant. Interestingly, *LRG1* poly(A) tail length was longer in *ccr4*Δ mutant but not in *puf5*Δ mutant. She unexpectedly found that *puf6*Δ mutation suppressed the growth defect caused by *ccr4*Δ *puf5*Δ mutation. Loss of ribosomal proteins Rpl43a and Rpl43b, the previously identified Puf6 interactors, also suppressed the growth defect of *ccr4*Δ *puf5*Δ mutant.

She then went on to examine the phosphorylation of Puf5 under 2 different conditions, medium containing glucose as a carbon source (glucose medium) and medium containing non-fermentable glycerol and lactate (gly-lac medium) as carbon sources, and observed different mobility shifts of Puf5 in two conditions. Additionally, Puf5 phosphorylation was dependent on two kinases, Snf1 and Pho85, since Puf5 phosphorylation was significantly decreased in *snf1Δ* and *pho85Δ* mutants in both conditions. Overexpression of *PUF5* efficiently suppressed the growth defect of *puf5Δ* mutant at high temperature, but overexpression of *PUF5* harboring phospho-defective mutations did not, and phospho-defective *PUF5* displayed stronger repression of *HO* repoter mRNA.

The author found that Pop2 was phosphorylated at serine 39 (S39) under unstressed conditions. The dephosphorylation of S39 occurred within 1 min after glucose depletion, and the addition of glucose to the glucose-deprived culture recovered this phosphorylation. Pop2 phosphorylation at S39 was involved in the expression of *HSP12* and *HSP26*, encoding small heat shock proteins. In medium supplemented with glucose, Pop2 might be phosphorylated at S39 by Pho85 kinase to repress the expression of *HSP12* and *HSP26*. Glucose starvation inactivated Pho85, which resulted in the derepression of *HSP12* and *HSP26*.

（考察　Discussion）

The author's results suggest that Puf5 regulates *LRG1* mRNA in a manner that is independent of deadenylation and Puf6 is also likely to be involved in cell growth regulation of Ccr4 and Puf5. In her results, multiple migrations of Puf5 with different patterns in glucose and glucose-deprived conditions were observed, indicating possible regulations by protein modifications in this process. Moreover, Pop2 is phosphorylated in a Pho85-dependent manner, and this phosphorylation contributes to the suppression of stress response genes, *HSP12* and *HSP26*. These results suggest an important role of Pop2 in stress responses in yeast.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The author's results provide valuable insights into the roles of two PUF RNA binding proteins, Puf5 and Puf6, in the growth of yeast cells. Her experiments utilized classic yeast genetics combined with modern whole-genome approaches, and were performed in an excellent manner. The results in this thesis implicate protein phosphorylation as a possible molecular mechanism by which Puf5 regulates *LRG1* mRNA. This mechanistic insight provides a direction for future studies, which should investigate the regulatory pathway of the phosphorylation and identify other mRNAs regulated by Puf5 in deadenylation independent-mechanisms. Overall, her work lays a solid foundation for further research on mRNA regulation in yeast cells.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on May 30, 2019. 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.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.