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学位の種類	博士（　医学　）
学位記番号	博甲第　　８８４７　　号
学位授与年月	平成　３０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	Cytoplasmic deadenylase Ccr4 is required for translational repression of *LRG1* mRNA in the stationary phase in *Saccharomyces cerevisiae* （ポリ A 分解酵素 Ccr4 は定常状態での LRG1 mRNA の翻訳抑制に関与する）
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The report of the Assessment of Mr. DUONG LONG DUY's Dissertation Defense

論文の要旨
Abstract of thesis

In this thesis, the author first described the roles of the poly(A) tail of mRNA in its stability and translation control. The relationship of poly(A) tail length and translation remains still ambiguous and it is unclear whether increasing poly(A) tail length would increase the efficiency of translation. In *Saccharomyces cerevisiae*, Ccr4 is the major cytoplasmic deadenylase that shortens the poly(A) tail of mRNAs. A previous report showed that an increase of poly(A) tail length of septin mRNA, which was caused by deletion of *CCR4*, does not affect the septin protein level in the log phase. To date, there is little evidence that supports the idea that poly(A) tail length positively correlates with translation efficiency. It has been also reported that Ccr4 negatively regulates expression of *LRG1* mRNA encoding a GTPase-activating protein (GAP) for Rho1 small GTPase, a component of cell wall integrity pathway, and deletion of *LRG1* suppresses the temperature sensitive growth defect of *ccr4*Δ mutant.

In this study, the author chose *LRG1* to examine the relationship of the poly(A) tail length and the efficiency of translation, and also to test the effect of Ccr4 on *LRG1* poly(A) tail length and its protein level in the log phase as well as in the stationary phase. In addition, the author aimed to clarify the involvement of Pbp1 in regulation of *LRG1* poly(A) tail length and the translation efficiency, because the slow growth and temperature sensitivity of the *ccr4Δ* mutant is suppressed by deletion of the *PBP1* gene. *PBP1* encodes a poly(A)-binding protein (Pab1)-binding protein 1, which is involved in regulation of poly(A) tail length by inhibiting the Pan2-Pan3 deadenylase complex.

The author employed PCR-based methods to generate yeast mutants, and the yeast mutants were grown in

liquid media from the log phase up to the stationary phase. The growth phases were determined based on cell density, glucose concentration, and ethanol concentration of the culture throughout the time course. Total RNAs were isolated from WT cells and mutant cells at the indicated time points and used for examining the *LRG1* mRNA level and its poly(A) tail length by qPCR and poly(A) tail length assay, respectively. The Lrg1 protein was fused with 3xFLAG tag at the N-terminus in order to detect its expression level by western blotting using an anti-FLAG antibody. The translational activities of the cells in different growth phases were analyzed by polysome analysis, and then RT-PCR was performed to detect the *LRG1* mRNA in polysome fractions. In addition to *LRG1*, the mRNA levels of other targets of Puf5 including *MCM2*, *MCM4*, *MCM7*, and *ELM1* were also examined.

The author showed that, in the log phase *ccr4*Δ mutant cells, the *LRG1* poly(A) tail length was longer and the *LRG1* mRNA level was increased more than that in wild-type (WT) cells. He observed, however, that the Lrg1 protein level was similar to that in WT cells. In the stationary phase, the *LRG1* poly(A) tail length was still longer, and the *LRG1* mRNA level was still higher in the *ccr4*Δ mutant as compared to those in WT cells. In addition, the Lrg1 protein level in the *ccr4*Δ mutant was much higher than that in WT cells in the stationary phase. The relative ratio of the Lrg1 protein level to the *LRG1* mRNA level was higher in *ccr4*Δ mutant cells than that in WT cells in the stationary phase, suggesting that Ccr4 represses the translation of *LRG1* in the stationary phase. The author also found that the number of actively translating ribosomes was diminished in WT cells but remained high in *ccr4*Δ mutant cells, which may cause the aberrant translation of *LRG1*. Loss of *PBP1* reduced the *LRG1* poly(A) tail length as well as the *LRG1* mRNA and protein levels in the stationary phase *ccr4*Δ mutant cells but did not suppress the aberrant translation. The author also observed similar expression patterns with other targets of Puf5, including *MCM2*, *MCM4*, *MCM7*, and *ELM1*.

In summary, the author provides the evidence that *LRG1* poly(A) tail length positively correlates with *LRG1* mRNA and protein levels in the stationary-phase cells, but not in the log-phase cells, in the yeast *Saccharomyces cerevisiae*. His study also suggests that cytoplasmic deadenylase Ccr4 is required not only for translational repression of Puf5 target genes but also for global translational repression in the stationary phase when the amount of nutrients is low condition. Thus, the author concludes that Ccr4 and Pbp1 play important roles in the post-transcriptional control of gene expression through regulating the poly(A) tail length and translational efficiency in the stationary-phase cells.

審査の要旨
Abstract of assessment result

【批評 Review】

The author showed that the poly(A) tail length of the *LRG1* mRNA positively correlates with *LRG1* mRNA and protein levels in the stationary-phase yeast cells. His research also revealed functional roles for Ccr4 and Pbp1 in regulating the efficiency of translation when yeast cells are in the stationary phase. These investigations by the author provide novel findings into the role of translational regulation in yeast cells, especially in the stationary phase when the amount of nutrients is limited. The author utilized elegant genetic analyses as well as some biochemical assays to provide insights into translational regulation, and his findings raise interesting questions to be addressed in future research in this field.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on June 12, 2018. 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.