氏　　名	Valderrama, Arvin Lapiz
学位の種類	博士（人間生物学）
学位記番号	博甲第　10133　号
学位授与年月	令和　3　年　9　月　24　日
学位授与の要件	学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Pbp1 mediates the aberrant expression of genes involved in growth defect of *ccr4*Δ and *pop2*Δ mutants in yeast *Saccharomyces cerevisiae* (Pbp1 は *ccr4*Δ および *pop2*Δ 変異株の増殖不全に関わる遺伝子発現の異常を引き起こす)

	（職名）	（学位）	（氏名）
主　　査	筑波大学教授	博士（医学）	川口　敦史
副　　査	筑波大学助教	博士（薬学）	船越　祐司
副　　査	筑波大学准教授	博士（薬学）	木村　圭志
副　　査	筑波大学教授	博士（理学）	入江　賢児

論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Mr. Arvin Lapiz Valderrama describes the functional interaction between Ccr4/Pop2 and Pbp1, which are proteins important for RNA metabolism. The summary is as follows:

（目的　Purpose）

CCR4 and *POP2* genes encode the catalytic subunits of the Ccr4-Not complex involved in shortening mRNA poly(A) tail in *Saccharomyces cerevisiae*. These genes are evolutionary conserved from yeast to human and play crucial roles in the regulation of gene expression. It has been known that the *ccr4*Δ and *pop2*Δ mutants exhibit pleiotropic phenotypes such as slow and temperature sensitive growth and abnormal cell wall synthesis. The growth defect of the *ccr4*Δ and *pop2*Δ mutants is caused by aberrant expression of Lrg1, a GTPase activating protein of Rho1, which is the master regulator of the cell wall integrity (CWI) pathway. Although the abnormal phenotypes are suppressed by deletion of the *PBP1* gene, which encodes poly(A)-binding protein (Pab1) binding protein 1, the underlying mechanism for this phenotype suppression has remained unclear. The author investigated the functional relationship between Ccr4/Pop2 and Pbp1.

（**対象と方法**　Materials and Methods）

To elucidate the influence of *PBP1* deletion on the pleiotropic phenotypes in *ccr4*Δ and *pop2*Δ mutants, the changes in gene expression in *ccr4*Δ and *pop2*Δ single mutants and *ccr4*Δ *pbp1*Δ and *pop2*Δ *pbp1*Δ double mutants were measured. Microarray analysis revealed specific genes that were regulated by Pbp1.

（**結果**　Results）

The author confirmed that the growth defect of the *pop2*Δ mutant was recovered by deleting the *PBP1* gene. To identify genes involved in the pleiotropic phenotypes caused by *ccr4*Δ and *pop2*Δ mutations, the author carried out the microarray analysis with wild-type, *ccr4*Δ, and *ccr4*Δ *pbp1*Δ mutants. The expressions of various environmental sensing and stress response genes, including *HSP12*, *HSP26*, *PIR3*, *FUS1*, and *GPH1*, increased in *ccr4*Δ mutant. The author confirmed that the expression of these genes was also upregulated in the *pop2*Δ mutant. The author also found that the *pbp1*Δ mutation not only restored the growth defect but also reduced the increased expressions of these genes observed in the *ccr4*Δ and *pop2*Δ mutants. The author next constructed the *ccr4*Δ mutant overexpressing *PBP1*, and the expression levels of *HSP12*, *HSP26*, *PIR3*, and *FUS1* were upregulated, while the expressions of *GPH1* and *LRG1* were not increased. The cell growth was also exacerbated by *PBP1* overexpression in the *ccr4*Δ mutant. To further understand the molecular mechanism, the author focused on Msn2 and Msn4, which are transcription factors for *HSP12* and *HSP26* genes. The author revealed that the gene expressions of *HSP12* and *HSP26* increased in the *ccr4*Δ mutant, but decreased in the *ccr4*Δ *msn2*Δ *msn4*Δ mutant, indicating that the expressions of *HSP12* and *HSP26* in the *ccr4*Δ mutant strain are dependent on Msn2 and Msn4. Further, the transcriptional regulation of *HSP12* and *HSP26* genes was regulated by Ccr4 and Pop2 in a Pbp1-dependent manner. Based on these findings, the author proposed that Pbp1 regulates the function of Ccr4-Not complex through modulating its deadenylase activity and an unknown transcriptional mechanism.

（**考察**　Discussion）

The author shows that the pleiotropic phenotypes of the *ccr4Δ* and *pop2*Δ mutants are caused by the abnormal gene expression via Pbp1. It has been reported that the poly(A) tail of mRNA is longer in the *ccr4*Δ mutant compared to the wild-type. This longer poly(A) chain could have accommodated the binding of more of Pab1 and Pbp1 species, resulting in abnormal protein synthesis. The previous study reported that the Lrg1 protein level is up-regulated in the stationary-phase *ccr4*Δ mutant cells, but not in the log-phase *ccr4*Δ mutant cells, and that loss of *PBP1* reduces the Lrg1 protein levels. In this study, the author also showed that the Lrg1 protein level is also up-regulated in the stationary-phase *pop2*Δ mutant cells, and that loss of *PBP1* reduced the Lrg1 protein levels. Thus, the author thought the possibility that the abnormal protein synthesis caused by their longer poly(A) tails and excess recruitment of Pab1 and Pbp1 proteins leads to the increased expression of *HSP12, HSP26, PIR3,* and *FUS1*. Since Lrg1 is not involved in the increased expression of *HSP12, HSP26, PIR3, and FUS1,* the increased gene expression is not a consequence of a change in Rho1 signaling, which is negatively regulated by Lrg1. The author speculate that additional proteins accumulate in the *ccr4*Δ and *pop2*Δ mutants, which increase the expression of *HSP12, HSP26, PIR3,* and *FUS1*. The author's findings do not explain all the actions of the Ccr4-Not complex together with Pbp1. By taking Pbp1 into consideration, the detailed mechanism of action of the Ccr4-Not complex that has been a mystery

may become clear.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

This study found that, in addition to *LRG1* gene which is involved in the CWI pathway regulation, a subset of genes involved in environmental sensing and stress response is aberrantly expressed in *ccr4*Δ and *pop2*Δ mutants. This abnormal expression is facilitated by Pbp1, a poly(A)-binding protein (Pab1) binding protein, and Pbp1 contributes to the pleiotropic phenotypes of the *ccr4*Δ and *pop2*Δ mutants. Ccr4 and Pop2 are evolutionary conserved key enzymes in RNA metabolism, and it is important to understand their physiological roles and the regulation of the activities of Ccr4 and Pop2. Thus, this study is highly expected to have a certain impact in this field.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on June 18th, 2021. 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 Human Biology.