

Title	Breeding of <i>Saccharomyces cerevisiae</i> with high RNA content by molecular and genome engineering technology
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Osaka University

Synopsis of Thesis

Title: Breeding of *Saccharomyces cerevisiae* with high RNA content by molecular and genome engineering technology

(リボ核酸高含有出芽酵母の分子およびゲノム工学育種)

Name of Applicant Fahmida Khatun

Chapter 1: General Introduction

Saccharomyces cerevisiae is an important microorganism as preferred source of ribonucleic acid (RNA)-related compounds such as 5'-GMP (5'guanosine monophosphate) and 5'-IMP (5'-inosine monophosphate) which are used as flavor enhancers and food additives. Because main source of RNA in *S. cerevisiae* cells is ribosomal RNA (rRNA), yeast strains producing large amounts of rRNA are desired. Transcription of rRNA genes in yeast is known to be regulated by Pol I pre-initiation complex which consists of four factors such as upstream activation factor (UAF), core factor (CF), TATA-binding protein (TBP), and Rrn3p. *RRN10* gene, one of UAF complex subunits, is indispensable to promote high level transcription of rRNA gene. Although the Δ *rrn10* disruptant is viable, mutant showed severe defects on growth phenotype and RNA content. Seven suppressor strains (SupA-SupG) were isolated which showed the ability to suppress the defects caused by *rrn10* disruption (Chuwattanakul et al. 2011). In this study, I investigated the suppression mechanism of one of a suppressor strains, named SupE, in which mutation is dominant and consist of multiple mutations collectively designated as *SUPE*. I found that *SUPE* mutation showed the ability to increase the transcription level of rDNA gene and assumed to affect feedback inhibition of rDNA transcription which in turn increase the total RNA content in *S. cerevisiae*.

Chapter 2: Increased transcription of *RPL40A* and *RPL40B* is important for the improvement of RNA production in *S. cerevisiae*

Efficient transcription of rDNA is very important to construct *S. cerevisiae* strains with high RNA content. To get more insight into the suppression mechanism in the SupE strain, a gene library was constructed from the SupE strain and introduced into the Δ *rrn10* disruptant strain. I found that *RPL40A* gene, a protein of large subunit of ribosome, conferred suppression effect to the Δ *rrn10* disruptant strain. Since there were no base changes in this gene as compared with the parental Δ *rrn10* strain, it was suggested that an additional copy of *RPL40A* suppress the defects caused by Δ *rrn10* disruption. When multiple copies of *RPL40A* were combined with *SUPE* mutation on an *RRN10*⁺ background, the resultant SupE strain displayed 92% higher RNA content than wild-type strain. I also found that *SUPE* mutation increased the transcription of ribosomal protein genes such as *RPL40A* and *RPL40B*. Increased transcription of both genes helped to recover the defects of rDNA transcription and increase RNA content in the SupE strain.

Chapter 3: Increase in rRNA content in an *S. cerevisiae* Δ *rrn10* suppressor strain by rDNA cluster duplication

To investigate the effect of increased copy number of the rDNA gene under Δ *rrn10* *SUPE* background, I constructed a SupE strain (SupE-2X) and a wild-type strain (WT-2X) with two copies of the rDNA cluster (ca. 300 rDNA genes) by chromosome splitting technology. Transcription level of the rDNA genes was increased in the SupE-2X although WT-2X did not show any significant difference irrespective of their rDNA copy number. Total RNA content of the SupE-2X strain was increased by 41% compared to that of WT-2X strain. Further increase of 47% in RNA content was achieved by multicopy expression of the *RPL40A* gene in the SupE-2X

strain. These observations suggest that *SUPE* mutation is effective to increase the transcription level of the rDNA gene in an *S. cerevisiae* strain with two copies of the rDNA cluster under $\Delta rrn10$ *SUPE* background. I also measured RNA content of the SupE-2X strain on *RRN10⁺* background and the resultant strain showed 95% higher RNA content compared to the WT-2X with *RRN10⁺* background. The above results suggest that the strategy of increasing rDNA copy number under $\Delta rrn10$ *SUPE* background provides an effective approach to construct *S. cerevisiae* strain with high RNA content.

Chapter 4: General discussion and conclusion

Here I described the effect of *SUPE* mutation on transcription level of rDNA gene and also on the increased copy number of the rDNA gene. I obtained increased rRNA level in the strain having $\Delta rrn10$ *SUPE* background. Although the identities of genes involved in *SUPE* mutation are still unknown, we assumed that following mechanisms might be involved in *SUPE* mutation. The first possible mechanism is that suppressor mutation might cause the relaxation of chromatin structure and consequently increased the accessibility of Pol I to promoter of rDNA thereby accelerating rDNA transcription. The second possibility is that *SUPE* mutation might occur in other components of UAF which largely influences the transcription level of the rDNA gene. The third possibility is that *SUPE* mutation might occur in some genes related with rDNA transcription which affects the feedback inhibition mechanism of rDNA transcription. It is known that Pol I mediated rDNA transcription is a finely tuned process with highly cooperative nature of the interactions among rDNA transcription, rRNA processing and others factors related to rDNA transcription, I assumed that the cumulative effects of all these components under $\Delta rrn10$ *SUPE* background increase the total RNA content in the SupE strain. In conclusion, utilization of *SUPE* mutation to construct *S. cerevisiae* strain with increased rDNA transcription and increased rDNA copy number are significantly effective and the knowledge of *SUPE* mutation will be useful for further improvement of RNA content in *S. cerevisiae*.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Saccharomyces cerevisiae is an important microorganism as preferred source of ribonucleic acid (RNA)-related compounds such as 5'-GMP (5'guanosine monophosphate) and 5'-IMP (5'-inosine monophosphate) which are used as flavor enhancers and food additives. Because main source of RNA in *S. cerevisiae* cells is ribosomal RNA (rRNA), yeast strains producing large amounts of rRNA are desired. Transcription of rRNA genes in yeast is known to be regulated by Pol I pre-initiation complex which consists of four factors such as upstream activation factor (UAF), core factor (CF), TATA-binding protein (TBP), and Rrn3p. *RRN10* gene, one of UAF complex subunits, is indispensable to promote high level transcription of rRNA gene. In chapter 1, Fahmida reviewed the previous findings that the Δ *rrn10* disruptant is viable, mutant showed severe defects on growth phenotype and RNA content. Seven suppressor strains (SupA-SupG) were isolated which showed the ability to suppress the defects caused by *rrn10* disruption (Chuwattanakul et al. 2011). In this study, Fahmida investigated the suppression mechanism of one of a suppressor strains, named SupE, in which mutation is dominant and consist of multiple mutations collectively designated as *SUPE*. She found that *SUPE* mutation showed the ability to increase the transcription level of rDNA gene and assumed to affect feedback inhibition of rDNA transcription which in turn increase the total RNA content in *S. cerevisiae*.

Efficient transcription of rDNA is very important to construct *S. cerevisiae* strains with high RNA content. To get more insight into the suppression mechanism in the SupE strain, a gene library was constructed from the SupE strain and introduced into the Δ *rrn10* disruptant strain. In chapter 2, Fahmida found that *RPL40A* gene, a protein of large subunit of ribosome, conferred suppression effect to the Δ *rrn10* disruptant strain. Since there were no base changes in this gene as compared with the parental Δ *rrn10* strain, it was suggested that an additional copy of *RPL40A* suppress the defects caused by Δ *rrn10* disruption. When multiple copies of *RPL40A* were combined with *SUPE* mutation on an *RRN10*⁺ background, the resultant SupE strain displayed 92% higher RNA content than wild-type strain. She also found that *SUPE* mutation increased the transcription of ribosomal protein genes such as *RPL40A* and *RPL40B*. Increased transcription of both genes helped to recover the defects of rDNA transcription and increase RNA content in the SupE strain. In chapter 3, Fahmida investigated the effect of increased copy

number of the rDNA gene under $\Delta rrn10$ *SUPE* background, she constructed a SupE strain (SupE-2X) and a wild-type strain (WT-2X) with two copies of the rDNA cluster (ca. 300 rDNA genes) by chromosome splitting technology. Transcription level of the rDNA genes was increased in the SupE-2X although WT-2X did not show any significant difference irrespective of their rDNA copy number. Total RNA content of the SupE-2X strain was increased by 41% compared to that of WT-2X strain. Further increase of 47% in RNA content was achieved by multicopy expression of the *RPL40A* gene in the SupE-2X strain. These observations suggest that *SUPE* mutation is effective to increase the transcription level of the rDNA gene in an *S. cerevisiae* strain with two copies of the rDNA cluster under $\Delta rrn10$ *SUPE* background. She also measured RNA content of the SupE-2X strain on *RRN10*⁺ background and the resultant strain showed 95% higher RNA content compared to the WT-2X with *RRN10*⁺ background. The above results suggest that the strategy of increasing rDNA copy number under $\Delta rrn10$ *SUPE* background provides an effective approach to construct *S. cerevisiae* strain with high RNA content.

In chapter 4, Fahmida discussed the effect of *SUPE* mutation on transcription level of rDNA gene and on the increased copy number of the rDNA gene. She obtained increased rRNA level in the strain having $\Delta rrn10$ *SUPE* background. Although the identities of genes involved in *SUPE* mutation are still unknown, she proposed that following mechanisms might be involved in *SUPE* mutation. The first possible mechanism is that suppressor mutation might cause the relaxation of chromatin structure and consequently increased the accessibility of Pol I to promoter of rDNA thereby accelerating rDNA transcription. The second possibility is that *SUPE* mutation might occur in other components of UAF which largely influences the transcription level of the rDNA gene. The third possibility is that *SUPE* mutation might occur in some genes related with rDNA transcription which affects the feedback inhibition mechanism of rDNA transcription. Pol I mediated rDNA transcription is a finely tuned process with highly cooperative nature of the interactions among rDNA transcription, rRNA processing and others factors related to rDNA transcription, it was assumed that the cumulative effects of all these components under $\Delta rrn10$ *SUPE* background increase the total RNA content in the SupE strain. Utilization of *SUPE* mutation to construct *S. cerevisiae* strain with increased rDNA transcription and increased rDNA copy number are significantly effective and the knowledge of *SUPE* mutation will be useful for further improvement of RNA content in *S. cerevisiae*. Judging from these achievements, this dissertation deserves the degree of Doctor of Engineering.