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論 文 内 容 の 要 旨

Chapter 1　General Introduction and objective of this study

Yeast strain such as *S. cerevisiae* is an important microorganism as preferred source for ribonucleic acid (RNA)-related compounds such as 5'-GMP (5'guanosine monophosphate) and 5'-IMP (5' inosine monophosphate) for flavour enhancers and food additives as well as a model eukaryote for the study of basic bioscience, especially genetics. Because main sources of RNA in yeast cells are ribosomal RNA (rRNA), yeast strains with producing large amounts of rRNA are desired. Objective of this study is to breed an effective yeast strain which is able to produce a high amount of RNA. For this purpose, one approach that I took in this study is to elevate the high level of transcription of rDNA gene in *S. cerevisiae*.

The regulation of rRNA synthesis is related to the cell growth rate and to RNA polymerase I (Pol I) activity in the transcribing rDNA gene. Transcription of rDNA genes in yeast begins with the formation of a Pol I pre-initiation complex at the promoter, and requires four major transcription factors: upstream activating factor (UAF), core factor (CF), TATA-binding protein (TBP), and Rrn3p. UAF directly associates with a region of rDNA located ～100 bp upstream of the transcription initiation site called the upstream element (UE). CF centrally localizes to the core element of the promoter. TBP interacts with both UAF and CF, bridging the two factors. Rrn3p is required for transcription of rDNA by Pol I and involved in recruitment of Pol I to rDNA. *RRN10* gene, one of UAF complex subunits, is indispensable to promote high level of transcription of rRNA gene. Although the Δ*rrn10* disruptant is viable, mutant shows severe slow growth and forms tiny-colony due to the defect of rRNA transcription. By taking this advantage we took Δ*rrn10* disruptant strain as a starting material because it is easier to find suppressors showing faster growth due to the improvement of rRNA transcription than using wild-type strain as a starting material. Suppressors may suppress the slow growth defect of Δ*rrn10* disruptant due to increased transcription of rDNA which may lead to more rRNA content when combined with *RRN10* wild-type background. Investigation of these suppressors by cloning genes responsible for the suppression of the Δ*rrn10* disruptant could provide useful information to further create superior yeast strain producing higher amount of RNA.

Chapter 2 Isolation and characterization of suppressors of the Δ*rrn10* disruptant and breeding of *S. cerevisiae* strain with a high level of RNA

We developed novel breeding strategy creating yeast strain with the ability to synthesize a large amount of RNA by three-step procedure. i) First, an *S. cerevisiae* Δ*rrn10* disruptant that displayed severe growth retardation due to a defect in rDNA transcription was created. ii) Subsequently, suppressors showing a normal growth phenotype were isolated after mutagenesis of the Δ*rrn10* disruptant. iii) Finally, the wild-type *RRN10* gene was integrated into chromosome V of the suppressors, with the expectation that the resultant strain would have an RNA content higher than that of the wild-type level. Based upon this idea, we isolated seven suppressors from the Δ*rrn10* disruptant which we named SupA-SupG and revealed that these suppressors had RNA content 32-56% higher than parental strain, i.e., Δ*rrn10* disruptant. Genetic analysis revealed that these suppressors are dominant and harbored multiple mutations. To see whether suppressor mutation lead to increased amount of RNA in $RRN10^{+}$ background, *RRN10* gene was integrated into chromosome of original suppressors harboring the Δ*rrn10* disruption and integrants were measured for the RNA content. Result of measurement revealed that integrants were able to produce RNA 1.4- to 2.3- fold higher than that of wild-type strain harboring no suppressor and Δ*rrn10* disruption mutations.

Chapter 3 Increased transcription of *NOP15*, involved in ribosome biogenesis in *S. cerevisiae*, enhances the production yield of RNA

To investigate molecular mechanism of suppression, cloning of one of genes responsible for the suppression was attempted using a genomic library from the SupD strain. *NOP15*, a gene involved in ribosome biogenesis, was found to be responsible for suppressing the growth defect of the Δ*rrn10* disruptant. The isolated *NOP15* allele (designated $NOP15^{T\text{-}279C}$) possessed a single T to C substitution at nucleotide position -279 of *NOP15*. The transcription level of $NOP15^{T\text{-}279C}$ in the originally isolated SupD strain was 2-fold higher than that in the Δ*rrn10* disruptant. Furthermore, increased transcription level of *NOP15* in the Δ*rrn10* disruptant by adding $NOP15^{T\text{-}279C}$ allele led to increase of total amount of RNA 1.3-fold compared with the Δ*rrn10* disruptant constructed by adding $NOP15^{WT}$. Introduction of the $NOP15^{T\text{-}279C}$ allele into the wild-type strain increased total RNA content by 1.4-fold. All of these observations indicate that the transcription level of *NOP15* is an important determinant of the productivity of RNA and that its increased transcription provides an effective approach to obtain higher RNA yields in yeast.

Chapter 4 General discussion and conclusion

Nop15p is a RNA-binding protein required for rRNA processing. Possible mechanism for increased RNA content that we could imagine is that $NOP15^{T\text{-}279C}$ may produce more inappropriately processed pre-rRNA intermediates that could not be utilized for ribosome biogenesis. This possibility likely occurs because introduction of $NOP15^{T\text{-}279C}$ allele enhanced total RNA content but did not improve the growth to the level of wild-type. If this is the case, inappropriate processed pre-rRNA is likely to be degraded and contribute to increased amount of total RNA.

論文審査の結果の要旨

Yeast strain such as *S. cerevisiae* is an important microorganism as preferred source for ribonucleic acid (RNA)-related compounds such as 5'-GMP (5'guanosine monophosphate) and 5'-IMP (5' inosine monophosphate) for flavour enhancers and food additives as well as a model eukaryote for the study of basic bioscience, especially genetics. Because main sources of RNA in yeast cells are ribosomal RNA (rRNA), yeast strains with producing large amounts of rRNA are desired. Objective of this study is to breed an effective yeast strain which is able to produce a high amount of RNA. For this purpose, one approach that Chuwattanakul took in this study is to elevate the high level of transcription of rDNA gene in *S. cerevisiae*.

She developed novel breeding strategy creating yeast strain with the ability to synthesize a large amount of RNA by three-step procedure. i) First, an *S. cerevisiae* Δ*rrn10* disruptant that displayed severe growth retardation due to a defect in rDNA transcription was created. ii) Subsequently, suppressors showing a normal growth phenotype were isolated after mutagenesis of the Δ*rrn10* disruptant. iii) Finally, the wild-type *RRN10* gene was integrated into chromosome V of the suppressors, with the expectation that the resultant strain would have an RNA content higher than that of the wild-type level. Based upon this idea, Chuwattanakul isolated seven suppressors from the Δ*rrn10* disruptant which she named SupA-SupG and revealed that these suppressors had RNA content 32-56% higher than parental strain, i.e., Δ*rrn10* disruptant. Genetic analysis revealed that these suppressors are dominant and harbored multiple mutations. To see whether suppressor mutation lead to increased amount of RNA in $RRN10^{+}$ background, *RRN10* gene was integrated into chromosome of original suppressors harboring the Δ*rrn10* disruption and integrants were measured for the RNA content. Result of measurement revealed that integrants were able to produce RNA 1.4- to 2.3- fold higher than that of wild-type strain harboring no suppressor and Δ*rrn10* disruption mutations.

Molecular mechanism of suppression was further investigated by cloning of one of genes responsible for the suppression of Δ*rrn10* disruptant using a genomic library from the SupD strain. *NOP15*, a gene involved in ribosome biogenesis, was found to be responsible for suppressing the growth defect of the Δ*rrn10* disruptant. The isolated *NOP15* allele (designated $NOP15^{T\text{-}279C}$) possessed a single T to C substitution at nucleotide position -279 of *NOP15*. The transcription level of $NOP15^{T\text{-}279C}$ in the originally isolated SupD strain was 2-fold higher than that in the Δ*rrn10* disruptant. Furthermore, increased transcription level of *NOP15* in the Δ*rrn10* disruptant by adding $NOP15^{T\text{-}279C}$ allele led to increase of total amount of RNA 1.3-fold compared with the Δ*rrn10* disruptant constructed by adding $NOP15^{WT}$. Introduction of the $NOP15^{T\text{-}279C}$ allele into the wild-type strain increased total RNA content by 1.4-fold. All of these observations indicate that the transcription level of *NOP15* is an important determinant of the productivity of RNA and that its increased transcription provides an effective approach to obtain higher RNA yields in yeast. In conclusion, the data presented in this study should not only contribute to understanding of transcriptional regulation of rDNA gene in yeast as well as higher eukaryotes but also provide valuable knowledge for application to food industries for developing superior yeast strains displaying higher amount of RNA content. Judging from these achievements, this dissertation deserves the degree of Doctor of Engineering.