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Increased transcription of *NOP15*, involved in ribosome biogenesis in *Saccharomyces cerevisiae*, enhances the production yield of RNA as a source of nucleotide seasoning

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Yeast RNA is a good source of nucleotide seasoning, and more than half of yeast RNA consists of ribosomal RNA (rRNA). Previously, we reported the development of a *Saccharomyces cerevisiae* strain displaying a 1.4- to 2.3-times higher RNA content than the wild-type strain through the isolation of dominant suppressors (designated SupA to SupG strains) from a Δ*rrn10* disruptant showing decreased rRNA transcription. In the present study, the cloning of one of the 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, a dose-dependent relationship between the transcription level of *NOP15* and total amount of RNA in the Δ*rrn10* disruptant was observed: the enhanced transcription due to the $NOP15^{T\text{-}279C}$ allele is involved in the suppression mechanisms in the SupD strain. Introduction of the $NOP15^{T\text{-}279C}$ allele into the wild-type strain increased the total RNA content by 1.4-fold. These results 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.

[**Key words:** *Saccharomyces cerevisiae*; Ribosomal RNA; *RRN10*; *NOP15*; Suppressor]

Yeast (*Saccharomyces cerevisiae*) is among the premier industrial microorganisms as important sources of RNA, which is an attractive source of 5′-ribonucleotides used as flavor enhancers in the food industry (1,2). The majority (80%) of RNA is ribosomal RNA (rRNA) (3). The rRNA of *S. cerevisiae* is encoded by the *RDN1* locus, an ∼1.5 Mb region consisting of ∼150 tandemly repeated copies of a 9.1 kb unit, on chromosome XII. Each single 9.1-kb unit consists of 2 transcribed regions, the 35S precursor rRNA and 5S rRNA (4); the 35S precursor rRNA is transcribed by RNA polymerase I (Pol I) and post-transcriptionally processed into 18S, 5.8S and 25S rRNAs (5).

Transcription of the 35S rRNA gene in *S. cerevisiae* 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 (6). UAF contains six subunits, Rrn5p, Rrn9p, Rrn10p, Uaf30p, and histones H3 and H4 (7), and directly associates with a region of rDNA located ∼100 bp upstream of the transcription initiation site called the upstream element (UE). CF is a complex of three subunits, Rrn6p, Rrn7p, and Rrn11p, and centrally localizes to the core element of the promoter (8). TBP interacts with both UAF and CF, bridging the two factors (9), and Rrn3p is required for the recruitment of Pol I to rDNA (10).

Rrn10p, one of the components of UAF, is essential to promote a high level of transcription of the 35S rRNA gene; therefore, the disruption of the *RRN10* gene causes a specific defect in the Pol I-mediated transcription of rDNA, leading to extremely slow growth and a small-colony phenotype (11). To create a strain that shows a higher RNA content for the food industry, suppressors were isolated from the Δ*rrn10* disruptant and genetically categorized into seven groups (SupA to SupG) (11). Our previous study revealed that all of the suppressors contained dominant and multiple mutations (hereafter, those multiple mutations in the SupA to SupG strains are collectively designated *SUPA* to *SUPG*), each of which were required to suppress the growth defect caused by the disruption of the *RRN10* gene and conferred the phenotype of a higher RNA content than the Δ*rrn10* disruptant strain (11). Importantly, the introduction of the *RRN10* gene into the originally isolated suppressors that carried the Δ*rrn10* disruption led to 1.4- to 2.3-fold higher RNA content than the wild-type strain, demonstrating that our strategy was effective for the development of a strain capable of producing a higher amount of RNA (11).

In the present study, we attempted to clone the genes responsible for the suppression of the Δ*rrn10* disruptant to analyze the molecular

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basis for the increased RNA content. We isolated *NOP15*, which encodes a protein involved in 60S ribosomal subunit biogenesis that partially suppressed the growth defect of the Δ*rrn10* disruptant, from the SupD genomic library. Sequence analysis revealed that a single T to C substitution occurred in its promoter region at nucleotide −279 of *NOP15* (designated $NOP15^{T\text{-}279C}$); this base change was found to lead to the increased transcription of *NOP15*, improving the yield of total RNA. Furthermore, the amount of total RNA was enhanced 1.4-fold with the introduction of $NOP15^{T\text{-}279C}$ into the wild-type strain. To our knowledge, this is the first report showing that the amount of total RNA can be effectively increased by specific genetic modification in *S. cerevisiae*. Because higher yields of yeast RNA are in demand for the food industry, our study is significant for the efforts to improve the productivity of nucleotide seasoning sources.

MATERIALS AND METHODS

***S. cerevisiae* and *Escherichia coli* strains, growth conditions, and oligonucleotide primers** The *S. cerevisiae* strains used in this study are described in Table 1. Strain SupD (SH6975; Δ*rrn10 SUPD*) was isolated as a suppressor of the growth defect of the Δ*rrn10* disruptant (SH6789) (11). Yeast strains were grown at 30°C in yeast peptone dextrose adenine (YPDA) medium or synthetic complete (SC) medium, as described by Amberg et al. (12). *E. coli* strain DH5α was used as a host for plasmid preparation. The oligonucleotide primers used in this study are listed in Table 2.

Construction of the SupD genomic library, cloning, and sequencing The ligation mixture for the construction of the genomic library was prepared by Takara Bio Dragon Genomics Center (Takara Bio, Inc., Shiga). Briefly, the genomic DNA of SH6975 (SupD) was mechanically random sheared and frationated by agarose gel electrophoresis. DNA fragments greater than 5 kb in size were purified from the agarose gel and treated with T4 DNA polymerase to convert both ends into blunt ends. The blunt-ended DNA fragments were ligated into bacterial alkaline phosphatase (BAP)-treated YCp50, a centromere-based vector containing *URA3* that was digested by *Nru*I. Then, *E. coli* DH10B competent cells (Invitrogen, Carlsbad, CA, USA) were transformed with the ligation mixture by electroporation using a GenePulser (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Cells of ampicillin-resistant colonies were selected, and pools of plasmid DNA were prepared. After transformation of the host strain SH6789 (Δ*rrn10*) with the genomic library, the transformants were screened for faster growth. Sequencing was performed according to the protocol of the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

Plasmid construction A plasmid, p3380 (YCp-$NOP15^{T\text{-}279C}$), was constructed by ligation of the *Sma*I-linearized pRS316 (13) vector with a PCR fragment of the $NOP15^{T\text{-}279C}$ allele comprising 500 bp and 337 bp of its upstream and downstream regions, respectively; this fragment was prepared using plasmid p3375 as the template and ORFNOP15-F and ORFNOP15-R (Table 2) as the forward and reverse primers, respectively. Similarly, plasmid p3379 (YCp-$NOP15^{WT}$) was constructed by ligation of the *Sma*I-linearized pRS316 vector with a PCR fragment of the $NOP15^{WT}$ gene prepared using SH6446 genomic DNA (14) as the template and ORFNOP15-F and ORFNOP15-R (Table 2) as the forward and reverse primers, respectively.

Quantification of *NOP15* mRNA by real-time RT-PCR and measurement of the total RNA content using the perchloric acid method Yeast cells were collected at log phase ($OD_{660} = 1.0$), and the RNA was extracted using the hot phenol method (15). To quantify the *NOP15* mRNA, real-time RT-PCR was performed using a 7300 Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions. The SYBR green RT-PCR reactions were performed by two-step RT-PCR using the High-Capacity cDNA Archive kit (Applied Biosystems) and SYBR Green Universal PCR Master Mix (Applied Biosystems). NOP15 mRNA-F and NOP15 mRNA-R (Table 2) were used as the forward and reverse primers, respectively. As an internal control, *ACT1* mRNA was detected using ACT1-F and ACT1-R (Table 2) as the forward and reverse primers, respectively. The total amount of RNA in the yeast cells was measured using the perchloric acid method according to Chuwattanakul et al. (11).

RESULTS AND DISCUSSION

Slow-growth phenotype of the Δ*rrn10* disruptant is partially restored by a *NOP15* allele with a single nucleotide substitution in its promoter region The Δ*rrn10 SUPD* mutant (designated as the SupD strain) was isolated after EMS mutagenesis of the Δ*rrn10* disruptant as suppressing the slow growth and small-colony phenotypes of the Δ*rrn10* disruptant (11). It was subsequently found that the rRNA transcription defect was also suppressed in the SupD strain (11). To clarify the molecular mechanism of the suppression of the defect in rRNA Pol I transcription in the Δ*rrn10* disruptant, we intended to clone the *SUP* genes responsible for the suppression of the Δ*rrn10* defect from the SupD strain. We have reported previously that suppressor mutations in the SupD strain are dominant and consist of multiple mutations collectively designated here as *SUPD*. The conclusions are based on the observation that a heterozygous diploid of SupD (SH6811: Δ*rrn10 SUPD*) and the parental strain (SH6968: Δ*rrn10*) segregated tetrads that exhibited a variety of medium-sized colonies in addition to those of large (similar to the colony size of the SupD strain) and small sizes (similar to the colony size of the Δ*rrn10* strain) (data not shown). Thus, we reasoned that it would be possible to clone any of the suppressor genes constituting the *SUPD* mutations by screening for Δ*rrn10* transformants displaying medium sized-colony phenotypes using a SupD genomic library. Accordingly, we attempted to clone the genes responsible for the suppression of the small-colony phenotype of the Δ*rrn10* disruptant.

A genomic library was constructed using chromosomal DNA from the SupD strain (SH6975) and a single-copy vector, YCp50. Pooled DNA from this library was introduced into the Δ*rrn10* disruptant, and the transformants were selected on uracil-lacking plates supplemented with the required amino acids after

TABLE 1. Yeast strains and plasmids used in this study.

Strain	Genotype	Remarks
S. cerevisiae		
SH6446	*MAT***a** *ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Δfob1::HIS3*	14
SH6789	*MATα ura3-52 or ura3Δ851 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Δfob1::HIS3 Δrrn10::CgLEU2*	11
SH6968	*MAT***a** *ura3-52 or ura3Δ851 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Δfob1::HIS3 Δrrn10::CgLEU2*	*MAT***a** convertant of SH6789 with *HO*
SH6811 (SupD)	*MATα ura3-52 or ura3Δ851 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Δfob1::HIS3 Δrrn10::CgLEU2 SUPD*	Suppressor mutant from SH6789
SH6975 (SupD)	*MAT***a** *ura3-52 or ura3Δ851 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Δfob1::HIS3 Δrrn10::CgLEU2 SUPD*	*MAT***a** convertant of SH6811 with *HO*
SH8846	Ura^+ transformant of SH6789 with p562 (= pRS316)	This study
SH8862	Ura^+ transformant of SH6789 with p3379	This study
SH8863	Ura^+ transformant of SH6789 with p3380	This study
SH6472	Ura^+ transformant of SH6446 with p562	This study
SH6473	Ura^+ transformant of SH6446 with p3379	This study
SH6475	Ura^+ transformant of SH6446 with p3380	This study
Plasmid		
p562 (=pRS316)	YCp-*URA3*	13
p3375	YCp-*URA3* having a fragment of chromosome XIV 417,269−422,308	This study
p3379	YCp-*URA3*-$NOP15^{WT}$	This study
p3380	YCp-*URA3*-$NOP15^{T\text{-}279C}$	This study