A secretory defect leads to transcriptional repression of both ribosomal protein and rRNA genes in yeast. To elucidate the mechanism of the signaling, we previously isolated rrs mutants that were unable to respond to a secretory defect, and we cloned RRS1 encoding a nuclear protein that was required for ribosome biogenesis (Tsuno, A., Miyoshi, K., Tsujii, R., Miyakawa, T., and Mizuta, K. (2000) Mol. Cell. Biol. 20, 2066–2074). We identified duplicated genes encoding ribosomal protein L11, RPL11B as a wild-type allele complementing the rrs2 mutation, and RPL11A in two-hybrid screening using RRS1 as bait. Rpl11p was copurified with Rrs1p in immunoprecipitation analysis. Ultracentrifugation analysis revealed that Rrs1p associated fairly tightly with 60 S preribosomal subunits. These results suggest that signaling in response to a secretory defect requires the normal assembly of 60 S ribosomal subunits including Rrs1p and Rpl11p.

Yeast ribosome consists of 4 rRNAs and 78 ribosomal proteins (RP). The amounts of the components are coordinately regulated corresponding to cell growth mainly at the level of transcription (1, 2). Furthermore, ribosome synthesis appears to be coupled with membrane synthesis; a secretory defect causes specific and significant repression of transcription of both RP and rRNA genes (3–5). To elucidate the molecular causes specific and significant repression of transcription (1, 2). Furthermore, ribosome synthesis appears to be coupled with membrane synthesis; a secretory defect causes specific and significant repression of transcription of both RP and rRNA genes due to a secretory defect (7, 8). Rap1p is involved in both transcriptional activation of many genes including most RP genes and silencing such as the telomere position effect. It is unknown how Rap1p is responsible for the repression due to a secretory defect. Furthermore, it has recently been proposed that plasma-membrane stretch caused by the elevated internal turgor pressure is a trigger of the signaling (5) and that protein kinase C (Pkc1p) is required for the signaling (9). However, mitogen-activated protein kinase cascade of the cell integrity pathway was not required for this response, and components downstream of Pkc1p remain unknown.

In this study, we cloned RPL11B, which encodes ribosomal protein L11 (Rpl11p), as a responsible gene for the rrs2 mutation. We also isolated RPL11A in yeast two-hybrid screening using RRS1 as bait. Rpl11p is encoded by the duplicated genes RPL11A (YPR102c) and RPL11B (YGR085c), formerly named RPL16B and RPL16A (10–12), respectively (for the nomenclature, see Ref. 13 and the Saccharomyces Genome Data base, genome-www.stanford.edu/Saccharomyces/). The RPL11A gene is expressed at twice the level of RPL11B (10). Rpl11Ap and Rpl11Bp differ only at their third amino acid residues, alanine and threonine, respectively (14, 15), and there is no functional distinction between the Rpl11Ap and Rpl11Bp (10). Rpl11p is necessary for the assembly of 60 S ribosomal subunits and is localized near the top surface of the central protuberance, where the 60 S subunit potentially contacts the 40 S subunit (16).

We demonstrate that disruption of either the RPL11A or RPL11B gene attenuates transcriptional repression of both RP and rRNA genes in response to a secretory defect. Rpl11p shows physical interaction with Rrs1p, which may have a central role for the signaling in response to a secretory defect. These results suggest that 60 S ribosomal subunit assembly machinery containing Rpl11p and Rrs1p is essential for the signaling.

**MATERIALS AND METHODS**

**Yeast Strains, Media, and a Library—**The yeast strains used in this study are listed in Table I. Deletion-insertion mutations of RPL11A or RPL11B were constructed in diploids, W303 or K1007, as described by Rotenberg et al. (10). Yeast cells were grown inYPD-rich medium, synthetic complete medium containing 2% glucose (SC), or SC dropout medium depending on the plasmid markers (17). Yeast transformation was performed by a lithium acetate procedure (18). A library consisting...
amplified DNA fragments were digested with contactaaagcccaaaaaccctatgc and 5'-tttctcgagattatttatcgagcacatcagcg), and /H11032 strain cells. Leu
mids for the production of lexA binding domain-fusion proteins and activation domain-Rpl11Ap and -Rpl11Bp fusion proteins. Two plas-
digested fragments were introduced into pACTII to produce Gal4p
/H11032 amplified by PCR using two sets of primers (5'-H11032)
was transformed into a temperature-sensitive CYH2 by the promoter of the ribosomal protein gene A) in which the
insert of pACT in the cells was characterized.

| Strain | Genotype | Source |
|--------|----------|--------|
| W303   | MATa/MATa his3-11,15 his3-11,15 ade2-1/ade2-1 ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 can1-100/can1-100 | Rothstein, R |
| W303a  | MATa his3-11,15 ade2-1 ura3-1 leu2-3,112 trp1-1 can1-100 sby1 | (3) |
| KM003  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 sby1 | (3) |
| KM007  | MATa/MATa his3-11,15/+ adel-2/adel-2 ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/+ can1-100/can1-100 sby1/sby1 | This study |
| KM148  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 rs1::LEU2 pRS313-RA-RS1 (ARS/CEC/HA-RS1 HIS3) | (6) |
| KM201  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 sby1 sry2 | This study |
| KM202  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 sby1 rspl::LEU2 | This study |
| KM203  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 sby1::URA3 | This study |
| KM204  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 rpl11a::LEU2 | This study |
| KM205  | MATa adel-2 ura3-1 leu2-3,112 trp1-1 can1-100 rpl11b::URA3 | This study |
| KM330  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 rps4A::LEU2 pRS313-RRS1-HA YCplac22-RPL11A-myc | This study |
| KM331  | MATa his3-11,15 adel-1 ura3-1 leu2-3,112 trp1-1 can1-100 rs1::LEU2 pRS313-RRS1-HA YCplac22-RPL11A-myc TRP1 | This study |
| J130A  | MATa his3-11,15 adel-2 ura3-1 leu2-3,112 trp1-1 can1-100 rps4A::LEU2 | (22) |
| J130C  | MATa his3-11,15 adel-2 ura3-1 leu2-3,112 trp1-1 can1-100 rps4C::HIS3 | (22) |
| KM314  | MATa his3-11,15 adel-2 ura3-1 leu2-3,112 trp1-1 can1-100 sby1 rspl::LEU2 | This study |
| KM319  | MATa his3-11,15 adel-2 ura3-1 leu2-3,112 trp1-1 can1-100 sby1::URA3 | This study |
| L40    | MATa his3Δ200 adel2 lys2-801am trp1-901 leu2-3,112 LYS2::lexA::His3 URA3::lexA::HIS3 | Sternglanz, R. |

of partial Sau3A fragments of Saccharomyces cerevisiae genomic DNA inserted into a single-copy yeast vector YCp60 was generously provided by M. D. Rose (19).

Isolation of Mutants—Mutants were isolated as described previously (6). Briefly, a plasmid (see Fig. 1A) in which the HIS3 gene was driven by the promoter of the ribosomal protein gene RPL28 (formerly called CYH2) was transformed into a temperature-sensitive his3 sby1 strain, which is defective in endoplasmic reticulum-to-Golgi trafficking (3). The cells are phenotypically His− at 25 °C, but at 31 °C, just below the non-permissive temperature for sby1, they grow extremely slowly on SC lacking histidine (SC−/His−) plates containing 3-aminoazotriazole because the HIS3 transcription is repressed. The cells were treated with ethyl methanesulfonate to a survival frequency of 25% and grown on SC−/His− plates containing 3-aminotriazole and incubated at 28 °C for 3 days. Co-immunoprecipitation and Immunoblotting Analysis—Yeast cells were grown in SC-selective media to a mid-log phase, collected by centrifugation, washed twice with ice-cold immunoprecipitate buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol, 30 mM NaCl, 0.05% Nonidet P-40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, and 1 µg/ml pepstatin A), and resuspended in 100 µl of IP buffer. The cells were broken with glass beads by using a vortex mixer at 4 °C. The homogenates were centrifuged twice at 15,000 rpm for 20 min at 4 °C in a microcentrifuge rotor. The superna-
tants were incubated with the anti-hemagglutinin (anti-HA, Roche Molecular Biochemicals) or anti-Myc (Berkeley Antibody) monoclonal antibodies and protein A-Sepharose beads (Sigma) overnight at 4 °C and then precipitated by centrifugation. The immunoprecipitates were washed five times with IP buffer, fractioned by SDS-PAGE, trans-
ferred to Hybond ECL membrane (Amersham Biosciences), and probed separately with the anti-HA or anti-Myc antibodies. Signals were visu-
alized with the ECL detection reagent (Amersham Biosciences) as in-
structed by the manufacturer.

Northern Blot and [methyl-3H]methionine Pulse-Chase Analyses—Northern blot and [methyl-3H]methionine pulse-chase analyses were carried out as described previously (3, 6).

Preparation of Ribosome Pellets from Yeast Lysates—After the addition of cycloheximide at the concentration of 100 µg/ml followed by the addition of crushed ice, yeast cells were harvested and resuspended in complex stabilization buffer (CSB) (21) containing 300 mM sorbitol, 20 mM HEPES-ROH, pH 7.5, 1 mM EGTA, 5 mM MgCl2, 10 mM KCl, 1% glycerol, 2 mM dithiothreitol, and 100 µg/ml cycloheximide. Glass-bead lysates of the cells were centrifuged at 5,000 rpm for 5 min, and the supernatant was centrifuged again at 15,000 rpm for 20 min at 4 °C in a microcentrifuge rotor. Supernatants (500 µl; 20 A260 units) were overlaid on 2 ml of sucrose cushions (CSB containing 500 mM sucrose instead of sorbitol) and centrifuged at 40,000 rpm for 2 h at 4 °C in a Beckman TLA100.3 rotor. Ribosome pellets were resuspended with CSB containing cycloheximide and various concentrations of LiCl, incubated for 30 min at 4 °C, and centrifuged at 40,000 rpm for 2 h at 4 °C in a Beckman TLA100.3 rotor. Ribosome pellets were resuspended in SDS sample buffer.

Polysome Analysis—Yeast cells were grown in 2 liters of medium to mid-log phase and harvested immediately following the addition of cycloheximide (100 µg/ml) and crushed ice. The pellet was washed twice with buffer A (10 mM Tris-HCl (pH 7.4), 100 mM NaCl, 30 mM MgCl2, 50 µg of cycloheximide/ml, 200 µg of heparin/ml) and suspended in buffer
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**rrs2** mutant. The construct of the reporter gene. The reporter gene consists of the coding sequence of the yeast HIS3 gene fused to the promoter of RPL28. B. Northern analysis of the rrs2 mutant under the secretory defect. Yeast KM003 (rrs2) and KM201 (sly1 rrs2) cells were grown in YPD medium to log phase (optical was carried out using 32P-labeled DNA probes specific for prepared and separated by gel electrophoresis. Northern blot analysis showed that the mutation is recessive; when the mutant cells B subunit assembly (6). In this study, protein essential for 25 S rRNA maturation and 60 S ribosomal loading. Small nucleolar RNA U3 was used as a marker to check equal washed with acetone, dried, and resuspended in SDS sample buffer.

**RESULTS**

**Isolation of Yeast Mutants Unable to Respond to a Secretory Defect**—To elucidate the molecular mechanism of the signaling in response to a secretory defect, we obtained rrs (regulator of ribosome synthesis) mutants in which a secretory defect did not cause transcriptional repression of RP genes (6). A plasmid containing the reporter gene, shown in Fig. 1A, was introduced into a his3 sly1 strain, a temperature-sensitive mutant defective in endoplasmic reticulum-to-Golgi trafficking. Mutants were isolated on an SC/His plate containing 3-amino-triazole at the semipermissive temperature of sly1. Previously, we analyzed rrs1 mutant and showed that RRS1 encoded a nuclear protein essential for 25 S rRNA maturation and 60 S ribosomal subunit assembly (6). In this study, rrs2 was chosen for further analysis. In rrs2 sly1 cells, the transcriptional repression of RP genes was significantly attenuated when the cells were transferred to the restrictive temperature (Fig. 1B). Genetic analysis showed that the mutation is recessive; when the mutant cells were crossed to parental sly1 cells of the opposite mating type, RP genes RPL28 and RPL3 were significantly repressed at the restrictive temperature (data not shown).

**rrs2 Has a Mutation in RPL11B**—To isolate a gene complementing the rrs2 mutation, we examined phenotypes of the rrs2 mutant cells and found that rrs2 mutant cells exhibited cycloheximide sensitivity. The cycloheximide sensitivity segregated 2:2 in tetrads from RRS2/rrs2 diploid cells, and every cycloheximide-sensitive clone in three sets of tetrads showed the signaling defect detected by Northern blot analysis (data not shown). By using cycloheximide-sensitive phenotype of rrs2, we succeeded in cloning the RRS2 gene as described below. The rrs2 cells were transformed with a library of yeast genomic DNA constructed in a centromere-based vector, YCP50 (19). Of 1.3 × 104 Ura + transformants, 35 colonies could grow on a YPD plate containing 0.12 μg of cycloheximide/ml. In 11 of the 35 strains, the complementing activity was plasmid-linked. Restriction maps of the plasmid DNAs recovered from these transformants revealed that ten plasmids had an identical 3.8-kb insert (named pRT-1) and one plasmid had an 11.9-kb insert (named pRT-2). We determined a partial DNA sequence of the plasmids. To identify the region of pRT-1 required for complementation, three subclones were constructed, and their complementing activities for the cycloheximide-sensitivity of rrs2 were checked. The complementing activity was fully recovered in the plasmid containing RPL11B. Subcloning of pRT-2 revealed that RPL11A was responsible for the complementing activity. Northern analysis showed that the plasmids containing RPL11A or RPL11B also complemented the signaling defect of rrs2 (data not shown).

To determine the mutated allele in the rrs2 mutant, the RPL11A and RPL11B genes were amplified by PCR using chromosomal DNA prepared from the mutant cells as template. Sequence analysis revealed that RPL11B had only one nucleotide difference (a C-to-T transition) within codon 39, resulting in a stop codon, whereas RPL11A had no difference.

**Both the rpl11a- and the rpl11b-null Mutations Affect the Transcriptional Repression of RP Genes in Response to a Secretory Defect**—As the rrs2 mutant has a nonsense mutation in RPL11B, we determined the effect of the rpl11a- and the rpl11b-null mutations (10) on the transcriptional repression of RP genes in response to a secretory defect. Both the rpl11a- and the rpl11b-null mutations attenuated the transcriptional repression of RP genes due to a secretory defect, which was caused by either addition of tunicamycin, an inhibitor of the secretory pathway (Fig. 2A), or shifting sly1 cells to the restrictive temperature (Fig. 2B). The signaling defect in the rpl11a-null mutant was more striking than that in the rpl11b-null mutant, probably reflecting that the RPL11A gene is expressed at twice the level of RPL11B (10). On the other hand, the null mutations of RPS4A or RPS4B (22) that encode a ribosomal protein of the small subunit had little effect on the response to a secretory defect (Fig. 2, A and B), suggesting that the attenuation of transcriptional repression of ribosomal protein genes is specific to a defect in large ribosomal subunits.

The rpl11 Mutation Also Affects the Repression of rDNA Transcription in Response to a Secretory Defect—As a secretory defect causes transcriptional repression of rDNA as well as RP genes (3), we examined whether the rpl11 mutation had any effect on the transcriptional repression of rDNA. We monitored the synthesis and processing of rRNA by [methyl-3H]methionine pulse-chase analysis since newly synthesized precursor RNA is methylated immediately (23, 24). As shown in Fig. 2C, a secretory defect by shifting the sly1 cells to the restrictive temperature leads to strong repression of rDNA transcription (lanes 3 and 4), consistent with previous data (3). On the other hand, in both the Δrpl11a and Δrpl11b strain cells, repression of rDNA transcription due to a secretory defect was significantly attenuated (lanes 7, 8, 11, and 12). These results indicate that Rpl11p depletion affects the signal transduction from a secretory defect to the repression of rDNA transcription as well as that of RP gene transcription.

**Rrs1p Shows Physical Interaction with Rpl11p**—Interestingly, RPL11A was also cloned in two-hybrid screening by using RRS1 as bait. Rrs1p, an evolutionarily conserved nuclear protein, is required for prerRNA processing and proper assembly of ribosomal subunits in S. cerevisiae (8). RRS1 was origi-
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Rrs1p with a ribosome is rather stable, although it appeared to dissociate in a soluble fraction in buffer with low ionic strength. To confirm whether Rrs1p associates with ribosomal particle, we examined whether Rrs1p associates with ribosomal particle and was isolated as the gene responsible for the rrs1-1 mutation, which causes a defect in the secretory response. To identify proteins that physically interact with Rrs1p, we carried out yeast two-hybrid screening on a yeast cDNA library using RRS1 as bait. Among the 21 cDNA clones isolated in this screen, one cDNA encoded most of the sequence of Rpl11Ap. As we reported previously, three clones contained EBP2 cDNA (20). Fig. 3A shows two-hybrid interaction of Rrs1p with Rpl11p in yeast with a positive control of Rrs1p and Ebp2p. HIS3 reporter gene was activated in the presence of Gal4p activation domain-Rpl11Ap fusion protein and lexA binding domain-Rrs1p fusion protein. Rpl11Ap also showed two-hybrid interaction with Rrs1p. The results were reproducible, and the assay using lacZ reporter gene showed similar results (data not shown). The C-terminal truncated protein, rrs1-1, also showed two-hybrid interaction with Rpl11l (data not shown).

To confirm the interaction between Rrs1p and Rpl11p, we constructed a yeast strain in which both RRS1 and RPL11A on the chromosomes were disrupted and complemented with plasmids expressing HA-Rrs1p and Myc-Rpl11Ap. Co-immunoprecipitation analysis confirmed the interaction of Rrs1p with Rpl11l as shown in Fig. 3B.

Rrs1p Associates with Ribosomal Particle—Rrs1p has an important function in ribosome biogenesis, especially in the maturation of 25 S rRNA and assembly of 60 S ribosomal subunits (6). As Rrs1p had two-hybrid interaction with Rpl11p, we examined whether Rrs1p associates with ribosomal particles. Ultracentrifugation was carried out using cell extract from the strain expressing HA-Rrs1p, and ribosome pellet and supernatant fractions were analyzed by Western blotting. As shown in Fig. 4, approximately a half-amount of HA-Rrs1p was detected in the ribosome fraction, and the other half was detected in a soluble fraction in buffer with low ionic strength. To learn the stability of the interaction of Rrs1p with ribosomes, the ribosome fraction was treated with various concentrations of LiCl. Approximately a half-amount of Rrs1p associated with ribosome even at 0.5 M LiCl, and most of Rrs1p dissociated from ribosomes in 1.0 M LiCl. This suggests that the interaction of Rrs1p with a ribosome is rather stable, although it appeared to be less stable as compared with that of ribosomal protein L3.

Next, to determine the type of ribosome with which Rrs1p associates, we performed sucrose density gradient centrifugation followed by immunoblotting analysis (Fig. 5). In the first
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Previously, we isolated rrs mutants in which transcription of RP genes was not repressed in response to a secretory defect (6). We demonstrated that RRS1, the wild-type allele responsible for the rrs1–1 mutation, had an important function in 25 S rRNA maturation and 60 S ribosomal subunit assembly when the secretory pathway functions normally (6). In this study, we have cloned the duplicated genes encoding ribosomal protein L11 not only as functional genes complementing the rrs2 mutation that causes a defect in the secretory response but also in two-hybrid screening using RRS1 as bait. These results suggest that Rrs1p and Rpl11p have a function in the same signaling pathway in response to a secretory defect and that protein–protein interaction of the two proteins may be important in the signaling. The C-terminal truncated form of Rrs1p, rrs1–1p, shows two-hybrid interaction with Rpl11p (data not shown), suggesting that the N-terminal half of Rrs1p is required for the interaction with Rpl11p and that the signal is transduced through the C-terminal half of Rrs1p. In the same two-hybrid screening, we previously identified Ehp2p and demonstrated that Ehp2p had a similar role to Rrs1p in 25 S rRNA maturation and 60 S ribosomal subunit assembly (20). We proposed that ribosome assembly machinery is responsive to the signaling, and in this study, we showed that the rpl11 mutation caused a defect in the signaling and that Rrs1p interacts with Rpl11p, strengthening our model.

Amino acid sequence analysis and immunological analysis revealed that Escherichia coli L5, Bacillus stearothermophilus L5, and Halobacterium cutribrum L19 are homologues of yeast Rpl11p (16). These three homologues were shown to associate with 5 S rRNA (25–27). Although it is not shown directly that yeast Rpl11p associates with 5 S rRNA, Rpl11p is localized at a position similar to that of its homologue L5 in E. coli ribosomes, near the top surface of the central protuberance of the 60 S subunit (10, 16). Recent analysis by docking of atomic models into a lower resolution cryo-electron microscopy map suggested that Rpl11p was involved in the formation of a bridge with the 40 S subunit via the interaction with Rps13p and that it also interacted with the 5 S rRNA-binding protein Rpl5p and the elbow of the P site-bound tRNA (28).

Our data suggest that approximately a half-amount of Rrs1p associates with ribosomal particles and that the binding is fairly tight. Rrs1p is localized mainly in the nucleolus and also in the nucleoplasm (6). The nucleolus is the site of pre-rRNA synthesis, pre-rRNA processing, and ribosomal subunit assembly. Most ribosomal proteins bind pre-rRNA in the nucleolus, and pre-rRNA processing is tightly linked to ribosomal subunit assembly (29, 30). Early associating ribosomal proteins bind 35S-labeled pre-rRNA to form 90 S preribosome, from which 66 and 43 S preribosomal subunits containing the 27 and 20 S pre-rRNAs, respectively, are formed. The 66 S preribosomal subunit contains late associating ribosomal proteins, and cleavage of 27 S pre-rRNA to 25 S rRNA leads to the 60 S preribosomal subunit. Rrs1p is detected mainly in the 60 S ribosomal subunit fraction in sucrose density gradient ultracentrifugation analysis. Considering that Rrs1p localizes in the nucleus, this suggests that Rrs1p associates with the preribosomal particle. Although HA-tagged Rrs1p also appears to associate with a lower fraction corresponding 80 S monosomes in the first sucrose density gradient ultracentrifugation, the band disappears in the fraction in the second analysis. As 90 S preribosomal particles sediment at nearly the same fractions as 80 S monosomes, and the 60 S ribosomal subunit fraction contains 66 S preribosomal subunits, it seems that Rrs1p associates with 66 S and/or 60 S preribosomal subunits and associates little with the 90 S pre-ribosomal particle if any. As Rrs1p

density gradient, the centrifugation of crude ribosome, most of the strong signals for HA-Rrs1p were observed in the fractions corresponding to 60 S ribosomal subunits, and weak signals were found in the fractions corresponding to 80 S monosomes and polysomes (Fig. 5B). To clarify whether HA-Rrs1p associates with the lower fractions, a pooled fraction from 80 S fractions of the first gradient was analyzed in the second sedimentation. No signal for HA-Rrs1p was detected (Fig. 5C), indicating that Rrs1p associates with only fractions corresponding to 60 S subunits.

**DISCUSSION**

![Image 50x229 to 294x523]

**FIG. 4.** HA-Rrs1p associates with ribosome. A, yeast KM148 (HA-RRS1) cells were treated with cycloheximide, lysed, and centrifuged through low salt sucrose cushions. Equivalent amounts of ribosome pellet (lane 1) and supernatant (sup; lane 2) were subjected to SDS-PAGE and immunoblotting using anti-HA antibodies and anti-ribosomal protein L3 antibodies (kindly provided by J. R. Warner). ppt, precipitate. B, ribosome pellets were treated with increasing concentrations of LiCl and centrifuged again through sucrose cushions containing the same concentrations of LiCl. Ribosome pellets were subjected to SDS-PAGE and immunoblotting. Concentrations of LiCl were as follows: 0.01 m (lane 1), 0.1 m (lane 2), 0.25 m (lane 3), 0.5 m (lane 4), and 1.0 m (lane 5).

**Fig. 5.** HA-Rrs1p cosediments with free 60 S ribosomal subunits. A, polysome profile of ribosomes isolated from KM148 (HA-RRS1) cells and separated using an 8–45% sucrose gradient. B: fractions from the gradient shown in panel A were collected, and proteins were precipitated with 10% trichloroacetic acid and analyzed by SDS-PAGE and immunoblotting. C, fractions containing 80 S monosomes in the second gradient centrifugation shown in panel A were pooled, pelleted, resuspended, and sedimented through the second gradient centrifugation. Each fraction was analyzed by immunoblotting. Ribosomal protein L3 was used as a marker of 60 S ribosomal subunits.
localizes throughout the nucleus, including the nucleolus. Rrs1p might have a role in the transport of large ribosomal subunits from the nucleolus to the nucleoplasm as well as pre-rRNA processing/ribosomal subunit assembly. It has recently been reported that Noc proteins, Noc1, Noc2, and Noc3, are required for the maturation and intranuclear transport of preribosomes (31). The role of Rrs1p on intranuclear transport is now under investigation. Furthermore, we have found a novel nuclear protein that interacts with Rrs1p, suggesting that Rrs1p exists as a component of a large complex.

We have presented here that Rrs1p and Rpl11p are essential for the secretory response, but the molecular mechanism of transcriptional repression of both rRNA and RP genes in response to a secretory defect remains to be elucidated. As depletion of Rps4p, a protein of the small ribosomal subunit, had little effect on the secretory response, it is unlikely that a decline of protein synthesis caused by the depletion of Rrs1p or Rpl11p canceled a secretory defect. We propose a model in which the signal from a secretory defect might be transmitted to the large ribosomal subunit assembly machinery including Rrs1p and Rpl11p. However, it is also possible that a decline of 60 S ribosomal subunits affects the signaling in response to a secretory defect. In this case, the yeast cells should sense a very small defect in ribosome synthesis because rpl11 null mutation could cause a small defect in ribosome synthesis (10). We cannot distinguish between these two possibilities at present because a defect in a component of the ribosomal subunit could affect the assembly of other components. In fact, qsr1–1 and qsr1–24 (32, 33; kindly provided by B. L. Trumpower), both of which have mutations in RPL10, were also defective in the signaling due to a secretory response (data not shown). Although Rpl10p appears to assemble with a large ribosomal subunit at the last step in ribosome biogenesis, rpl10 mutations affect the earlier steps of pre-rRNA processing (34). Nevertheless, we prefer the former model for three reasons. First, RPL11 was isolated in two independent screens. Second, Rrs1p appears to be a key protein in the signaling as the rrs1–1 allele leads to an extremely strong defect in the signaling (6). Third, rrs1–1 cells still have the ability to repress ribosome synthesis by mild heat shock (6) or nitrogen starvation. It should be noted that the mechanism of the secretory response is different from that of mild heat shock or nitrogen starvation. According to our model, the mechanism of transcriptional repression in response to a secretory defect is tightly linked to the normal regulatory mechanism that maintains ribosome synthesis. It has recently been proposed that rRNA transcriptional machinery is closely linked to pre-rRNA processing machinery (35). This model supports our idea in which transcriptional repression of rRNA genes is mediated through ribosomal subunit assembly machinery, that is, pre-rRNA processing machinery. In addition, we have found that transcriptional repression of rRNA genes is an early event (8), consistent with our model.

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REFERENCES

1. Warner, J. R. (1999) Trends Biochem. Sci. 24, 437–440
2. Woolford, J. L., Jr., and Warner, J. R. (1991) in The Molecular and Cellular Biology of the Yeast Saccharomyces: Genome Dynamics, Protein Synthesis, and Energetics (Broach, J. R., Pringle, J. R., and Jones, E. W., eds), pp. 587–626, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3. Mizuta, K., and Warner, J. R. (1994) Mol. Cell. Biol. 14, 2493–2502
4. Li, B., and Warner, J. R. (1996) J. Biol. Chem. 271, 16813–16819
5. Li, Y., Moir, R. D., Sethy-Coraci, I. K., Warner, J. R., and Willis, I. M. (2000) Mol. Cell. Biol. 20, 3843–3851
6. Tsuno, A., Miyoshi, K., Tsuji, R., Miyakawa, T., and Mizuta, K. (2000) Mol. Cell. Biol. 20, 2066–2074
7. Mizuta, K., Tsuji, R., Warner, J. R., and Nishiyama, M. (1998) Nucleic Acids Res. 26, 1063–1069
8. Miyoshi, K., Miyakawa, T., and Mizuta, K. (2001) Nucleic Acids Res. 29, 3297–3303
9. Nierras, C. R., and Warner, J. R. (1999) J. Biol. Chem. 274, 13325–13341
10. Rotenberg, M. O., Moritz, M., and Woolford, J. L., Jr. (1998) Genes Dev. 12, 160–172
11. Moritz, M., Pulaski, B. A., and Woolford, J. L., Jr. (1991) Mol. Cell. Biol. 11, 5681–5692
12. Moritz, M., Paulovich, A. G., Tsay, Y.-F., and Woolford, J. L., Jr. (1990) J. Cell. Biol. 111, 2261–2274
13. Mager, W. H., Planta, R. J., Ballesta, J.-P. G., Lee, J. C., Mizuta, K., Suzuki, K., Warner, J. R., and Woolford, J. L., Jr. (1997) Nucleic Acids Res. 25, 4872–4875
14. Leer, R. J., Raamsdonk-Duin, M. M., Mager, W. H., and Planta, R. J. (1984) FEBS Lett. 175, 371–376
15. Teem, J. L., Abovich, N., Kaufer, N. E., Schwindinger, W. F., Warner, J. R., Levy, A., Woolford, J. L., Jr., Leer, R. J., van Raamsdonk-Duin, M. M. L., Mager, W. H., Planta, R. J., Schulte, L., Friesen, J. D., Fried, H., and Roshbash, M. (1984) Nucleic Acids Res. 12, 8295–8312
16. Tsay, Y.-F., Shankweiler, G., Lake, J., and Woolford, J. L., Jr. (1994) J. Biol. Chem. 269, 7579–7586
17. Kaiser, C., Michaelis, S., and Mitchell, A. (1994) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
19. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D., and Fink, G. R. (1987) Gene (Amst.) 60, 237–243
20. Tsuji, R., Miyoshi, K., Tsuno, A., Matsui, Y., Toh-e, A., Miyakawa, T., and Mizuta, K. (2000) Genes Cells 5, 543–553
21. Nelson, R. J., Ziegelhoeffer, T., Niolet, C., Werner-Washburne, M., and Craig, E. A. (1992) Cell 71, 97–105
22. Synetos, D., Dabeva, M. D., and Warner, J. R. (1992) J. Biol. Chem. 267, 3068–3071
23. Udum, S. A., and Warner, J. R. (1972) J. Mol. Biol. 65, 227–242
24. Chen-Schmeisser, U., and Garrett, R. A. (1977) FEBS Lett. 74, 287–291
25. Horne, J. R., and Erdmann, V. A. (1972) Mol. Gen. Genet. 119, 337–344
26. Smith, N., Matheson, A. T., Yaguchi, M., Willick, G. E., and Nazar, R. N. (1978) J. Cell Biol. 79, 501–509
27. Spierer, P., and Zimmermann, R. A. (1978) Biochemistry 17, 2474–2479
28. Spahn, C. M. T., Beckmann, R., Eswar, N., Penczek, P. A., Sali, A., Blobel, G., and Frank, J. (2001) Cell 107, 373–386
29. Venema, J., and Tollervey, D. (1999) Annu. Rev. Genet. 33, 261–311
30. Kressler, D., Linder, P., and de la Cruz, J. (1999) Mol. Cell. Biol. 19, 7897–7912
31. Milkereit, P., Gadal, O., Podtelejkov, A., Trumtel, S., Gas, N., Petfalski, E., Tollervey, D., Mann, M., Hurt, E., and Tschöchner, H. (2001) Cell 105, 499–509
32. Thierry Y, Yang M., Dick, F. A., Schmitt, M. E., and Trumpower, B. L. (1995) J. Biol. Chem. 270, 9961–9970
33. Eisinger, D. P., Dick, F. A., and Trumpower, B. L. (1997) Mol. Cell. Biol. 17, 5136–5145
34. Gadal, O., Strauß, D., Kessel, J., Trumpower, B. L., Tollervey, D., and Hurt, E. (2001) Mol. Cell. Biol. 21, 3405–3415
35. Fath, S., Milkereit, P., Podtelejkov, A. V., Bischler, N., Schultz, P., Bier, M., Mann, M., and Tschöchner, H. (2000) J. Cell Biol. 149, 575–589

2 D. Morita, K. Miyoshi, Y. Matsui, A. Toh-e, H. Shinkawa, T. Miyakawa, and K. Mizuta, unpublished data.
3 K. Miyoshi and K. Mizuta, unpublished data.
Normal Assembly of 60 S Ribosomal Subunits Is Required for the Signaling in Response to a Secretory Defect in *Saccharomyces cerevisiae*

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