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Differential Subcellular Localization of Ribosomal Protein L7 Paralogs in *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, ribosomal protein L7, one of the ~46 ribosomal proteins of the 60S subunit, is encoded by paralogous *RPL7A* and *RPL7B* genes. The amino acid sequence identity between Rpl7a and Rpl7b is 97 percent; they differ by only 5 amino acid residues. Interestingly, despite the high sequence homology, Rpl7b is detected in both the cytoplasm and the nucleolus, whereas Rpl7a is detected exclusively in the cytoplasm. A site-directed mutagenesis experiment revealed that the change in the amino acid sequence of Rpl7b does not influence its subcellular localization. In addition, introns of *RPL7A* and *RPL7B* did not affect the subcellular localization of Rpl7a and Rpl7b. Remarkably, Rpl7b was detected exclusively in the cytoplasm in *rpl7a* knockout mutant, and overexpression of Rpl7a resulted in its accumulation in the nucleolus, indicating that the subcellular localization of Rpl7a and Rpl7b is influenced by the intracellular level of Rpl7a. Rpl7b showed a wide range of localization patterns, from exclusively cytoplasmic to exclusively nucleolar, in knockout mutants for some rRNA-processing factors, nuclear pore proteins, and large ribosomal subunit assembly factors. Rpl7a, however, was detected exclusively in the cytoplasm in these mutants. Taken together, these results suggest that although Rpl7a and Rpl7b are paralogous and functionally replaceable with each other, their precise physiological roles may not be identical.

INTRODUCTION

In eukaryotes, ribosome biogenesis takes place largely within the nucleolus in a multistep process. Although eukaryotic ribosomes function in the cytoplasm, the synthesis, processing, and assembly of the 40S and 60S ribosomal subunits in *Saccharomyces cerevisiae* and higher eukaryotes occur in the nucleolus. A ribosome is composed of four rRNA species and ~80 ribosomal proteins that are distributed between the 40S and 60S subunits (Tate and Poole, 2004). The 18S, 5.8S, and 25S rRNAs are derived from a single 35S rRNA precursor that is synthesized by RNA polymerase I and then processed by a series of endonucleolytic cleavages (Venema and Tollervey, 1999). The 5S rRNA is synthesized separately by RNA polymerase III and associates with the 60S pre-ribosomal subunit early in assembly. The mature 40S ribosomal subunit contains the 18S rRNA and ~32 ribosomal proteins, while the 60S subunit is composed of the 5S, 5.8S, and 25S rRNAs and ~46 ribosomal proteins.

In *S. cerevisiae*, many ribosomal protein genes are duplicated and encode identical or nearly identical gene products. Remarkably, 59 of the 78 ribosomal proteins are encoded by two genomic copies. Following the initial discovery of duplicated ribosomal protein genes, growth rates were measured in knockout mutants for the ribosomal protein L16 to determine whether the paralogous *RPL16A* and *RPL16B* genes were functionally distinct. The correlation between fitness defects and expression levels of ribosomal proteins, together with the observation that overexpression of one ribosomal protein gene rescued the growth defect from the deletion of its paralog, led to the conclusion that duplicated ribosomal protein genes are functionally redundant with the more highly expressed paralog playing more significant roles in cells (Rotenberg et al., 1988).

However, recent studies reveal a more complex relationship between paralogous ribosomal proteins. Recent high-throughput analyses have suggested more subtle differences between duplicated ribosomal protein genes, including paralog-specific defects in sporulation (Enyenihi and Saunders, 2003), actin organization (Haarer et al., 2007), and bud-site selection (Ni and Snyder, 2001). Although these studies suggest the functional specificity of duplicated ribosomal protein paralogs, the underlying mechanisms remain unclear.

Protein localization information is a valuable resource in elucidating the function of eukaryotic proteins. Several studies have proven that alteration in subcellular localization is an important mechanism for regulating protein function. Based on the assumption that the functional specificity of duplicated ribosomal protein paralogs might be reflected in their subcellular localization, we checked whether duplicated ribosomal proteins show differential localization, using the protein localization information available from the Yeast GFP Fusion Localization Database (http://yeastgfp.ucsf.edu/) (Huh et al., 2003). From among several duplicated ribosomal proteins, we found that Rpl7a and Rpl7b show distinct subcellular localization. Rpl7b is

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detected in both the cytoplasm and the nucleolus, whereas Rpl7a is detected exclusively in the cytoplasm. In the present study, we sought to find out the factors that affect the distinct localization of Rpl7a and Rpl7b. We found that several factors can differentially affect the subcellular localization of Rpl7a and Rpl7b. Our results suggest that although Rpl7a and Rpl7b are paralogous and functionally replaceable with each other, their physiological roles may not be identical.

MATERIALS AND METHODS

Media and culture conditions

The yeast strains used in this study are listed in Table 1. Rich medium (1% yeast extract, 2% peptone, 2% glucose; YPD) and synthetic complete medium (0.67% yeast nitrogen base without amino acids, 2% glucose; SC medium) lacking appropriate amino acids for selection were prepared as described previously (Sherman, 2002). The cells were grown to the mid-logarithmic phase at 30°C as described previously (Lee and Lee, 2008).

Gene manipulation

Gene deletion was carried out by a one-step PCR-based method (Wach, 1996). The *URA3* or *KanMX4* gene was used as a selectable marker to replace the target gene. To construct chromosomally GFP-tagged strains, gene-specific oligonucleotide primers were synthesized, each of which had been designed to share sequences complementary to the GFP tag-marker cassette at the 3′ end and contain 40 bp of homology with a specific gene of interest to allow in-frame fusion of the GFP tag at the C-terminal coding region of the gene. Gene-specific cassettes containing a GFP tag and a selection marker were then generated by PCR using pFA6a-GFP(S65T)-His3MX6 or pFA6a-GFP(S65T)-KIURA3 as a template (Sung et al., 2008). Appropriate haploid yeast strains were transformed with the PCR products and selected on SC medium lacking histidine or uracil. Integration of the cassette by homologous recombination was verified by genomic PCR of samples from individual colonies with a primer internal to the GFP tag and a separate gene-specific primer designed to produce a product of approximately 500 bp.

Construction of plasmids

For cloning *RPL7A-GFP* into the pTZ18R vector (Pharmacia, USA), the *RPL7A-GFP* sequence was amplified by PCR using the genomic DNA of a yeast strain expressing GFP-tagged *RPL7A* as the template, a forward primer, 5′-CAGTCTCTAGAACGAGCTAATGTCTTATATCTC-3′, containing an *Xba*I enzyme recognition site, and a reverse primer, 5′-GTCAAAGCTTATTCGCTTATTTAGAAGTGG-3′, containing a *Hin*dIII enzyme recognition site. For cloning *RPL7B-GFP* into the pTZ18R vector, the *RPL7B-GFP* sequence was amplified by PCR using the genomic DNA of a yeast strain expressing GFP-tagged *RPL7B* as the template, a forward primer, 5′-CAGTCTCTAGACAATAACGTTCAGTTGTGGTTC-3′, containing an *Xba*I enzyme recognition site, and a reverse primer, 5′-GTCAAAGCTTATTCGCTTATTTAGAAGTGG-3′, containing a *Hin*dIII enzyme recognition site. The obtained PCR products were digested with *Xba*I and *Hin*dIII and cloned into the *Xba*I-*Hin*dIII site of the pTZ18R vector. To generate the *URA3* CEN plasmid for the expression of *RPL7A-GFP* and *RPL7B-GFP* in yeast cells, *Xba*I-*Hin*dIII fragments from pTZ18R-RPL7A-GFP and pTZ18R-RPL7B-GFP were cloned into the *Xba*I-*Hin*dIII site of the pRS416 vector (Sikorski and Hieter, 1989), yielding pRS416-RPL7A-GFP and pRS416-RPL7B-GFP, respectively. For expressing intronless *RPL7A-GFP*, the total RNA was extracted and reverse transcription PCR was performed using the forward primer 5′-CTGATCTAGAACCAAGCAAATTAAGATCAC-3′ and the reverse primer 5′-GTCAAAGCTTATTCGCTTATTTAGAAGTGG-3′. For expression of intronless *RPL7B-GFP*, the total RNA was extracted and reverse transcription PCR was performed using the forward primer 5′-AGACTCTAGATACAAATCTCCATCAACGTC-3′ and the reverse primer 5′-GTCAAAGCTTATTCGCTTATTTAGAAGTGG-3′. The obtained PCR products were digested with *Xba*I and *Hin*dIII and cloned into the *Xba*I-*Hin*dIII site of the p416ADH vector (Mumberg et al., 1995).

Site-directed mutagenesis

Rpl7b mutants were generated by the QuikChange Multi site-directed mutagenesis protocols (Stratagene, USA). To generate each mutant, a mutant strand synthesis reaction was performed using pTZ18R-RPL7B-GFP as the template and mutagenic oligonucleotide primers. The amplified products were subsequently digested with *Dpn*I to remove the parental template DNA and transformed into competent *Escherichia coli* TG1 cells. For amplification of the mutant plasmid, transformed TG1 cells were inoculated into 5 ml Luria broth with 100 μg/ml ampicillin. Plasmid DNA was purified from the overnight culture, using the Plasmid Mini Extraction Kit (Bioneer, Korea). Successful mutagenesis was confirmed by sequencing. This process was repeated for the 5 different amino acids between Rpl7a and Rpl7b (S2A, T3A, T16S, I26V, A242S).

Fluorescence microscopic analysis

Fluorescence microscopic analysis was performed as described previously (Sung and Huh, 2007). Yeast cells cultured to the mid-logarithmic phase in SC dropout medium were microscopically analyzed in 96-well glass bottom microplates (Whatman, UK) pre-treated with concanavalin A (Sigma, USA) to ensure cell adhesion. Microscopy was performed on a Zeiss Axiovert 200M inverted microscope with a Plan-NeoFluar 100×/1.3 NA oil immersion objective. Images were recorded on a Zeiss Axiocam MRm with 2 × 2 binning. Fluorescence images for GFP were captured using a standard fluorescein isothiocyanate filter set (excitation band pass filter, 450-490 nm; beam splitter, 510 nm; emission band pass filter, 515-565 nm).

RESULTS AND DISCUSSION

Rpl7a and Rpl7b show differential subcellular localization

It is believed that the *S. cerevisiae* genome carries 137 genes encoding the cytoplasmic ribosomal proteins (Planta and Mager, 1998). We retrieved the subcellular localization information for all the 137 ribosomal proteins available from the Yeast GFP Fusion Localization Database (http://yeastgfp.ucsf.edu/) (Huh et al., 2003) and classified the proteins according to their subcellular localization (Table 2). Except the 21 proteins for which subcellular localization has not been determined, most of the GFP-fused ribosomal proteins (107 out of 137) are detected in the cytoplasm. Among the 9 other proteins, 3 are detected in both the cytoplasm and the nucleolus, 2 in the nucleolus, 2 in the nucleus, 1 in both the cytoplasm and the nucleus, and 1 in both the nucleolus and the nucleus. Taking into account that the information available from the Yeast GFP Fusion Localization Database represents the steady-state localization, these localization patterns suggest that the majority of the GFP-fused ribosomal proteins are found in the cytoplasm, presumably as components of the mature ribosome.

Ribosomal protein L7 is encoded by two duplicated genes, namely, *RPL7A* (YGL076C) and *RPL7B* (YPL198W), in *S. cerevisiae* (Mizuta et al., 1992). *RPL7A* is located on chromo-

Table 1. Yeast strains used in this study

Strain	Genotype	Source
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Research Genetics
KY0101	BY4741 *RPL7A-GFP*::*His3MX6*	Huh et al. (2003)
KY0102	BY4741 *RPL7B-GFP*::*His3MX6*	Huh et al. (2003)
KY0103	BY4741 *RPL7B-GFP*::*His3MX6* (pRS416-NOP56-RFP)	This study
KY0111	BY4741 *rpl7bΔ*::*KanMX4* (pRS416-RPL7B(S2A)-GFP)	This study
KY0112	BY4741 *rpl7bΔ*::*KanMX4* (pRS416-RPL7B(T3A)-GFP)	This study
KY0113	BY4741 *rpl7bΔ*::*KanMX4* (pRS416-RPL7B(T16S)-GFP)	This study
KY0114	BY4741 *rpl7bΔ*::*KanMX4* (pRS416-RPL7B(I26V)-GFP)	This study
KY0115	BY4741 *rpl7bΔ*::*KanMX4* (pRS416-RPL7B(A242S)-GFP)	This study
KY0116	BY4741 *rpl7bΔ*::*KanMX4* (pRS416-RPL7B(S2A/T3A/T16A/I26V/A242S)-GFP)	This study
HY0622	BY4741 *rpl7bΔ*::*KlURA3 RPL7A-GFP*::*His3MX6*	This study
HY0623	BY4741 *rpl7aΔ*::*KlURA3 RPL7B-GFP*::*His3MX6*	This study
HY0624	BY4741 (pRS416-RPL7A-GFP)	This study
HY0625	BY4741 (pRS416-RPL7A-GFP)	This study
HY0628	BY4741 *rpl7aΔ*::*KanMX4* (p416ADH-RPL7A-GFP)	This study
HY0629	BY4741 *rpl7bΔ*::*KanMX4* (p416ADH-RPL7B-GFP)	This study
HY0636	BY4741 *kap120Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0637	BY4741 *kap120Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0638	BY4741 *kap123Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0639	BY4741 *kap123Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0640	BY4741 *nup170Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0641	BY4741 *nup170Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0642	BY4741 *cgr1Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0643	BY4741 *cgr1Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0646	BY4741 *kap114Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0647	BY4741 *kap114Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0648	BY4741 *loc1Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0649	BY4741 *loc1Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0650	BY4741 *los1Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0651	BY4741 *los1Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0652	BY4741 *msn5Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0653	BY4741 *msn5Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0654	BY4741 *npl3Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0655	BY4741 *npl3Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0658	BY4741 *nsr1Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0659	BY4741 *nsr1Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0660	BY4741 *nup2Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0661	BY4741 *nup2Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0682	BY4741 *nup42Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0683	BY4741 *nup42Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0684	BY4741 *nup84Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0685	BY4741 *nup84Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0686	BY4741 *nup100Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0687	BY4741 *nup100Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0688	BY4741 *nup120Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0689	BY4741 *nup120Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0690	BY4741 *nup133Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0691	BY4741 *nup133Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0692	BY4741 *puf6Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0693	BY4741 *puf6Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0694	BY4741 *slx9Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0695	BY4741 *slx9Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study
HY0696	BY4741 *sxm1Δ*::*KanMX4 RPL7A-GFP*::*KlURA3*	This study
HY0697	BY4741 *sxm1Δ*::*KanMX4 RPL7B-GFP*::*KlURA3*	This study

Table 2. Subcellular localization of ribosomal proteins in *S. cerevisiae*

Subcellular localization	Ribosomal proteins
Cytoplasm	Rps0a, Rps0b, Rps1a, Rps1b, Rps4a, Rps4b, Rps6a, Rps6b, Rps7a, Rps7b, Rps8a, Rps8b, Rps9a, Rps9b, Rps10a*, Rps10b, Rps11a, Rps11b, Rps16a, Rps16b, Rps17a, Rps17b, Rps18a, Rps18b, Rps19a, Rps19b, Rps21a, Rps21b, Rps23a, Rps23b, Rps24a, Rps24b, Rps25a, Rps25b, Rps26a, Rps26b, Rps27a, Rps27b, Rps28a, Rps28b, Rps29a, Rps29b, Rps30a, Rps30b, Rpp1a, Rpp1b, Rpp2a, Rpp2b, Rpl1a, Rpl1b, Rpl2a, Rpl3, Rpl4a, Rpl4b, Rpl5, Rpl6a, Rpl6b, Rpl7a, Rpl8a, Rpl8b, Rpl9a, Rpl9b, Rpl10, Rpl11a, Rpl11b, Rpl12a, Rpl12b, Rpl13a, Rpl13b, Rpl14a, Rpl14b, Rpl16a, Rpl17a, Rpl17b, Rpl18b, Rpl19a, Rpl19b, Rpl20a, Rpl20b, Rpl21a, Rpl21b, Rpl22a, Rpl22b, Rpl24a, Rpl24b, Rpl26a, Rpl26b, Rpl27b, Rpl29, Rpl31b, Rpl33b, Rpl34a, Rpl34b, Rpl35a, Rpl35b, Rpl36a, Rpl36b, Rpl37a, Rpl37b, Rpl38, Rpl40b, Rpl41a, Rpl41b, Rpl42a, Rpl42b, Rpl43a, Rpl43b
Cytoplasm/Nucleolus	Rps14b, Rpl7b, Rpl27a
Cytoplasm/Nucleus	Rpl40a
Nucleolus	Rps22a, Rps22b
Nucleus	Rpl23a, Rpl23b
Nucleolus/Nucleus	Rpl15b
Not determined	Rps2, Rps3, Rps5, Rps12, Rps13, Rps14a, Rps15, Rps20, Rps31, Rpp0, Rpl2b, Rpl15a, Rpl16b, Rpl18a, Rpl25, Rpl28, Rpl30, Rpl31a, Rpl32, Rpl33a, Rpl39

*Subcellular localization of Rps10a is wrongly assigned as "Nucleolus/Nucleus" in the Yeast GFP Fusion Localization Database (data not shown).

some VII, while *RPL7B* is located on chromosome XVI. Disruption of both these genes is lethal. Unlike other duplicated ribosomal protein genes, these genes are interrupted by two introns containing long conserved sequences. Two of the small nucleolar RNAs (snoRNAs), snR39 and snR59, are embedded in the introns of the *RPL7A* and *RPL7B* genes, respectively (Ghazal et al., 2005). The expression level of Rpl7a is about 14 times that of Rpl7b (Ghaemmaghami et al., 2003). A comparison of amino acid sequences revealed that Rpl7a and Rpl7b are 97% identical and differ only in 5 amino acids at residues 2, 3, 16, 26, and 242 (Fig. 1A). Interestingly, despite the high sequence homology between Rpl7a and Rpl7b, the Rpl7b-GFP fusion protein was detected in both the cytoplasm and the nucleolus, whereas Rpl7a-GFP fusion protein was detected exclusively in the cytoplasm (Fig. 1B).

The change in the amino acid sequence of Rpl7b does not influence its subcellular localization

We first assumed that the difference in the amino acid sequence might lead to the differential subcellular localization of Rpl7a and Rpl7b. To examine this possibility, the *RPL7B-GFP* sequence was cloned into the pTZ18R vector and site-directed mutagenesis was performed for the 5 amino acid residues of Rpl7b that are different from Rpl7a. The mutated *RPL7B-GFP* sequence was confirmed by sequencing, subcloned into the pRS416 vector, and expressed in the *rpl7b*Δ cells. Unexpectedly, none of the five mutants (Rpl7b(S2A), Rpl7b(T3A), Rpl7b(T16S), Rpl7b(I26V), or Rpl7b(A242S)) showed alteration in subcellular localization (Fig. 2A). The Rpl7b mutants derived from the double, triple, or quadruple site-directed mutagenesis also did not show alteration in subcellular localization (data not shown). Next, we mutated all the 5 amino acid residues of Rpl7b so that the amino acid sequence of the mutated Rpl7b could be identical to that of Rpl7a. Notably, even this Rpl7b mutant, with the same amino acid sequence as that of Rpl7a, continued to show the same localization pattern as wild-type Rpl7b (Fig. 2A). This result

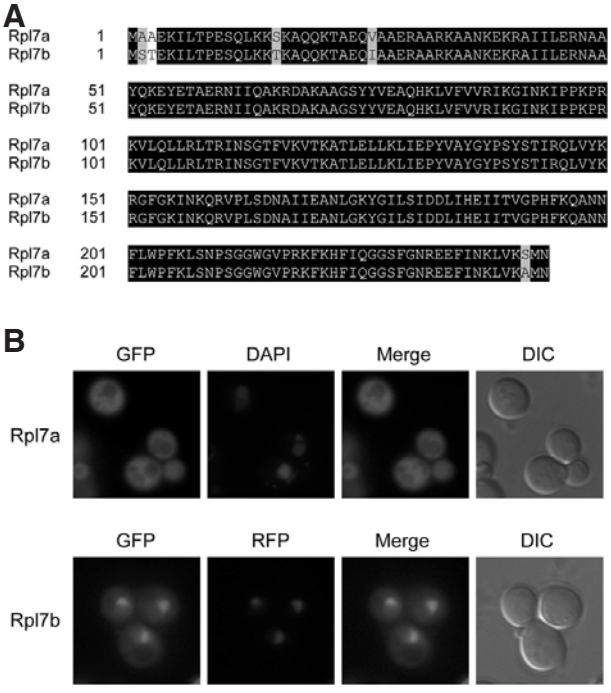

Fig. 1. Rpl7a and Rpl7b share high sequence homology but show differential subcellular localization. (A) Alignment of the amino acid sequences of Rpl7a and Rpl7b. The numbers on the left side refer to the amino acid positions in each sequence. Identical residues are shown against a black background and similar residues are shaded in gray. (B) Fluorescence microscopic analysis of subcellular localization of Rpl7a and Rpl7b. RFP-tagged Nop56 was used as a nucleolar marker (Huh et al., 2003). Fluorescence microscopic images for Rpl7a-GFP and Rpl7b-GFP were captured using a standard fluorescein isothiocyanate filter set and merged with images for DAPI or Nop56-RFP.

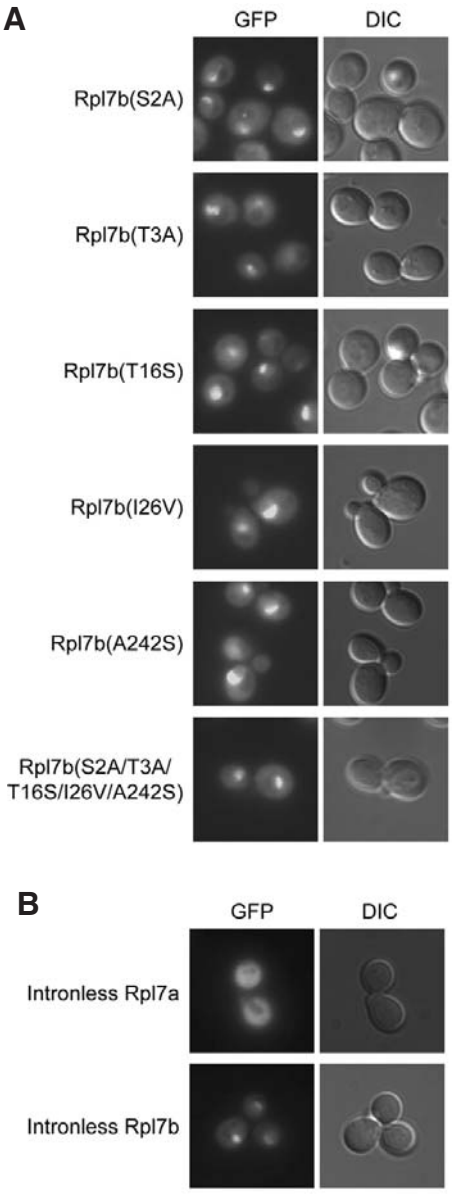

Fig. 2. The change in the amino acid sequence of Rpl7b does not influence its subcellular localization. (A) Fluorescence microscopic analysis of the subcellular localization of site-directed mutagenized Rpl7b. The mutated Rpl7b-GFP was expressed under its own promoter on pRS416 in *rpl7b*Δ cells. (B) Fluorescence microscopic analysis of the subcellular localization of intronless Rpl7a and Rpl7b. cDNAs for Rpl7a-GFP and Rpl7b-GFP were expressed under the *ADH1* promoter on pRS416 in *rpl7a*Δ and *rpl7b*Δ cells, respectively.

suggested that Rpl7a can have the localization characteristic similar to that of Rpl7b when Rpl7a is expressed in the environment of *RPL7B* gene.

Introns of *RPL7A* and *RPL7B* do not affect subcellular localization of Rpl7a and Rpl7b

Unlike other duplicated ribosomal protein genes, the *RPL7A* and *RPL7B* genes are interrupted by two introns, which contain snoRNAs-snR39 and snR59, respectively (Ghazal et al., 2005). To determine if the presence of the introns or snoRNAs em-

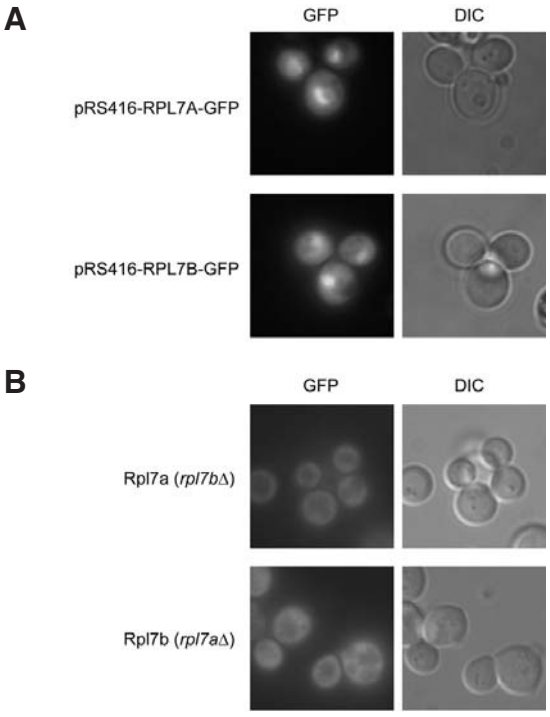

Fig. 3. The expression level of Rpl7a affects its subcellular localization and that of Rpl7b. (A) Fluorescence microscopic analysis of subcellular localization of overexpressed Rpl7a and Rpl7b. Rpl7a-GFP and Rpl7b-GFP were expressed under their own promoters on pRS416 in wild-type cells. (B) Fluorescence microscopic analysis of subcellular localization of Rpl7a and Rpl7b in mutants for their respective paralog. Subcellular localization of Rpl7a and Rpl7b was monitored in *rpl7b*Δ and *rpl7a*Δ cells, respectively.

bedded in the introns might affect the subcellular localization of Rpl7a and Rpl7b, we cloned and expressed the cDNAs of *RPL7A* and *RPL7B* under the *ADH1* promoter in *rpl7a*Δ and *rpl7b*Δ cells, respectively. These intronless Rpl7a and Rpl7b still showed the same localization patterns as wild-type Rpl7a and Rpl7b, respectively; intronless Rpl7a was detected in the cytoplasm, and intronless Rpl7b was detected in both the cytoplasm and the nucleolus (Fig. 2B). This result indicated that the presence of introns or snoRNAs embedded in the introns does not affect the subcellular localization of Rpl7a and Rpl7b nor does it have any bearing on their differential subcellular localization.

The expression level of Rpl7a affects its subcellular localization and that of Rpl7b

Although the amino acid sequences of Rpl7a and Rpl7b are almost identical, their expression levels are quite different; the level of Rpl7a is about 14 times that of Rpl7b (Ghaemmaghami et al., 2003). We checked if the expression levels of Rpl7a and Rpl7b could affect their localization. The *RPL7A-GFP* sequence was cloned into the pRS416 vector and expressed under its own promoter in wild-type cells so that Rpl7a could be expressed at a level about twice the normal. Remarkably, in these Rpl7a-overexpressing cells, Rpl7a was detected not only in the cytoplasm but also in the nucleolus (Fig. 3A, upper panel). We also expressed *RPL7B-GFP* sequence under its own promoter in wild-type cells so that Rpl7b could be about twice more expressed than its natural level. These Rpl7b-overex-

Table 3. Factors affecting subcellular localization of Rpl7b

rRNA-processing factors	Nuclear pore proteins	Nuclear transport factors	Ribosomal large subunit assembly factors
Mutants in which Rpl7b was exclusively localized to the cytoplasm			
	*los1*Δ, *nup2*Δ, *nup170*Δ		
Mutants in which Rpl7b was exclusively localized to the nucleolus			
*cgr1*Δ, *nsr1*Δ, *slx9*Δ			*loc1*Δ, *puf6*Δ
Mutants in which subcellular localization of Rpl7b was not altered			
	*nup42*Δ, *nup84*Δ, *nup100*Δ, *nup120*Δ, *nup133*Δ	*kap114*Δ, *kap120*Δ, *kap123*Δ, *msn5*Δ, *npl3*Δ, *sxm1*Δ	

pressing cells showed the same localization pattern as the cells with normal Rpl7b levels (Fig. 3A, lower panel). Next, we examined if the absence of Rpl7a or Rpl7b could affect the subcellular localization of its paralog. We first deleted the *RPL7B* gene in cells chromosomally expressing *RPL7A-GFP* and monitored the subcellular localization of Rpl7a. Rpl7a in these *rpl7b*Δ cells showed the same localization pattern as that in wild-type cells (Fig. 3B, upper panel). We then deleted the *RPL7A* gene in cells chromosomally expressing *RPL7B-GFP* and monitored the subcellular localization of Rpl7b. Remarkably, in these *rpl7a*Δ cells, Rpl7b was detected exclusively in the cytoplasm (Fig. 3B, lower panel). Taken together, these results demonstrated that the expression level of Rpl7a, the major form of ribosomal protein L7, influences its own subcellular localization and that of Rpl7b.

Rpl7a appears to be more preferentially incorporated into the 60S ribosomal subunit than Rpl7b

To check whether the GFP-fused ribosomal protein L7 is incorporated into the ribosome complex, the growth rate of the cells expressing Rpl7a-GFP or Rpl7b-GFP was measured and compared with that of *rpl7a*Δ or *rpl7b*Δ cells. As expected, *rpl7a*Δ cells showed significant retarded growth, while *rpl7b*Δ cells showed a growth pattern indistinguishable from wild-type cells (data not shown). This observation correlated with the fact that the endogenous protein level of Rpl7a is about 14 times higher than that of Rpl7b (Ghaemmaghami et al., 2003). The cells expressing Rpl7b-GFP also showed a growth pattern similar to that of wild-type cells. Surprisingly, the cells expressing Rpl7a-GFP showed very retarded growth; their growth was even slower than that of *rpl7a*Δ cells. This result suggested that the GFP fusion interferes with the function of Rpl7a and thus inhibits the activity of ribosome. It appears that the GFP-fused Rpl7a is incorporated into the mature ribosome complex, because if Rpl7a-GFP is not incorporated into the ribosome complex and thus does not affect the ribosomal function, the cells expressing Rpl7a-GFP would show a growth pattern similar to that of *rpl7a*Δ cells.

Ribosomal protein genes are responsible for nearly 40% of the RNA polymerase II transcription initiation events and the most coordinately regulated cluster of genes in *S. cerevisiae* (DeRisi et al., 1997; Gasch et al., 2000). Through tight regulation, ribosomal proteins are supposedly synthesized on an equimolar basis. The newly synthesized ribosomal proteins are imported into the nucleus; they then accumulate in the nucleolus and assemble with the rRNAs to form ribosome subunits. A recent study reported that ribosomal proteins are expressed at high levels, beyond those required for typical ribosomal subunit production and accumulate in the nucleolus more quickly than other nucleolar components. This is balanced by continual degradation of the unassembled ribosomal proteins in the nucleoplasm, thereby providing a mechanism for cells to ensure that ribosomal protein levels are never rate-limiting for the efficient assembly of ribosome subunits (Lam et al., 2007). Our results correlate with this report. When overexpressed, Rpl7a was detected not only in the cytoplasm but also in the nucleolus (Fig. 3A, upper panel). Detection of overexpressed Rpl7a in the nucleolus as well as the cytoplasm seems to be due to the accumulation of Rpl7a in the nucleolus as it was synthesized beyond the normally required level. In contrast, Rpl7b, whether overexpressed or not, was detected in the cytoplasm and the nucleolus (Fig. 3A, lower panel). We hypothesize that Rpl7a is more preferentially incorporated into the 60S ribosomal subunit than Rpl7b. This hypothesis explains why Rpl7b is detected exclusively in the cytoplasm in *rpl7a*Δ cells (Fig. 3B, lower panel). In wild-type cells where both Rpl7a and Rpl7b are normally expressed, Rpl7a is preferentially incorporated into the 60S subunit and the redundant Rpl7b is accumulated in the nucleolus. In *rpl7a*Δ cells, however, Rpl7a is not synthesized and Rpl7b can now be incorporated into the 60S subunit as a sole ribosomal protein L7 present in cells.

The subcellular localization of Rpl7b is affected by several factors

A recent study showed that paralogous ribosomal proteins exhibit differences in their subcellular localization in mutants for ribosomal assembly factors (Komili et al., 2007). This paralog-specific effect among ribosomal proteins led us to question whether the subcellular localization of Rpl7a and Rpl7b would also be affected by various extrinsic factors, such as rRNA-processing factors, nuclear pore proteins, nuclear transport factors, and large ribosomal subunit assembly factors. To address this question, *RPL7A-GFP* and *RPL7B-GFP* were chromosomally expressed under their own promoters in a panel of mutant strains for the above mentioned factors (Table 3) and the effect of each mutation was monitored on the subcellular localization of Rpl7a and Rpl7b. In all the mutants, Rpl7a was detected exclusively in the cytoplasm as in wild-type cells (Fig. 4A; data not shown), indicating that the subcellular localization of Rpl7a is not influenced by these mutations. However, Rpl7b showed a wide range of localization patterns, from exclusively cytoplasmic to exclusively nucleolar, in these mutants. For example, in a knockout mutant for Nup170, an abundant subunit of the nuclear pore complex, Rpl7b was detected exclusively in

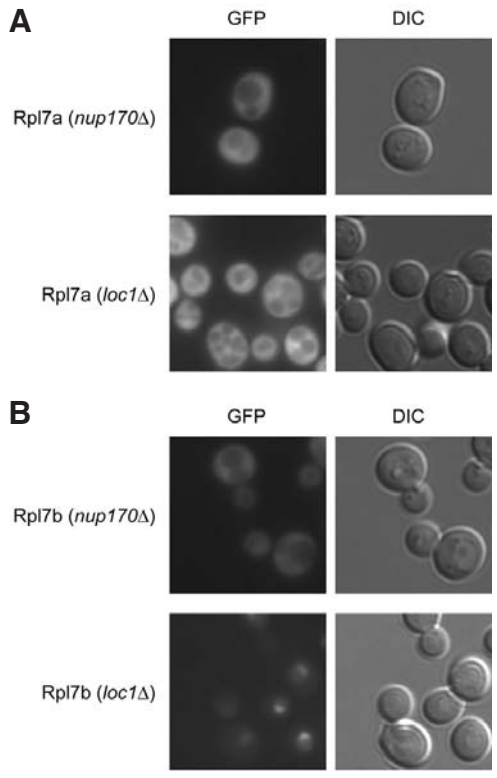

Fig. 4. The subcellular localization of Rpl7b is affected by several factors. (A) Fluorescence microscopic analysis of subcellular localization of Rpl7a in mutants for Nup170 and Loc1. Subcellular localization of Rpl7a was monitored in *nup170*Δ (upper panel) and *loc1*Δ cells (lower panel). (B) Fluorescence microscopic analysis of the subcellular localization of Rpl7b in mutants for Nup170 and Loc1. Subcellular localization of Rpl7b was monitored in *nup170*Δ (upper panel) and *loc1*Δ cells (lower panel).

the cytoplasm (Fig. 4B, upper panel). In contrast, Rpl7b was detected almost exclusively in the nucleolus in a knockout mutant for Loc1, a large ribosomal subunit assembly factor (Fig. 4B, lower panel). The effects of all mutations on the subcellular localization of Rpl7b are summarized in Table 3.

It is surprising that the subcellular localization of Rpl7b was affected by several factors, whereas that of Rpl7a was not. Our results show that some rRNA-processing factors, nuclear pore proteins, and large ribosomal subunit assembly factors can differentially affect the subcellular localization of ribosomal protein L7 paralogs. This raises the possibility that nuclear transport, interaction with rRNA, or ribosomal assembly of Rpl7a may occur in a manner different from that of Rpl7b and although Rpl7a and Rpl7b are paralogous, their precise physiological roles may not be identical. Several recent studies supporting this notion have reported that paralogous ribosomal proteins are functionally distinct (Degenhardt and Bonham-Smith, 2008; Enyenihi and Saunders, 2003; Haarer et al., 2007; Ni and Snyder, 2001). Furthermore, a line of evidence suggests that several ribosomal proteins have extraribosomal functions other than conventional protein synthesis (Amsterdam et al., 2004; Draptchinskaia et al., 1999; Dresios et al., 2006; Enerly et al., 2003; Lohrum et al., 2003; Mazumder et al., 2003; Wool, 1996). Thus, a further study into the functional specificity of duplicated ribosomal proteins would provide a new level of understanding of the functions of ribosomal proteins.

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