We have identified a yeast gene encoding a protein structurally similar to mammalian nucleolin. The gene was previously cloned as a cold shock-inducible gene and found to be identical to yeast NSR1 gene, which encodes a protein that has been reported to bind sequences required for nuclear localization of protein. The carboxyl-terminal half of NSR1, consisting of two tandemly repeated putative RNA-binding domains and a glycine/arginine-rich domain, has 37% amino acid sequence identity with the same part of mammalian nucleolin, while no sequence similarities are found between their amino-terminal regions. Although a null mutation of the NSR1 gene was not lethal, it caused a severe defect on growth. Pulse-labeling analysis revealed that the nsrl strain had reduced levels of 18 S rRNA and accumulated 35 S pre-rRNA compared with the wild-type strain. The level of 25 S rRNA was also slightly reduced in the nsrl strain. Pulse-chase labeling experiments showed slow processing of 35 S pre-rRNA and impaired methylation of 18 S rRNA. The ratio of 40 S to 60 S ribosomal subunits in the nsrl strain is significantly reduced and is consistent with impaired synthesis of 18 S rRNA. The results indicate that NSR1 is involved in pre-rRNA processing and ribosome biosynthesis in yeast.

Eukaryotic ribosome biosynthesis occurs in the nucleolus, a subnuclear organelle where pre-ribosomal RNA (pre-rRNA) is first synthesized. The pre-rRNA is processed to smaller mature rRNAs, while assembling into ribosomal subunits for transport to the cytoplasm (see reviews by Warner, 1989; Reeder, 1990; Hernandez-Verdun, 1991). As the rRNA is transcribed and processed, it assembles with a large number of proteins. Most of them are ribosomal proteins and are found in the mature ribosomal subunits. Several other proteins specifically located in the nucleolus but not a part of the ribosomal subunits have been identified and characterized. They are associated with the rRNA and likely to be involved in transcription and processing of pre-rRNA, as well as packaging and transport of ribosomal particles (Reeder, 1990; Hernandez-Verdun, 1991). Nucleolin is a major nucleolar protein and has been extensively studied (see review by Jordan, 1987). The expression of nucleolin in the cell is correlated directly with rRNA transcription (Bouche et al., 1987; Caircigues-Ferrer et al., 1989). In vitro experiments suggested that nucleolin is associated with pre-rRNA (Bugler et al., 1987) and involved in its transcriptional control (Bouche et al., 1984) as well as chromatin decondensation (Brand et al., 1988).

In yeast Saccharomyces cerevisiae, SSBl and NOP1 have been identified as nucleolar proteins (Jong et al., 1987; Schimang et al., 1989; Henriquez et al., 1990). NOP1 is an essential gene encoding a protein similar to fibrillarin, a mammalian nucleolar protein, and is required for pre-rRNA processing (Tollervey et al., 1991). SSBl is a dispensable gene encoding an RNA-binding protein, which is associated with small nuclear RNAs (Jong et al., 1987; Clark et al., 1990). In this report, we describe a gene of S. cerevisiae encoding a protein that has structural similarity to mammalian nucleolin and demonstrate that it is involved in pre-rRNA processing and ribosome production. This gene was originally identified as a cold shock-inducible gene by differential hybridization screening of a yeast genomic library (Kondo and Inouye, 1991), and it was found that its increased expression is required for normal pre-rRNA processing after cold shock (Kondo et al., 1992). The sequence was found to be identical to the yeast NSR1 gene which was identified to encode a nuclear localization sequence-binding protein (Lee et al., 1991).

**Materials and Methods**

**Strains and Growth Conditions**—S. cerevisiae S288C (a mal gal2) was used for DNA preparations. SP1 (a his3 ura3 trpl leu2 ade8 gal82 can1) and KN1 (a/q his3 his3 ura3/ura3 trpl1 trpl1 leu2/leu2 ade8/+ /ade8 lys2/+) were used as hosts for transformation. Transformation was carried out by the method of Ito et al. (1983). The strains constructed in this study have the following genotypes: KN101 (a his3 ura3 trpl1 leu2 ade8 gal82 can1' ura3::URA3), KN102 (a his3 ura3 trpl1 leu2 ade8 gal2 can1' ura3::URA3), and KN2 (a his3 trpl1 leu2 ade8 gal2 can1' ura3::URA3). KN2 was constructed by transformation of strain SP1 with plasmid Ylp5 (Scherer and Davis, 1979) linearized by digestion with Stul and used for labeling of RNA with [3H]uracil. YPD medium and synthetic complete (SC) medium were prepared as described by Rose et al. (1990). Cells were grown under the conditions previously described (Kondo and Inouye, 1991).

**Hybridization Analysis**—Preparation of DNA from strain S288C was previously described (Kondo and Inouye, 1991). DNA from the transformants was prepared using glass beads according to the method described by Rose et al. (1990). Southern hybridization analysis was carried out as previously described (Kondo and Inouye, 1991).

**DNA Sequencing**—DNA sequencing was performed by using the dyeoxyxynucleotide chain termination method (Sanger et al., 1977) using Sequenase (U. S. Biochemical Corp.) according to the instructions of the supplier.

**Deletion of Chromosomal NSR1 Gene**—A strain carrying a mutation in the NSR1 gene was constructed by the y-transformation method as described by Sikorski and Hieter (1989). A vector pRS306 (Sikorski and Hieter, 1989) was used to construct plasmid pIC516.
Yeast Gene Encoding Nucleolin-like Protein

for deletion of the NSR1 gene. The 0.7-kb HpaI-BglII fragment containing a 5′ end region of the ORF (see Fig. 1A) was cloned into pUC19 HindIII and BamHI sites. The resulting plasmid was digested with HindIII and KpnI, which generated a 0.7-kb fragment containing a 5′ region of the ORF. The HindIII-KpnI fragment and a 0.6-kb XbaI-HindIII fragment containing a short 3′-untranslated region of the ORF and 3′-untranslated region (see Fig. 1A) were cloned between the XbaI and KpnI sites of the pRS806 to create pICS16. Yeast haploid strain SP1 and diploid strain KN1 were transformed with plasmid pICS16 linearized by digestion with HindIII. This resulted in the replacement of a 0.35-kb BglII-XbaI fragment in the middle of the ORF with the vector sequence containing the URA3 gene.URA+ transformants were selected on SC plates lacking uracil. There was no significant difference in the efficiency of transformation between the two strains.

Recombinant Plasmids—Plasmid pYCB3 was constructed by inserting a 3.5-kb HindIII fragment, containing approximately a 2-kb 5′-untranslated region and the entire NSR1 gene, into the HindIII site of a centromeric plasmid pGS65 (kindly provided by J. Broach, Princeton University; the URA3 gene on plasmid YCP50 was replaced with the LEU2 gene). The HindIII fragment was prepared from plasmid pKCB20, which was isolated from a yeast genomic library by hybridization screening using the NSR1 gene as a probe.

Labeling of RNA and Electrophoresis—Labeling of RNA was performed by adding 50 μCi of [3H]uracil (1 μCi/μl, Amersham Corp.) into a 2-ml culture growing in SC medium lacking uracil at OD600 of 0.6. The labeling was carried out for 30 min at 30 °C. Cells were centrifuged and frozen until use. For pulse-chase labeling of RNA, unlabeled [methyl-3H]methionine, a 3-ml culture growing in SC medium lacking methionine were labeled at OD600 of 1.0 with 150 μCi of [methyl-3H]methionine (5 μCi/μl, Amersham Corp.) for 3 min at 30 °C. Unlabeled methionine was then added to a final concentration of 100 μg/ml, and aliquots (1 ml) were taken after 0, 2, and 5 min of chase and quickly centrifuged at room temperature. Pulse-chase labeling of RNA with [3H]uracil was carried out by adding 500 μCi of [3H]uracil (1 μCi/μl, Amersham Corp.), which was concentrated about 2-fold by lyophilization before use, to 5 ml of culture growing in SC medium lacking uracil at OD600 of 1.0 at 30 °C. After 5 min, unlabeled uracil was added to a final concentration of 200 μg/ml, and aliquots (1 ml) were taken after 0, 5, 10, 30, and 60 min of chase. Cell pellets were stored at –20 °C until used. RNA was prepared by the method described by Köhrer and Domdey (1991). Glass beads were used to improve the recovery of RNA. RNA was separated on a 1.2% agarose/6% formaldehyde gel (Kondo and Inouye, 1991) or on a 2% polyacrylamide/8M urea gel. RNA was separated on 10% polyacrylamide gels in the presence of 8M urea (Kondo and Inouye, 1991). RNA was separated on 10% polyacrylamide gels in the presence of 8M urea (Kondo and Inouye, 1991). RNA was separated on 10% polyacrylamide gels in the presence of 8M urea (Kondo and Inouye, 1991). RNA was separated on 10% polyacrylamide gels in the presence of 8M urea (Kondo and Inouye, 1991). RNA was separated on 10% polyacrylamide gels in the presence of 8M urea (Kondo and Inouye, 1991).

RESULTS

Sequence of the Gene Isolated as Cold Shock-inducible Gene and Comparison of the Amino Acid Sequence with Other Proteins—We previously performed a differential hybridization screening of a yeast genomic DNA library in order to identify the genes whose expression levels were changed by shifting the growth temperature from 30 to 10 °C. One of the genes, TIP1, whose expression was induced by cold and heat shock was found to encode a putative membrane protein of 210 amino acid residues (Kondo and Inouye, 1991). In this report we characterize a gene responsible for a cold shock-inducible 1.6-kb transcript. Restriction maps of the two plasmids (pDCSS2 and pDCSS84) containing the gene for the transcript are shown in Fig. 1A. The approximate position of the gene corresponding to the 1.6-kb transcript was determined by Southern blot hybridization of the plasmid DNA fragments with a 32P-labeled cDNA probe prepared from cold-shocked cells. From this analysis (data not shown), the region

\[\text{The abbreviations used are: kb, kilobase(s); ORF, open reading frame.}\]
ogous to that of mammalian nucleolin (Fig. 1B). There are two tandem repeats of 80 residues in the center of NSR1 (169-248, 268-347) exhibiting significant identity with the RNA recognition motifs of a number of RNA-binding proteins including nucleolin (Bandziulis et al., 1989; Query et al., 1989). Each repeat contains two highly conserved segments (RNP1 and RNP2, indicated in Fig. 1B) which are common in the RNA-binding domains of these proteins (Bandziulis et al., 1989). In addition, the glycine/arginine-rich domain at the carboxyl terminus (351-391) shows a high similarity with the carboxyl terminus of both proteins are rich in α-helices, and a predicted secondary structure indicates that the amino-terminal region of both proteins are rich in α-helices, and there are similarities in the domain arrangements and hydropathy profiles between the two proteins (see "Discussion"). These results suggest that NSR1 is evolutionarily related to nucleolin.

Effect of the nsrl Gene Deletion on Growth—To investigate the function of NSR1, a strain carrying a deletion of the NSR1 gene was constructed as described under "Materials and Methods." Transformations were performed using diploid and haploid strains as hosts, but no significant difference in the efficiency of transformation was detected (data not shown), indicating that the NSR1 gene is not essential for yeast. Southern analysis was carried out to verify disruption of the chromosomal NSR1 gene (Fig. 2). Digestion of genomic DNA of the haploid strain with HindIII produced a unique 3.5-kb fragment that hybridized with the NSR1 gene probe as shown in Fig. 2A, lane 1. In Ura+ transformants, the 3.5-kb fragment disappeared, and a new 7.5-kb band was detected due to an integration of plasmid DNA at the NSR1 locus (lanes 2 and 3, Fig. 2A). Hybridization using the vector plasmid pRS306 as a probe demonstrated that the 4.8- and 2.1-kb fragments derived from the chromosomal ura3 gene did not change after the transformation (Fig. 2B), while the 7.5-kb fragment was detected only in Ura+ transformants (lanes 2 and 3). Northern hybridization analysis demonstrated that there was no detectable transcript of the NSR1 gene in the nsrl deletion strain (data not shown).

The nsrl deletion strain grew more slowly than the wild-type strain in SC and YPD liquid medium and on agar plates at 30 °C. The doubling time of exponentially growing nsrl cells in SC medium was 3.2 h, which is 1.6-fold longer than that of the wild-type cells. When cells in the late stationary phase were diluted into fresh SC medium, a lag period of approximately 4 h was observed in both strains before cells resumed growth (Fig. 3). Reintroduction of the wild-type NSR1 gene on a centromeric plasmid vector restored the growth defect of the deletion strain, indicating that the slow growth of the nsrl cells was due to the absence of the NSR1 gene product (Fig. 3).

Synthesis of rRNA and Ribosomal Subunits in the nsrl Strain—Because of the sequence similarity of NSR1 to nucleolin, we attempted to examine the effect of the nsrl deletion on ribosome synthesis. First, we compared the rates for RNA and protein synthesis of the nsrl strain with those of the wild-type strain. Cells in log-phase were labeled with [3H] uracil or tranaS-label for a short time, and the radioactivity in the trichloroacetic acid precipitates were counted (Table I). The total incorporation of radioactivity into RNA and protein in the nsrl strain fell to about 50 and 80% of the levels of the wild-type strain, respectively. This result suggests that the rate of RNA synthesis was specifically reduced in the nsrl strain compared with the reduction of protein synthesis rate and growth rate of the nsrl strain. Synthesis of rRNA was examined by analyzing total RNA prepared from cells

![Fig. 2. Deletion of chromosomal NSR1 gene. DNA purified from the wild-type strain (SP1) (lane 1) and two independent Ura+ transformants (KN101) (lanes 2 and 3) were digested with HindIII, electrophoresed on an agarose gel, and transferred to a nylon filter. The filters were probed with the 0.6-kb Hpal-EcoRI fragment of the NSR1 gene (A) (see Fig. 1A) or with the vector plasmid pRS306 (B). The sizes of the HindIII fragments are denoted on the right of the autoradiograms.](image-url)

![Fig. 3. Effect of the nsrl deletion on growth rate. Wild-type cells (SP1) (open circles), nsrl cells (KN101) (solid circles), and nsrl cells harboring centromeric plasmid containing the NSR1 gene (plasmid pYCB3) (KN102) (triangles) were first grown until reaching late stationary phase in SC medium (SP1, KN101) or SC medium lacking leucine (KN102) at 30 °C. Cells were then diluted approximately 100-fold in fresh medium. Cell growth at 30 °C was monitored by measuring OD600.](image-url)
Comparison of protein and RNA synthesis in \textit{nsr1} and wild-type strains

Cells were grown in SC medium lacking methionine and uracil to OD\textsubscript{600} of 0.6 at 30 °C. Duplicates of 100 μl of culture were incubated with 1 μl of tran\textsuperscript{35}S-label (1 μCi/μl) or 2 μl of \textsuperscript{3}H]uracil (1 μCi/μl) at 30 °C for 2 or 4 min. After incubation, an equal volume of cold 20% trichloroacetic acid was added to stop the reaction, precipitates were collected by centrifugation, and washed with acetone. Average counts/OD\textsubscript{600} was then determined.

<table>
<thead>
<tr>
<th>Strain</th>
<th>\textit{NSR1} gene</th>
<th>\textsuperscript{35}S\textsuperscript{S} Trichloroacetic acid precipitable counts/OD\textsubscript{600} (%)</th>
<th>\textsuperscript{3}H]Trichloroacetic acid precipitable counts/OD\textsubscript{600} (%)</th>
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<td></td>
<td>2 min</td>
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<tr>
<td>KN2</td>
<td>+</td>
<td>5282 (100)</td>
<td>7565 (100)</td>
</tr>
<tr>
<td>KN101</td>
<td>−</td>
<td>4237 (80)</td>
<td>6086 (80)</td>
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Densitometric scanning of the film showed that the amounts of 25 S and 18 S rRNAs of the \textit{nsr1} strain were 70 and 20% of those of the wild-type strain, respectively, and the ratio of 18 S to 25 S rRNA decreased from 0.45 for the wild-type to 0.15 for the \textit{nsr1} strain.

To determine whether the differences observed above reflect kinetic alterations in processing, pulse-chase labeling of pre-rRNA with \textit{methyl-}\textsuperscript{3}H]methionine or \textit{[\textsuperscript{3}H]}uracil was performed. Since 35 S pre-rRNA is known to be methylated during or immediately after its synthesis (Warner, 1991), wild-type and \textit{nsr1} cells were labeled with \textit{methyl-}\textsuperscript{3}H]methionine for a short time (3 min) and chased with an excess amount of cold methionine for 0, 2, and 5 min (Fig. 5A). In the wild-type cells, pre-rRNA processing was very rapid so that the 35 S pre-rRNA was not detected even at time 0 under the present conditions (lane 1). In contrast, the unprocessed 35 S pre-rRNA was detected at time point 0 in the \textit{nsr1} cells (lane 4). In the wild-type cells, 20 S pre-rRNA was clearly observed in lane 1 and was further processed to 18 S rRNA (lanes 2 and 3). In the \textit{nsr1} cells, however, both 20 S and 18 S rRNAs were barely detectable throughout the chase experiments, while 27 S and 25 S rRNA were clearly detected (lanes 4–6). The slow processing of 35 S pre-rRNA and the greatly reduced levels of 18 S/20 S rRNAs in the \textit{nsr1} strain are consistent with the result shown in Fig. 4, although the ratio of 18 S to 25 S rRNA is significantly lower in Fig. 5A than the value obtained by the pulse labeling experiment in Fig. 4. We performed another pulse-chase labeling experiment using \textit{[\textsuperscript{3}H]}uracil for further understanding of rRNA processing (Fig. 5B). In this experiment, cells were labeled for 5 min and chased for 0, 5, 10, and 30 min. As observed in the pulse-chase experiment using \textit{methyl-}\textsuperscript{3}H]methionine (Fig. 5A), pre-rRNA processing was very rapid so that the 35 S pre-rRNA was hardly detected at time 0 in the wild-type cells, while 27 S and 20 S precursors were clearly observed (lane 1). In contrast, in the \textit{nsr1} cells the unprocessed 35 S pre-rRNA was detected until 10 min after chase (lanes 5–7), indicating the slow processing of the 35 S pre-rRNA. The band of 27 S pre-rRNA was clearly detected until 5 min after chase (lanes 5 and 6), but 20 S pre-rRNA was hardly detected throughout the chase experiment in the \textit{nsr1} cells. This observation is in agreement with the result of Fig. 5A. However, accumulation of 18 S rRNA was clearly detected in the \textit{nsr1} strain (Fig. 5B, lanes 5–8), whereas 18 S rRNAs were barely detectable.

FIG. 4. Effect of the \textit{nsr1} deletion on rRNA synthesis. Panel A, polyacrylamide/agarose gel electrophoresis of RNA labeled with \textit{[\textsuperscript{3}H]}uracil. Wild-type cells (KN2), \textit{nsr1} cells (KN101), and \textit{nsr1} cells harboring plasmid YCB3 (KN102) were pulse-labeled with [\textsuperscript{3}H]uracil for 30 min. Equal counts (2 × 10\textsuperscript{4} cpm) of labeled RNA were analyzed by 2% polyacrylamide/0.5% agarose gel electrophoresis. An autoradiogram of the dried gel is shown. Panel B, an autoradiogram after short exposure of the same gel is shown. Panel C, schematic representation of the pathway of pre-rRNA processing in \textit{S. cerevisiae} (Klootwijk and Planta, 1989).

FIG. 5. rRNA processing in wild-type and \textit{nsr1} strains. Panel A, wild-type strain (SP1) (lanes 1–3) and \textit{nsr1} strain (KN101) (lanes 4–6) were pulse-labeled with \textit{methyl-}\textsuperscript{3}H]methionine for 3 min and chased with an excess amount of unlabeled methionine for 0, 2, and 5 min at 30 °C. Equal counts (5 × 10\textsuperscript{4} cpm) of labeled RNA were analyzed on a 1.2% agarose/formaldehyde gel. An autoradiogram of the dried gel is shown. Panel B, the wild-type strain (KN2) (lanes 1–4) and the \textit{nsr1} strain (KN101) (lanes 5–8) were pulse-labeled with \textit{[\textsuperscript{3}H]}uracil for 5 min and chased with an excess amount of unlabeled uracil for 0, 5, 10, and 30 min at 30 °C. Equal counts (2 × 10\textsuperscript{4} cpm) of labeled RNA were analyzed on a 1.2% agarose/formaldehyde gel.
throughout the chase experiments when RNA was labeled with [methyl-\(^{3}\)H]methionine (Fig. 5A, lanes 4–6). The results indicate that the very low level of 18 S rRNA observed in Fig. 5A is likely due to alteration of methylation in the \(nsl1\) strain and not to an absence of 18 S rRNA itself. The ratio of 18 S to 25 S rRNA when labeled with \([\text{H}]\)uracil was 0.3 in the \(nsl1\) strain and 0.5 in the wild-type strain. Note that the ratio of 18 S to 25 S rRNA did not change 60 min after the chase in the \(nsl1\) strain (data not shown), indicating that the mature 18 S rRNA is not unstable in the \(nsl1\) strain. Since all the pre-rRNAs and mature rRNAs originate from the 35 S pre-rRNA, the impaired formation of 18 S rRNAs in the \(nsl1\) strain is likely due to instability of 20 S pre-rRNA coupled with its slow processing.

In order to examine ribosome production in the \(nsl1\) strain, we performed sucrose density gradient centrifugations of cell extracts. Cells were labeled with trans\(^{35}\)S-label for 15 min, and cell extracts were prepared in a buffer containing a high concentration of salt to dissociate the 40 S and 60 S ribosomal subunits. These subunits were separated on a 10–40% sucrose density gradient as shown in Fig. 6. The ratio of 40 S and 60 S subunits was measured by counting radioactivity of pooled fractions corresponding to each peak (data not shown). The amount of the 40 S subunit, containing 18 S rRNA, in the \(nsl1\) cells was significantly reduced compared with that in the wild-type cell. The ratio of the 40 S subunit to the 60 S subunit, which contains 25 S, 5.8 S, and 5 S rRNAs, decreased from 0.8 in the wild-type strain to 0.5 in the \(nsl1\) strain. The imbalanced synthesis of the 40 S and 60 S ribosomal subunits in the \(nsl1\) strain is consistent with the disproportionate formation of 18 S and 25 S rRNA described above.

**Southern Hybridization Analysis**—Because of the fact that the \(NSR1\) gene is not absolutely essential for cell growth, we attempted to search for other genes homologous to \(NSR1\) in the yeast genome, which may complement the function of \(NSR1\). Three probes containing various portions of the ORF were used for Southern analysis (Fig. 7): 0.6-kb \(HpaI\)-\(EcoRI\) fragment, 1.0-kb \(HpaI\)-\(XbaI\) fragment, and 0.8-kb \(EcoRI\)-\(SspI\) fragment corresponding to polypeptide residues from 1 to 187, from 1 to 324, and from 188 to 414 of \(NSR1\), respectively (see Fig. 1A). The temperature used for hybridization (55 °C) was 41–42 °C below the calculated \(T_m\) of the probes, allowing the detection of sequences with at least 60% homology to the probes (Beltz, et al., 1983). Fig. 7 shows the autoradiograms of the filters after washing in 1 × SSC at 51 °C (32–33 °C below the \(T_m\) of the probes). When the 0.6-kb \(HpaI\)-\(EcoRI\) fragment was used as a probe, several very faint bands in addition to one major band corresponding to the \(NSR1\) gene were detected on each lane (Fig. 7A). An identical pattern was observed with the 1.0-kb \(HpaI\)-\(XbaI\) fragment (Fig. 7B). These faint bands disappeared when the washing temperature was raised to 56 °C (data not shown). However, when the 0.8-kb \(EcoRI\)-\(SspI\) fragment was used as a probe (Fig. 7C), 2 or 3 relatively major bands and several very faint bands were detected on each lane. The positions of the submajor bands are distinctly different from those of the faint bands observed in Fig. 7, A and B. These submajor bands were still detected after the wash at 56 °C but disappeared at 61 °C (data not shown). These results indicate that there is no sequence with significant homology with the entire \(NSR1\) gene, while the yeast genome contains two or three other genes that have 70–80% homology only to the glycine/arginine-rich domain at the carboxyl terminus of \(NSR1\).

**DISCUSSION**

In the present study, we showed that one of a few cold shock-inducible genes in yeast S. cerevisiae is identical to yeast \(NSR1\) gene and that the strain carrying the \(nsl1\) gene deletion led to defects in pre-rRNA processing and ribosome synthesis. Pulse-labeling experiments revealed that the levels of 18 S rRNA and its 20 S precursor were greatly reduced with concomitant accumulation of unprocessed 35 S pre-rRNA in the \(nsl1\) strain compared with the wild-type strain. The level of 25 S rRNA was also slightly decreased in the \(nsl1\) deletion strain. It should be noted that Northern hybridization analysis using oligonucleotide probes that were designed to hybridize to pre-rRNA revealed that the amount of 27 S pre-rRNA was reduced in the \(nsl1\) strain in addition to 20 S pre-rRNA (Kondo et al., 1992). A pulse-chase labeling study using \([\text{H}]\)uracil demonstrated that 20 S pre-rRNA was hardly detected in the \(nsl1\) strain throughout the chase, although a reduced level of 18 S rRNA was clearly observed (Fig. 5B). The result suggests that the impaired synthesis of 18 S rRNA is likely due to rapid turnover of 20 S pre-rRNA. On the other hand, 18 S rRNA was hardly detected in the \(nsl1\) strain when RNA was labeled with [methyl-\(^{3}\)H]methio-
mature rRNA sequence is likely impaired in the nslr1 strain. Therefore, NSR1 may directly be involved in the methylation reaction or alternatively may indirectly affect the reaction by altering the association of pre-rRNA with the preribosomal particle. Inhibition of methylation of pre-rRNA has been shown to inhibit RNA processing in HeLa cells (Vaughan et al., 1967). The impaired methylation in the nslr1 strain could alter the processing of 20 S pre-rRNA to 18 S rRNA and lead to rapid turnover of the 20 S precursor. The disproportionate amount of 40 S and 60 S ribosomal subunits agreed with the imbalanced ratio of 18 S to 25 S rRNA. The imbalanced synthesis of ribosomal subunits decreases the production of functional 80 S ribosomes, and this defect in ribosome synthesis may be limiting for growth.

A number of yeast genes whose deletion affect rRNA processing have been identified. Most of them are genes for ribosomal proteins and the conditional deletion or deletion of the genes causes inefficient processing of rRNA associated with the protein as well as impaired production of the corresponding ribosomal subunits (Nam and Fried, 1986; Rotenberg et al., 1988; Finley et al., 1989; Sachs and Davis, 1989; Moritz et al., 1990). In addition to these examples, deletion of some of small nucleolar RNAs localized in the nucleolus, such as snR10, U14, and U3 (Tollervey, 1987; Li et al., 1990; Hughes and Ares, 1991), and NOP1, the homologue of mammalian fibrillarin (Tollervey et al., 1991) has been shown to cause inefficient processing of pre-rRNA. It is unlikely that NSR1 is part of the mature ribosome since ribosomal proteins of this molecular weight have not been identified, and the localization of NSR1 was shown to be in the nucleus and probably in the nucleolus (Lee et al., 1991). Therefore, it is possible that NSR1 is associated with the preribosomal particle in the nucleolus. Tollervey et al. (1991) reported that a conditional deletion of the nucleolar protein NOP1 causes impairment of rRNA processing, resulting in reduction of rRNA species and cell death. They revealed that the synthesis of the 18 S rRNA was particularly defective. It is noteworthy that NSR1 and NOP1 share a similar arginine/glycine-rich domain with 78% identity, although this domain locates at the amino-terminal region in NOP1 while it locates in the carboxyl-terminal region in NSR1. NSR1 and NOP1 may have similar functions, but NOP1 is probably more directly involved in ribosome synthesis since a nolp1 deletion was lethal (Tollervey, 1991). At present, one cannot rule out the possibility that NSR1 may be associated with the pre-rRNA processing.

The carboxyl-terminal half of NSR1, consisting of two tandemly repeated putative RNA-binding domains and a glycine/arginine-rich domain, has a good sequence similarity to the carboxyl-terminal part of the nucleolin (37% identity in 249 amino acids) (Fig. 1B). Nucleolin is a major nucleolar protein that is ubiquitous in higher eukaryotes and has been extensively studied. It has been shown that the amino-terminal domain, containing four acidic stretches, induces chromatin decondensation by interacting with histone H1 (Erard et al., 1988), and four RNA-binding domains are responsible for interaction with nascent pre-rRNA (Bugler et al., 1987). The glycine/arginine-rich domain at the carboxyl-terminal region may be involved in protein-protein interaction (Chung and Wooley, 1986). Nucleolin is thus likely to be multifunctional and appears to coordinate the multiple roles of the nucleolus for pre-rRNA transcription and processing as well as ribosome maturation and assembly (Jordan, 1987). In contrast to the high similarity in the carboxyl-terminal regions, there are no significant sequence similarities in the amino-terminal regions between NSR1 and human nucleolin. However, there are similarities in the domain arrangements and hydropathy profiles between the two proteins (Fig. 8). The amino-terminal sequences of both proteins are lysine-rich: 10 lysine residues within the NSR1 sequence from residue 1 to 26 (38%) and 6 residues within human nucleolin sequence from residue 1 to 22 (27%). It is interesting to note that almost all basic amino acid residues in the amino-terminal half of both NSR1 and nucleolin are lysine; 24 out of 25 in NSR1 and 50 out of 51 in human nucleolin. The NSR1 lysine-rich sequence is followed by a serine and acidic amino acid-rich domain from residues 29 to 166 (containing 65 serine and 49 acidic residues, 47 and 36%, respectively). In this domain, there are two short acidic stretches each containing 9 acidic residues (residues 98-107 and 144-153). Although human nucleolin has no serine-rich domain, it contains four acidic stretches containing 13, 25, 24, and 38 acidic residues (residues 25–40, 143–169, 185–209, and 234–271, respectively (Srivastava et al., 1989)).

It appears that NSR1 lacks the first two acidic sequences of human nucleolin together with the relatively hydrophobic region between them (Fig. 8), where one can find a repetition motif, TPXKK identified as a cdc2 kinase phosphorylation site sequence (Belenguer et al., 1990; Peter et al., 1990). NSR1 and nucleolin also differ in the number of RNA-binding domains; there are two domains in NSR1 in contrast to four in nucleolin (Fig. 8). All these differences found in the amino-terminal regions between the two proteins may not be significant enough to rule out the possibility that NSR1 is a nucleolin homologue in yeast because sequence diversity in the amino-terminal region of nucleolins has been previously reported (Caiergues-Ferrer et al., 1989). A comparison of Xenopus and hamster nucleolins has shown that the acidic
domains located at the amino-terminal half of the protein are much shorter in Xenopus than in rodents (the lengths of the third and fourth acidic domains are reduced from 25 to 11 and 33 to 20, respectively). Furthermore, the first and second RNA-binding domains are less conserved (42 and 52% identity, respectively) compared with the third and fourth domains (65 and 87% identity, respectively) (Caizergues-Ferrer et al., 1989). The lack of the region encompassing the first two acidic sequences and the first two RNA-binding domains in NSR1 as well as the substantial difference in the amino-terminal half between NSR1 and nucleolin may be explained as diversity during evolution. The structural similarity of NSR1 with nucleolin and the involvement of NSR1 in ribosome biosynthesis strongly suggest that NSR1 is functionally related to nucleolin. The differences in the amino-terminal regions between the two proteins, however, suggest that NSR1 may have other functions different from nucleolin.

A deletion mutation of the NSR1 gene resulted in growth defects although NSR1 was not an essential gene. The very slow growth observed for the nsrl cells after the initial lag period indicates that NSR1 is required for optimal cell growth (Fig. 3). Thus, it appears that NSR1 plays an important role as an auxiliary factor for efficient ribosome production, particularly in cells where the requirement of protein synthesis is suddenly shifted from a low to a very high level. It was reported that NSR1 binds nuclear localization sequences of yeast histone H2B (Lee and MleÁEse, 1989). Yeast ribosomal proteins have been shown to contain nuclear localization sequences for their transport into the nucleus (Moreland et al., 1985). NSR1 may be involved in the localization of ribosomal proteins to the nucleus and also the transport of ribosomal subunits from the nucleus to the cytoplasm. It is interesting to note that nucleolin shuttles between the nucleus and the cytoplasm and may function in mediating transport of ribosomal proteins and ribosomal subunits across the nuclear membrane (Borer et al., 1989). The finding that NSR1 is not essential for cells and may function as an auxiliary factor for ribosome biosynthesis in yeast is surprising because nucleolin has been known to play a key role in ribosome biosynthesis. We cannot rule out a possible existence of another nucleolin homologue in yeast, although Southern analysis indicates that such a protein, if any, has a low sequence homology to NSR1. It is of great interest to investigate the role of the increase of NSR1 during cold shock since the NSR1 gene was originally cloned as a cold shock inducible gene. We have found that the nsrl deletion caused a more severe defect on pre-rRNA processing during cold shock and report that in the following paper (Kondo et al., 1992).

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