The SSD1 gene has been isolated as a single copy suppressor of many mutants, such as sit4, slk1/bck1, pde2, and rpc31, in the yeast Saccharomyces cerevisiae. Ssd1p has domains showing weak but significant homology with RNase II-related proteins, Cyt4p, Dss1p, VacB, and RNase II, which are involved in the modification of RNA. We found that Ssd1p had the ability to bind RNA, preferably poly(rA), as well as single-stranded DNA. In contrast, RNase II-related proteins was not necessary for interaction with RNA. Indirect immunofluorescence staining with anti-Ssd1p antibody revealed that Ssd1p was detected mainly in the cytoplasm. Furthermore, sucrose gradient sedimentation analysis demonstrated that Ssd1p was not cofractionated with polyribosomes, suggesting that Ssd1p is not particularly bound to a translationally active subpopulation of mRNA in the cytoplasm.

Also reported that there are two alleles of the SSD1 gene; one is called ssd1-d (dead) and the other is called SSD1-V (viable). They described that SSD1-V could suppress the double mutations of ssd1-d and sit4 (6). We have also isolated the SSD1 gene as the MCS1 gene involved in stable maintenance of the minichromosome (12). The SSD1/MCS1 gene product was detected as a ~160-kDa protein in certain wild type strains bearing SSD1-V, such as KA31 or RAY-3A, whereas a protein of this size was not detected in another wild type strain bearing ssd1-d, such as YPH499 (7). These findings indicate that SSD1-V is simply a wild type gene and ssd1-d is a defective gene. However, the functions of SSD1 have not yet been clarified.

In recent years, it has been reported that SSD1 has a weak but significant similarity with dis3" of Schizosaccharomyces pombe (6, 13), DSS1 of S. cerevisiae (14), vacB of Shigella flexneri (15), cyt4 of Neurospora crassa (16), zam of Synchytrium PCC 6803 (17), and rnb of Escherichia coli (18). Some of these genes are known, or implied, to be involved in the modification of RNAs: 1) cyt4 is required for the mitochondrial rRNA splicing and processing reaction; 2) DSS1 is a multicyclic suppressor of the disruptant of SUV3 encoding a putative RNA helicase-like protein; 3) the vacB mutation reduces the level of the virulence antigens, IpaB, IpaC, IpaD, and VirG, at the post-transcriptional level; and 4) the RNase II encoded by rnb has a 3'-to-5' exoribonuclease activity. However, there have been no reports describing direct interaction with RNA in these gene products, except for RNase II of E. coli. Here we report the biochemical characterization and cellular localization of the Ssd1 protein.

MATERIALS AND METHODS

Strains and Culture Conditions—RAY-3A (MATa ura3 leu2 trp1 his3) and YKM1H (RAY-3A ssd1Δ::HIS3) were used for the ribonuclease assay, nucleotide binding studies, metabolic labeling with [32P]orthophosphate, and sucrose gradient fractionation of cell extract. KA31–2A (MATa ura3 leu2 trp1 his3) (19) and YKM1H (KA31–2A ssd1Δ::HIS3) were used for testing growth rates and for indirect immunofluorescence microscopy. The SSD1 disruption was performed by using pYK907 plasmid as described previously (12). Culture media, includingYPD (1% yeast extract, 2% peptone, and 2% glucose) and synthetic minimal SD (0.7% yeast nitrogen base without amino acid and 2% glucose) with amino acid supplements, were prepared according to Rose et al. (20). SRaf contained 0.7% Difco yeast nitrogen base without amino acids, 2% raffinose, and appropriate supplements.

Plasmid Construction—Plasmid pPF4 was constructed by inserting the 6-kilobase BamHI fragment bearing the SSD1 gene derived from the original clone, pPF2CU (12), into pUC13. For overexpression of the SSD1 gene, a plasmid pSSD1.0 was made as follows. pYES2 (Invitrogen), a high copy number plasmid carrying the URA3 marker and the inducible GAL1 promoter, was digested with HindIII, and the DNA ends were made flush with a Klenow fragment of DNA polymerase I; digested with BamHI, and then ligated with the 5.2-kilobase BamHI fragment bearing the SSD1 gene derived from pPF4. Translational initiation of Ssd1p is started at the ATG at position 52 in the open reading frame of SSD1. Therefore, Ssd1p is expressed from pSSD1.0 plasmid encodes the protein lacking 17 amino acids of the N terminus. pSSD1.1 was constructed as follows. After pSSD1.0 was

Received for publication, April 25, 1997
digested with XhoI and HindIII, the DNA ends were made flush and ligated. pSSD1.2 was constructed by digestion of pSSD1.0 with XhoI, and the resulting large fragment was recircularized. Plasmid pFK1CU or plasmid pFK5EU contains SSD1 on YCUP4 or YEUP3 vector (constructed in Fujita), respectively (12).

**RNA Binding Experiments—**YRM1 cells carrying pFK1CU or pSSD1.0, or pSSD1.2 were grown in SRAf-Ura to early log phase and transferred to SRAf-Ura containing 0.5% galactose followed by incubation at 30 °C for 4 h. For the preparation of cellular extract, 5 × 10^7 cells were harvested by centrifugation and washed with ice-cold lysis buffer (100 mM Tris-HCl, pH 7.5, 200 mM NaCl, 1 mM EDTA, 0.5% glycerol, 0.5 mM DTT, 10 μg/ml each of leupeptin, pepstatin A, and antipain). Cells were resuspended in 150 μl of lysis buffer and lysed by shaking four times with an equal amount of glass beads for 30-s intervals. An additional 200 μl of lysis buffer was added, and mixtures were shaken again for 30 s. Extract was pipetted out and centrifuged at 12,000 × g for 15 min to remove cell debris. Immunoprecipitations of each Ssd1p derivative, gel electrophoresis, and immunoblotting experiments were performed as described previously (6). An RNA binding experiment was carried out by incubating the electroblotted proteins with 32P-labeled RNA as described previously (21). Radiolabeled total RNA were prepared from yeast cells by using PUREscript™ RNA isolation kits (Gentra system Co., Ltd), and treated with RNase-free DNase (Sigma).

**Exoribonuclease Assay—**Yeast cells of YRM1 carrying pFK1CU or YCUP4 were grown in SD-Ura to midlog phase at 25 °C. Early log phase cells of YRM1 carrying pSSD1.0 grown in SRAf-Ura were transferred to SRAf-Ura containing 0.5% galactose or 2% glucose and incubated for 4 h at 25 °C. Cellular extracts (200 μl) were prepared from approximately 5 × 10^7 cells as described above, and immunoprecipitations were performed using 20 μl of anti-Ssd1p antibody, as described previously (6). Immunoprecipitants were washed twice with buffer for measurement of exoribonuclease and resuspended in 75 μl of the same buffer, and exoribonuclease assays were carried out. The assays of exoribonuclease were based on the release of acid-soluble radioactivity from [3H]poly(A) or [32P]-labeled total RNA according to the procedure as described previously (27–29).

**Ribohomopolymer Binding Analysis and ssDNA1 Chromatography—**For preparation of yeast lysates, RAY-3A cells were grown in YPD to midlog phase, and early log phase cells of YRM1 carrying pSSD1.0, pSSD1.1, or pSSD1.2 were grown in SRAf-Ura containing 0.5% galactose for 4 h at 25 °C. Approximately 1 × 10^8 cells were lysed by shaking with glass beads in 300 μl of binding buffer (10 mM Tris (pH 7.4), 1.5 mM MgCl2, 100 mM NaCl, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1% aprotinin, and 5 μg/ml each of leupeptin, pepstatin A, and antipain), an additional 200 μl of binding buffer was added, and cellular extracts were prepared by glass beads shearing as described above. Using this extract, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) chromatography, and ribohomopolymer (Sigma) binding assay, were performed as described previously (22).

**Indirect Immunofluorescent Photomicroscopy—**Indirect immunofluorescence microscopy of yeast cells was performed with a modification of previously published procedures (23). KA31–2A cells carrying pFK5EU, an multicopy plasmid carrying the SSD1 gene, were grown to early log phase in SD-Ura, and formaldehyde was directly added to a final concentration of 5%. After incubation for 1 h at room temperature, cells were washed twice with 100 mM KH2PO4, pH 7.5, and resuspended in 1 ml of buffer S (100 mM KH2PO4, pH 7.5, 1.2 mM sorbitol) containing 20 μg of zymolyase 100T (Seikagaku) and 0.1% 2-mercaptoethanol and incubated for 30 min at 30 °C. Cells were rinsed twice with buffer S and twice with phosphate-buffered saline containing 3% bovine serum albumin. Cells were resuspended in 200 μl of the same buffer containing 2 μl of anti-Ssd1p antiserum (100:1) and incubated for 4 h at 30 °C. Cells were applied to polylisine-coated glass slides for 0.5 h and washed five times with phosphate-buffered saline containing 0.1% bovine serum albumin. Cells were incubated with the same buffer containing fluorescein isothiocyanate-conjugated goat anti-rabbit antibody (100:1) for 1 h at 30 °C. Samples were washed five times with phosphate-buffered saline and added with 0.5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). For the preparation of low salt extract, approximately 3 × 10^8 early log phase cells were lysed by vortexing with glass beads in 400 μl of standard extraction buffer A (20 mM Tris-HCl (pH 7.4), 2 mM MgCl2, 10 mM NaCl, 1 mM dithiothreitol, 1 M phenylmethylsulfonyl fluoride, 1% aprotinin, and 5 μg/ml each of leupeptin, pepstatin A, and antipain). Cell debris were removed by centrifugation for 10 min at 5,000 × g. Then one half (150 μl) of the supernatant was incubated with RNase A (10 μg/ml) plus micrococcal nuclease (200 μg/ml) at 30 °C for 30 min, and the other half (150 μl) was incubated under the same condition without RNase A. The sample was layered onto 12 ml of a continuous 10–30% sucrose gradient and centrifuged at 35,000 rpm in a Beckman SW-40 rotor for 18 h at 4 °C. For a polysome preparation, 1.5 × 10^9 early log phase cells were treated with cycloheximide and were processed as described previously (25). For the preparation of extract that contains deacylating polysome, 1.5 × 10^8 midlog phase cells were collected, and cell extracts were prepared by glass beads shearing (26). Samples were fractionated (24). Each sample was layered onto 12 ml of a continuous 15–50% sucrose gradient by centrifugation at 40,000 rpm in a Beckman SW-40 rotor for 2.5 h at 4 °C. A 254 of the gradient fraction was monitored using the Pharmacia FPLC system and Buchler Auto DensiFlow IIIC.

**RESULTS**

**Amino acid Sequence Similarity among Ssd1p and RNase II-related Proteins—**It has been reported that the Ssd1 protein shows weak similarity with the Cyt4 protein, which is related to a mitochondrial RNA splicing and processing factor of N. crassa (16). Another report demonstrated the conserved domains in several proteins involving not only Cyt4p but also RNase II, a 3′ to 5′ exoribonuclease, encoded by rnb of E. coli (14). Therefore, we compared the amino acid sequence of Ssd1p with those of other proteins. Computer search analysis using the GENETYX program (Software Development Co., Ltd.) revealed that Ssd1p has similarities with SpDis3p of S. pombe (23.1% identity in 565 amino acids of Ssd1p), ScDis3p of S. cerevisiae (24.2% identity in 505 amino acids) (27), Dss1p of S. cerevisiae (12.4% identity in 290 amino acids), Cyt4p of N. crassa (21.0% identity in 372 amino acids), VaeC of Shigella flexneri (20.1% identity in 387 amino acids), and Rnb of E. coli (20.9% identity in 373 amino acids). The homology search demonstrated that similarities are restricted to the C-terminal half of Ssd1p and that three conserved domains exist in this region (Fig. 1A). The first domain of Ssd1p was located around a region from 689 to 785 (domain 1, D1), the second was a region from 880 to 910 (domain 2, D2), and the last was a region from 983 to 1014 (domain 3, D3) in Ssd1p. Of these domains, domain 3 is the most highly conserved among these proteins (Fig. 1B). In addition to the proteins shown in Fig. 1, the protein predicted from the sequence of F48Es.6 of Caenorhabditis elegans (BLAST network service) also has these domains. These findings indicate that domains 1, 2, and 3 are conserved from prokaryote to eukaryote.

**Domain Analysis of SSD1—**To test whether these conserved domains are necessary for the function of SSD1 or not, we constructed several SSD1 derivatives (Fig. 2A): SSD1.0, encoding a protein lacking 17 amino acids of the N terminus of Ssd1p expressed under the GAL1 promoter on a multicopy vector (see “Materials and Methods”), could complement the temperature sensitivity of YKM1H, the ssd1 disruptant of the KA31–2A strain; probably, it was expressed from the GAL1 promoter on SRAf medium containing glucose. This result indicated that SSD1.0 encoded a functional protein (Fig. 2B, left panel, 37 °C). In contrast to this, the gene lacking both domains 2 and 3 (SSD1.1) or all of domains 1, 2, and 3 (SSD1.2) could not complement the temperature sensitivity of YKM1H (Fig. 2B, left panel, 37 °C). To our surprise, overexpression of SSD1.0 in the existence of galactose inhibited the growth of YKM1H at 25 °C, while SSD1.1 or SSD1.2 did not (Fig. 2B, right panel). Thus, the SSD1 gene requires the region including domains 2 and 3 for its function. In the case of dis3 of S. pombe, a similar result indicating that the most conserved region is necessary for its activity has been described previously (13). The growth-inhibited cells did not show any characteristic morphology.

1 The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.
This growth inhibition was more remarkable in the strain KA31 or W303 than in another wild type strain, RAY-3A (data not shown).

RNA Binding Activity of Ssd1p—Since the sequence of Ssd1p has similarity with that of the RNase II of E. coli as described above, we tested whether Ssd1p had an exoribonuclease activity. The immunoprecipitants were prepared by using anti-Ssd1p antibody from the lysates of the wild type, the ssd1 disruptant, and the SSD1-overexpressing cells. Using these immunoprecipitants, we examined an ability of Ssd1p to degrade 3H-labeled poly(rA) or 32P-labeled total RNA extracted from yeast cells under the following several assay conditions of RNase II of E. coli (28), 5' to 3' exoribonuclease (Xrn1p) of S. cerevisiae (29), or mitochondrial 5' to 3' exoribonuclease of S. cerevisiae (30). However, we were unable to detect any exoribonuclease activity with these immunoprecipitants.

We next examined whether Ssd1p could bind RNA in vitro. Ssd1.0p and derivatives from it were immunoprecipitated from extracts of yeast cells overexpressing each SSD1 derivative using anti-Ssd1p antibody and electrophoresed on nitrocellulose. The molecular masses of Ssd1.0p, Ssd1.1p, and Ssd1.2p were about 160, 120, and 95 kDa, respectively when detected by immunoblotting analysis (Fig. 3, left panel). The molecular weights of Ssd1.0p, Ssd1.1p, and Ssd1.2p deduced from amino acid sequences were 140, 95, and 76 kDa, respectively, suggesting that all of these proteins are modified and that the modified regions may reside in the N-terminal half of Ssd1p, which does not show any significant similarity with other proteins. The filter was incubated with 32P-labeled total RNA extracted from yeast cells. Only Ssd1.1p of 120 kDa lacking both D2 and D3 could bind to 32P-labeled RNA, while Ssd1.0p and Ssd1.2p could not (Fig. 3, right panel). This result indicates that Ssd1.1p can associate with RNA directly without any binding proteins and that the region containing D1 is necessary for RNA binding. The reason why Ssd1.0p did not bind to RNA is

This growth inhibition was more remarkable in the strain KA31 or W303 than in another wild type strain, RAY-3A (data not shown).

RNA Binding Activity of Ssd1p—Since the sequence of Ssd1p has similarity with that of the RNase II of E. coli as described above, we tested whether Ssd1p had an exoribonuclease activity. The immunoprecipitants were prepared by using anti-Ssd1p antibody from the lysates of the wild type, the ssd1 disruptant, and the SSD1-overexpressing cells. Using these immunoprecipitants, we examined an ability of Ssd1p to degrade 3H-labeled poly(rA) or 32P-labeled total RNA extracted from yeast cells under the following several assay conditions of RNase II of E. coli (28), 5' to 3' exoribonuclease (Xrn1p) of S. cerevisiae (29), or mitochondrial 5' to 3' exoribonuclease of S. cerevisiae (30). However, we were unable to detect any exoribonuclease activity with these immunoprecipitants.

We next examined whether Ssd1p could bind RNA in vitro. Ssd1.0p and derivatives from it were immunoprecipitated from extracts of yeast cells overexpressing each SSD1 derivative using anti-Ssd1p antibody and electrophoresed on nitrocellulose. The molecular masses of Ssd1.0p, Ssd1.1p, and Ssd1.2p were about 160, 120, and 95 kDa, respectively when detected by immunoblotting analysis (Fig. 3, left panel). The molecular weights of Ssd1.0p, Ssd1.1p, and Ssd1.2p deduced from amino acid sequences were 140, 95, and 76 kDa, respectively, suggesting that all of these proteins are modified and that the modified regions may reside in the N-terminal half of Ssd1p, which does not show any significant similarity with other proteins. The filter was incubated with 32P-labeled total RNA extracted from yeast cells. Only Ssd1.1p of 120 kDa lacking both D2 and D3 could bind to 32P-labeled RNA, while Ssd1.0p and Ssd1.2p could not (Fig. 3, right panel). This result indicates that Ssd1.1p can associate with RNA directly without any binding proteins and that the region containing D1 is necessary for RNA binding. The reason why Ssd1.0p did not bind to RNA is

**Fig. 1.** Amino acid sequence homology among RNase II-related proteins. A, schematic presentation of the proteins, Ssd1p of S. cerevisiae (6), SpDis3p of S. pombe (13), ScDis3p of S. cerevisiae (27), Dss1p of S. cerevisiae (14), Cyt4p of N. crassa (16), VacB of S. flexneri (15), and RNase II of E. coli (18). Conserved domains 1, 2, and 3 are shown as D1, D2, and D3, respectively. B, alignment of amino acid sequences of domains 1, 2, and 3. Residues identical in at least two proteins are shown in a lightly shaded box. Residues identical over six proteins are shown in a darkly shaded box. The consensus sequence consists of residues identical in all of the proteins.
Ssd1p Is an RNA-binding Protein

**FIG. 2. Construction and overexpression of Ssd1p derivatives.** A, schematic representation of Ssd1p derivatives. Ssd1.0p indicates the protein lacking 17 amino acids of the N terminus. Ssd1.1p or Ssd1.2p indicates the protein lacking the domain 2 and 3 regions, or lacking domains 1, 2, and 3 (D1, D2, and D3), derived from Ssd1.0p, respectively. All of these proteins were expressed under the control of the GAL1 promoter (pSSD1.0, pSSD1.1, and pSSD1.2, respectively). The thick line indicates pYES2 vector. Hp, HpaI; Xb, XhoI; H, HindIII; B, BamHI; X, XhoI. B, effect of overexpression of Ssd1p derivatives on growth of the ssd1 disruptant. Yeast cells of YRM1H(KA31-2a/δ3/δ4/ΔHIS3) carrying pYES2, pFK1CU, pSSD1.0, pSSD1.1, or pSSD1.2 were streaked onto a SRaf-Ura plate containing 0.5% galactose (left) or a SRaf-Ura plate containing 0.5% galactose (right) and incubated for 2 days at 37 °C (top) or for 3 days at 25 °C (bottom).}

**FIG. 3. RNA binding analysis of Ssd1p.** Proteins were immuno-precipitated with anti-Ssd1p serum from extracts of YRM1H (RAY-3A/ssd1Δ/HIS3) carrying pYES2 (lane 1), pSSD1.0 (lane 2), pSSD1.1 (lane 3), or pSSD1.2 (lane 4), subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes. One membrane was probed with anti-Ssd1p serum (left), and the other was probed with 32P-labeled total RNA (right).

unclear at this moment. We cannot exclude a possibility that native Ssd1.0p and Ssd1.2p can bind RNA; therefore, it is important to test whether the native Ssd1p has an ability to bind RNA.

**Nucleic Acid Binding Property of Ssd1p**—To detect an ability of native Ssd1p to bind polynucleotide, we carried out DNA-cellulose chromatography. The same amount of extracts from the yeast cells overexpressing Ssd1.0p or its derivatives was mixed with dsDNA- and ssDNA-cellulose in 0.1 or 1.0 M NaCl, and proteins were eluted with 2 M NaCl. Ssd1.0p was able to bind to ssDNA at 0.1 M NaCl (Fig. 4A, lane 1) but not to dsDNA efficiently (Fig. 4A, lane 7), whereas Ssd1.2p lacking all conserved domains weakly bound ssDNA but not dsDNA (Fig. 4A, lanes 3, 4, 9, and 10). Thus, Ssd1.0p has an ability to bind single-stranded DNA, and the conserved regions seem to be necessary for interaction. However, Ssd1.1p lacking the most highly conserved region, domain 3, could bind ssDNA more efficiently than Ssd1.0p (Fig. 4A, lanes 1 and 5). These results indicate that Ssd1p can bind ssDNA without the most highly conserved region, as seen in the case of Fig. 3, and that a region, other than the conserved regions, may bind ssDNA. Interestingly, Ssd1.1p could bind dsDNA as well as ssDNA (Fig. 4A, lane 11), suggesting that the region including both domains 2 and 3 is necessary for specific binding to single-stranded polynucleotides.

To further characterize the RNA binding property of Ssd1p, we examined whether Ssd1p or its derivatives was bound to four ribohomopolymers. This binding assay has been successful in distinguishing the specificities of a variety of RNA-binding proteins (22). Extracts from the wild type cells were mixed with poly(rA), poly(rU), poly(rG), and poly(rC)-agarose in 0.1 or 1.0 M NaCl. The ribohomopolymer-binding proteins were eluted with SDS sample buffer. Basically, Ssd1p bound efficiently to the all kinds of ribohomopolymers in 0.1 M NaCl, whereas it bound to poly(rA) more efficiently than to the other polynucleo-
Ssd1p Is an RNA-binding Protein

Localization of Ssd1p—It is important to determine the localization of Ssd1p because the nucleic acid-binding proteins exert their functions in their respective compartments. Indirect immunofluorescence microscopy using anti-Ssd1p antibody was carried out to determine the subcellular localization of Ssd1p. To facilitate detection of the immunofluorescence, the SSD1 gene was cloned on a multicopy YEUP3 vector (pFK5EU) to overexpress SSD1 in cells. The affinity-purified anti-Ssd1p antibody revealed an intense signal in cytoplasm and a weak signal in the nucleus in the ssd1 disruptant cells (YKM1H) carrying pFK5EU. The signal was always observed in the cytoplasm, irrespective of budded or unbudded cells (Fig. 5, A and C), indicating that Ssd1p mainly stays in the cytoplasm throughout the cell cycle. As a reference, signals were not observed in the ssd1 disruptant cells carrying YEUP3 vector (Fig. 5E). These observations suggest that Ssd1p may associate with RNA in the cytoplasm but not DNA or RNA in the nucleus; therefore, Ssd1p may play some roles in the stability or turnover of cytoplasmic RNA rather than its maturation.

Sucrose Density Gradient Sedimentation of Ssd1p—To know whether Ssd1p can associate with cellular RNA or not, low salt extracts prepared from the wild type cells were fractionated on a 10–30% continuous sucrose density gradient, and each fraction was analyzed by immunoblotting using anti-Ssd1p antibody. Ssd1p showed a broad distribution in fractions 7–19 (Fig. 6, upper panel). However, after treatment of extracts with RNase A and micrococcal nuclease, Ssd1p was mainly sedimented in the upper fractions 7 and 8, where the monomeric Ssd1 protein (160 kDa) was expected to sediment (Fig. 6, lower panel). The fact that Ssd1p in the extract treated with nuclease also sedimented in the faster sedimenting fractions indicates that some population of Ssd1p may form complexes with certain protein or nucleic acids. Thus, we presumed that Ssd1p associated with RNA in vivo.

Cytoplasmic mRNAs exist either in translationally active form or translationally inactive form. In mammalian cells, the histone mRNA degradation occurs at the 3' terminus and appears to be catalyzed by a polyribosome-associated 3' to 5' exoribonuclease. In Saccharomyces cerevisiae, two major pathways for mRNA degradation have been identified: one is the so-called deadenylation-dependent pathway, and the other is the deadenylation-independent pathway (33, 34). Both degradation pathways require XRN1 encoding a 5' to 3' exoribonuclease (35–37). Subsequently, the study using the xrn1 mutant showed the existence of 3' to 5' exoribonuclease (33). In mitochondria of S. cerevisiae, an NTP-dependent 3' to 5' exoribonuclease has also been isolated and characterized (30, 38). However, the gene encoding 3' to 5' exoribonuclease of cytoplasm has not been identified yet. There are no reports identifying a gene encoding a 3' to 5' exoribonuclease, except for rnhB of E. coli. The SGD (Saccharomyces Genome Database) project revealed that three Saccharomyces genes, DSS1, YOL021C/ScDIS3, and SSD1, encode
Ssd1p Is an RNA-binding Protein

...show immunoblot analysis of Ssd1p. Lower parts were collected from top (fractionated through sucrose gradient sedimentation) and from RAY-3A cells without treatment of cycloheximide (B).

...domains showing weak similarities with RNase II of E. coli. These gene products may be candidates of the 3' to 5' exoribonuclease of S. cerevisiae. However, we could not detect exoribonuclease activity of Ssd1p using poly(rA) or total RNA as a substrate.

...Highly purified preparations of mitochondrial 3' to 5' exoribonuclease of S. cerevisiae have shown to form a complex and require NTP-dependent RNA helicase for its activity (30, 39). It contains three major polypeptides estimated to be 75, 90, and 110 kDa, which is predicted to be the gene product of DSS1 (29). In addition, the Dis3 protein of S. pombe has also reported to be part of a 250–350-kDa oligomer (13). Thus, Ssd1p may also need another component for an exoribonuclease activity. As shown in Fig. 6, most of Ssd1p was in monomeric fraction after treatment with RNase, and a small fractions of Ssd1p remained as stable complexes. Ssd1p may form a stable complex not only with proteins but also with certain RNA molecules, and formation of such a complex may be necessary for expressing exoribonuclease activity. Post-translational modification of Ssd1p also may contribute to the expression of exoribonuclease activity: Ssd1p is phosphorylated in vivo (12), suggesting that the yet unidentified ribonuclease activity of Ssd1p may be tightly regulated by a certain protein kinase.

Ssd1.1p, lacking the highly conserved region domain 3, could bind RNA more efficiently than Ssd1.0p as shown in Fig. 3, therefore, domain 3 seems to inhibit the association of Ssd1p with RNA. If Ssd1p is exoribonuclease, domain 3 may be necessary for its exoribonuclease activity rather than RNA binding, because the SSD1 gene requires domain 3 for its entire function as shown in Fig. 2. The major known RNA-binding motifs (5) are not seen in the sequence of Ssd1p. Interestingly, the region necessary for interaction with RNA seems to be a region having domain 1 showing a weak similarity but not a region having highly conserved domain 3. Therefore, the region containing domain 1 of Ssd1p may be a new RNA binding motif.

The DSS1 gene has been isolated as a multicopy suppressor of the disruptant of SUV3, encoding putative RNA helicase, involved in mitochondrial RNA metabolism. It has been reported that the amino terminus of Dss1p is predicted to have a mitochondrial targeting sequence and that the dss1 disruptant is viable but does not grow in a glycerol medium (14). From these findings, Dss1p seems to localize in mitochondria. On the other hand, the SpDis3p of S. cerevisiae shows a high identity with the SpDis3p of S. pombe throughout the length (27), while Ssd1p and Dss1p show low identities. In addition, Naguchi et al. (27) have also reported that ScDIS3 was able to rescue the dis3 mutant of S. pombe, while Kinoshita et al. (13) have reported that Ssd1 could not. These findings indicate that ScDIS3, but not Ssd1, is a counterpart of dis3" of S. pombe. The dis3" gene product of S. pombe localizes mainly in the nucleus (13), suggesting that the ScDIS3 gene product localizes in nucleus. Intracellular localization of Dss1p and ScDis3p is in a clear contrast to that of Ssd1p, which was mainly localized in the cytoplasm as shown in Fig. 5. These observations suggest an interesting possibility that these three proteins of S. cerevisiae having similarity with RNase II of E. coli, Dss1p, ScDis3p, and Ssd1p associate with mitochondrial RNA, nuclear RNA, and cytoplasmic RNA, respectively.

The fact that Ssd1p was mainly localized in cytoplasm suggests that the target of Ssd1p could be RNA in cytoplasm including rRNA, mRNA, or others. It has been reported that the cyt4 mutant in N. crassa had several defects including maturation of rRNA in mitochondria (40). However, rRNA prepared from the sgd1 disruptant did not show any remarkable difference in its maturation or concentration, in comparison with rRNA prepared from wild type cells (data not shown). In addition, Ssd1p was not cofractionated either with ribosomes or with polysomes, as shown in Fig. 7. Thus, the target of Ssd1p does not seem to be rRNA. We could not detect Ssd1p in...
UV-cross-linked polyadenylated RNA-RNPs (data not shown). Therefore, we have no evidence to indicate direct interaction of Ssd1p with mRNA.

Immunoblotting analysis using anti-Ssd1p antibody recognizing its N-terminal region revealed that the Ssd1-d2 protein in W303 used as wild type strain was detected as an 83-kDa protein (data not shown). This protein may be a C-terminal truncated protein lacking all of the domain 1, 2, and 3 regions, suggesting that the Ssd1-d2 protein may also be a nonfunctional protein. The Ssd1-d protein of another wild type strain, YPH499, was also the same size as that of W303 (data not shown). These results suggest that a ssd1-d mutation is widely spread among many laboratories’ strains. The ssd1-d mutation results in a subtle phenotype, such as caffeine-sensitive (6) and leaky temperature-sensitive. However, when it is combined with another mutation such as sit4, the double mutation causes a severer temperature-sensitive phenotype (synthetic lethal) than either of the single mutations (6–11). It is reasonable that the Ssd1p gene has been obtained as a single copy suppressor in various screens. Mutations that show synthetic lethality with ssd1-d seem to be involved in the transcriptional regulation of certain genes: 1) sit4 (41), 2) bcy1 (42–45), 3) mpk1 (46), and 4) rpb1, rpc31, and rpc53 (7). These genetic characteristics displayed by the ssd1 mutation, in addition to the fact that Ssd1p is a cytoplasmic RNA-binding protein, suggest that SSD1 may be involved in the expression of various genes at the post-transcriptional level by controlling RNA metabolism.

REFERENCES
1. Atwater, J. A., Wisdom, R., and VermaI, I. M. (1990) Annu. Rev. Genet. 24, 519–541
2. Bandziulis, R. J., Swanson, M. S., and Dreyfuss, G. (1989) Genes & Dev. 3, 431–457
3. Hargrove, J. L., Hulsey, M. G., and Beale, E. G. (1991) BioEssays 6, 667–674
4. Sutton, A., Immanuel, D., and Arndt, K. T. (1991) Mol. Cell. Biol. 11, 2133–2149
5. Stettler, S., Chinnikulchhai, N., Denmat, S. H., Lalo, D., Lacroute, F., Sentenac, A., and Thuriaux, P. (1993) Mol. & Gen. Genet. 239, 169–176
6. Costigan, C., Gehring, S., and Snyder, M. (1992) Mol. Cell. Biol. 12, 1162–1178
7. Lee, K. S., Irie, K., Gotoh, Y., Watanabe, Y., Araki, H., Nishida, K., Matsumoto, K., and Levin, D. E. (1993) Mol. Cell. Biol. 13, 3067–3075
8. Kinosita, N., Goeb, M., and Yanagida, M. (1991) Mol. Cell. Biol. 11, 5389–5447
9. Dmochowska, A., Golik, P., and Stepien, P. P. (1995) Curr. Genet. 28, 108–112
10. Tobe, T., Sakawaka, C., Okada, H., Noma, Y., and Yoshikawa, M. (1992) J. Bacteriol. 174, 6359–6367
11. Turcq, B., Dohinse, K. F., Bertzaw, N., and Lambowitz, A. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1676–1680
12. Beuf, L., Bedu, S., Cami, B., and Jostet, F. (1995) Plant Mol. Biol. 27, 779–788
13. Zilhao, R., Camelo, L., and Arraianno, C. M. (1993) Mol. Microbiol. 8, 43–51
14. Irie, K., Takase, M., Lee, K. S., Levin, D. E., Araki, H., Matsumoto, K., and Oshima, Y. (1993) Mol. Cell. Biol. 13, 3076–3083
15. Rose, M. D., Winston, F., and Hieter, P. (1990) Methods in Yeast Genetics: A Laboratory Course Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
16. Lee, J.-F. S., and Mass, J. (1993) J. Biol. Chem. 268, 15080–15087
17. Matunis, M. J., Matunis, E. L., and Dreyfuss, G. (1993) Mol. Cell. Biol. 13, 6114–6121
18. Pringle, J. R., Preston, R. A., Adams, A. E. M., Stearns, T., Drubin, D. G., Haarer, B. K., and Jones, E. W. (1989) Methods Enzymol. 31, 357–435
19. Liang, S., Hromi, M., Hu, Y., Liu, Y., and Tartakoff, A. M. (1996) Mol. Cell. Biol. 16, 5139–5146
20. Sachs, A. B., and Davis, R. W. (1989) Cell 58, 857–867
21. Kraig, E., and Haber, J. (1980) J. Bacteriol. 144, 1098–1112
22. Tanaka, K., Matsumoto, K., and Toh-e, A. (1988) Methods Enzymol. 13, 517–194
23. Beelman, C. A., Stevens, A., Caponigro, G., LaGrandeur, T. E., Hatfield, L., Fortner, D. M., and Paker, R. (1996) Nature 370, 578–581
24. Cherrel, S. J., Zilhaib, R., and Arraianno, C. M. (1995) EMBO J. 15, 5638–5656
25. Ma et al., S. (1990) J. Biol. Chem. 255, 3080–3085
26. Min, J., and Zassenhaus, H. P. (1989) J. Biol. Chem. 264, 3750–3757
27. Caruccio, N., and Ross, J. (1994) J. Bacteriol. 176, 31814–31821
28. Anderson, J. T., Paddy, M. R., and Swanson, M. S. (1993) Mol. Cell. Biol. 13, 6102–6113
29. Muhlrad, D., and Paker, R. (1994) Nature 370, 578–581
30. Beelman, C. A., Stevens, A., Caponigro, G., LaGrandeur, T. E., Hatfield, L., Fortner, D. M., and Paker, R. (1996) Nature 372, 642–646
31. Kenna, M., Stevens, A., McCammon, M., and Douglas, M. G. (1993) Mol. Cell. Biol. 13, 341–350
32. Larimer, F. W., and Stevens, A. (1990) Gene (Amst.) 85, 85–90
33. Ma et al., S. (1990) J. Biol. Chem. 255, 3080–3085
34. Min, J., and Zassenhaus, H. P. (1989) J. Biol. Chem. 264, 3750–3757
35. Margossian, S. P., Li, H., Zassenhaus, H. P., and Butow, R. A. (1996) Cell 84, 199–209
36. Garriga, G., Bertrand, H., and Lambowitz, A. M. (1984) Cell 36, 623–634
37. Fernandez-Sarabia, M. J., Sartol, A., Zhong, T., and Arndt, K. T. (1992) Genes & Dev. 6, 4173–4228
38. Tanaka, K., Matsumoto, K., and Toh-e, A. (1988) EMBO J. 7, 495–502
39. Marchger, G., Schuller, C., Adam, G., and Ruis, H. (1995) EMBO J. 14, 1977–2003
40. Cherry, J. R., Johnson, T. R., Doolin, C., Schuster, J. R., and Denis, C. L. (1989) EMBO J. 8, 605–612
41. Matsui, A., Tanaka, K., and Matsumoto, K. (1995) Mol. Cell. Biol. 15, 5740–5749
42. Watanabe, Y., Irie, K., and Matsumoto, K. (1995) Mol. Cell. Biol. 15, 5740–5749