Saccharomyces cerevisiae Nfs1p is mainly found in the mitochondrial matrix and has been shown to participate in iron-sulfur cluster assembly. We show here that Nfs1p contains a potential nuclear localization signal, RRRPR, in its mature part. When this sequence was mutated to RRGSR, the mutant protein could not restore cell growth under chromosomal NFS1-depleted conditions. However, this mutation did not affect the function of Nfs1p in biogenesis of mitochondrial iron-sulfur proteins. The growth defect of the mutant was complemented by simultaneous expression of the mature Nfs1p, which contains the intact nuclear localization signal but lacks its mitochondrial-targeting presequence. These results suggest that a fraction of Nfs1p is localized in the nucleus and is essential for cell viability.

The NifS protein is found in various organisms and is involved in iron-sulfur protein biosynthesis. In the diazotroph Azotobacter vinelandii, the nifS gene was first identified as a member of the nif operon that plays an essential role in the biosynthesis of nitrogenase (1). Extensive biochemical analyses of A. vinelandii NifS reveal that it is a PPLP-containing enzyme exhibiting desulfurase activity that produces elemental sulfur and L-alanine from L-cysteine (2). These findings suggest that the physiological function of NifS is to supply inorganic sulfur for the assembly of the iron-sulfur cluster in nitrogenase (3, 4).

Many eukaryotic NifS homologues have been identified, of which the Saccharomyces cerevisiae NifS homologue, Nfs1p, is the best characterized (5–9). The nfs1 null mutant is lethal (5, 9), and Nfs1p is localized mostly to the mitochondrial matrix (7–9). Mitochondrial Nfs1p mediates the assembly of the iron-sulfur cluster of both mitochondrial and cytosolic iron-sulfur proteins (6, 8) and also regulates mitochondrial and cytosolic iron homeostasis (9). Kolman and Söll (5) also show that a mutation in the NFS1 gene suppressed a certain tRNA-splicing mutant (5), although the role of Nfs1p in the process of tRNA splicing, which is thought to take place in the nucleus, remains unclear.

Mouse and human counterparts to Nfs1p (mNfs1 and hNfs1, respectively) have also been identified (7, 10). We showed that mNfs1 was found mainly in the mitochondrial matrix (7). The full-length hNfs1 protein also possesses a mitochondrial-targeting presequence and has been shown to be localized to mitochondria. Different sized hNfs1 proteins from a single transcript have also been detected in the cytosolic and nuclear fractions (10), but the physiological significance of these extramitochondrial hNfs1 proteins has not been elucidated.

The Escherichia coli NifS homologue, IcsS, is a multifunctional enzyme that possesses important physiological functions not only in iron-sulfur cluster assembly (11, 12) but also in sulfur transfer to some intracellular compounds (13–18). These results suggest that a fraction of Nfs1p may have some essential function other than iron-sulfur cluster assembly, possibly in a different subcellular location. In this study, we identified a potential nuclear localization signal (NLS) sequence in the mature domain of yeast Nfs1p and showed in vivo that this sequence was crucial for the physiological function of extramitochondrial Nfs1p.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—The S. cerevisiae strains used in this study are listed in Table I. A 2% galactose-containing medium (SC-Gal) was used to express a gene under the galactose-inducible GAL1 promoter. A 2% glucose-containing medium (SC-D) and a 2% lactate-containing medium (SC-lactate) were used to depress the GAL1 promoter (19).

Mutagenesis of the NFS1 Gene and Plasmid Construction—A 5′-terminal 500-base pair fragment of the NFS1 gene was amplified by PCR from genomic DNA isolated from the wild-type strain W303–1B (20). It was cloned into the URA3-containing vector pXO103 (Ingenius) downstream of the GAL1 promoter. This plasmid was linearized by cutting at a unique BlnI site in the cloned NFS1 fragment and was integrated by homologous recombination into the corresponding region in the chromosomal NFS1 locus in W303–1B cells. Recombinants were selected on uracil-depleted medium, and correct integration was confirmed by PCR. One recombinant whose chromosomal NFS1 expression was controlled by the inducible GAL1 promoter was named YN101 and used as a host strain to express the additional plasmid-borne Nfs1p (either wild-type or mutant Nfs1p).

To construct a plasmid that constitutively expresses the wild-type Nfs1p, the entire NFS1-coding region was amplified by PCR and then subcloned into a CEN4-based TRP1-containing plasmid pTT-GAP (21) so the inserted NFS1 could be expressed under the constitutive glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter. This plasmid was named pTT-NFS1. To express Nfs1p with a carboxyl-terminal hexahistidine peptide tag, the pTT-NFS1-h6 plasmid was also constructed in a similar manner to pTT-NFS1, except for the use of a (CATCAC)3-containing 3′-end primer for the initial PCR amplification.

The NLS-like sequence RRRPR found in the Nfs1p clone was changed to RRGSR by site-directed mutagenesis using a QuikChange site-directed mutagenesis kit (Stratagene). The resulting plasmid was named pTT-GSR-NFS1-h6, which expressed the mutant Nfs1p containing the RRGSR sequence instead of the intact RRRPR sequence.

According to the sequence alignment of NifS-like proteins, a methionine at residue 104 in yeast Nfs1p (Met104) was near the second methionine of hNfs1, and another methionine at residue 118 (Met118) was conserved among the three eukaryotic NifS-like proteins including hNfs1. Met104 and the Met118 in yeast Nfs1p were replaced with alanine by site-directed mutagenesis, and the resulting mutant NFS1-h6 genes were used in this study.
Rhodobacter sphaeroides (and the corresponding regions in other homologs are highlighted in a black box or mutant Nfs1p with or without a (histidine)_6-tag under the constitutive GAL1 promoter: pYK-NFS1 for the entire Nfs1p-EGFP, pYK-GSR-NFS1 for Nfs1p-EGFP with the altered RRGSR sequence in Nfs1p, pYK-mNfs1p for mNfs1p-EGFP, and pYK16-GSR-mNFS1 for mNfs1p-EGFP with the altered RRGSR sequence. These plasmids were transformed into S. cerevisiae YN101(pTT-GSR-NFS1-h6) by homologous recombination. ADE2^+ colonies were selected on the adenine-depleted SC-Gal, and cells with proper integration were used for further analyses.

One XhoI restriction site in the pYK16 plasmid (kindly provided by S. Nishikawa at Nagoya University) that was located downstream of the EGFP gene was removed, and the resulting plasmid was named pYK16EX. The following pYK16EX derivatives were used for the expression of various Nfs1p-EGFP fusion proteins under the yeast GAL1 promoter: pYK-NFS1 for the entire Nfs1p-EGFP, pYK-GSR-NFS1 for Nfs1p-EGFP with the altered RRGSR-sequence in Nfs1p, pYK-mNfs1p for mNfs1p-EGFP, and pYK16-GSR-mNFS1 for mNfs1p-EGFP with the altered RRGSR sequence. These plasmids were transformed into S. cerevisiae W303 (diploid strain), and transformants were selected on SC-Gal to ADE2 and grown in SC-Gal to 0.5 and fixed with 5% formaldehyde in 0.1 M potassium phosphate buffer (pH 6.5) for 2 h at room temperature. Cells were then resuspended in phosphate-buffered saline and mounted on poly-L-lysine-coated glass and observed by fluorescence microscopy (Nikon).

Subcellular Fractionation and Immunological Detection of Proteins—Yeast cells were grown in SC-Gal to A_600 to 0.5 and fixed with 5% formaldehyde in 0.1 M potassium phosphate buffer (pH 6.5) for 2 h at room temperature. Cells were then resuspended in phosphate-buffered saline and mounted on poly-L-lysine-coated glass and observed by fluorescence microscopy (Nikon).
Eukaryotic Nfs1 proteins (Fig. 1)

Sequence, which is similar to the NLS-like sequence found in the mature Nfs1p sequence (Fig. 1) amino acid sequence with the protein sorting signal searching found in bacterial NifS proteins (7, 10). Analysis of the Nfs1p targeting presequence followed by a conserved sequence also the known eukaryotic Nfs1 proteins have a mitochondrial-cysteine or threonine) instead of the basic residue in the third or the corresponding region, including one neutral residue (glycine or threonine) instead of the basic residue in the third or the fourth position of the sequence (Fig. 1B). However, both the E. coli and A. vinelandii IscS proteins possess the RRKPR sequence, which is similar to the NLS-like sequence found in eukaryotic Nfs1 proteins (Fig. 1B).

Ectopic mNfs1p-EGFP Lacking the Mitochondrial-targeting Sequence Localized to Both Nucleus and Cytosol but Not Mitochondria—Several attempts to detect endogenous Nfs1p in the nucleus by immunoblotting failed (7, 8), possibly because Nfs1p is only present at extremely small levels in the nucleus. We tried to visualize the intranuclear localization of Nfs1p by overexpressing Nfs1p-EGFP fusion protein with or without the mitochondrial-targeting presequence (Fig. 2). The fusion protein was expressed in wild-type yeast diploid cells, and cellular localization of the proteins was observed by fluorescent microscopy. The Nfs1p precursor protein, which contained the intact NLS-like sequence, was fused to the amino terminus of EGFP, the fusion protein was clearly localized to mitochondria—consistent with previous Western blotting analyses (7, 8), whereas only faint fluorescence was observed in the cytosol and nucleus (Fig. 2c). In contrast, when the mNfs1p-EGFP was ectopically expressed, weak fluorescence could be detected in the cytosol and nucleus but not in mitochondria (Fig. 2e). We also examined localization of the GSR-mNfs1p-EGFP fusion protein, which contained an RRGRS sequence in place of the intact NLS-like sequence in Nfs1p. This RRGRS sequence was designed to mimic the corresponding region of bacterial NifS proteins (Fig. 1B). The resulting GSR-mNfs1p-EGFP also remained in the cytosol (Fig. 2f). However, because of the very faint and scattered fluorescence, it was unclear whether the fused protein was excluded from the nucleus. Thus, we next took a more sensitive approach to exploring the possible localization of Nfs1p to the nucleus.

**RESULTS**

**Eukaryotic Nfs1 Proteins Have a NLS-like Sequence**—All of the known eukaryotic Nfs1 proteins have a mitochondrial-targeting presequence followed by a conserved sequence also found in bacterial NifS proteins (7, 10). Analysis of the Nfs1p amino acid sequence with the protein sorting signal searching program PSORT also identified an NLS-like sequence, RRKPR, in the mature Nfs1p sequence (Fig. 1A). Sequence alignment of three known eukaryotic (yeast, mouse, and human) Nfs1 proteins showed that this NLS-like sequence was completely conserved (Fig. 1B). In contrast, most bacterial NifS proteins, including A. vinelandii Nfs1p, have somewhat diverse sequences in the corresponding region, including one neutral residue (glycine or threonine) instead of the basic residue in the third or the fourth position of the sequence (Fig. 1B). However, both the E. coli and A. vinelandii IscS proteins possess the RRKPR sequence, which is similar to the NLS-like sequence found in eukaryotic Nfs1 proteins (Fig. 1B).

**Nuclear Transportation Trap Analysis Showed That a Fraction of Nfs1p Fusion Protein Is Present in the Nucleus**—Using the nuclear transportation trap method (22), we examined whether fusion of Nfs1p to another protein could mediate translocation of that protein into the nucleus. In this method, NES-LexAD, an engineered transcription factor containing a nuclear export signal, is used to test whether a protein of interest has the capacity to localize to the nucleus of yeast EGY48 cells (28), which harbor the LexAD-responsive LEU2 reporter gene. If the fusion protein contains an NLS, transformants are expected to form Leu” colonies. We constructed two mNfs1p fusion proteins, one with an intact RRKPR sequence (NES-LexAD-mNfs1p) and the other with a mutated RRGRS sequence (NES-LexAD-GSR-mNfs1p). When the expression vector encoding each of these fusion proteins was introduced into EGY48 cells, transformants were first selected for a His” phenotype. Expression of the fusion proteins was confirmed immunologically with anti-LexAD monoclonal antibody (data not shown). Then these transformants were further tested for the leucine prototroph (Fig. 3). If the Nfs1p contains a functional NLS, transformants were expected to form His”/Leu” colonies. As shown in Fig. 3C, transformants that expressed

![Fig. 2. Fluorescent microscopic analyses of yeast expressing various Nfs1p-EGFP proteins.](image-url)
the NES-LexAD-mNfs1p showed the Leu\(^+\) phenotype, whereas cells expressing the NES-LexAD-GSR-mNfs1p had the His\(^+\)/Leu\(^+\) phenotype (Fig. 3D). These results suggested that the RRRPR sequence potentially functions as an NLS in Nfs1p and that nuclear localization is lost when the sequence is altered to RRGSR.

**Mutation of the NLS Sequence in Nfs1p Induced a Growth Defect in Cells under Wild-type Nfs1p-depleted Conditions**—The \(\text{NFS1}\) gene has been shown to be essential for cell viability (5, 8). Therefore, a yeast strain named YN101 in which the chromosomal \(\text{NFS1}\) gene was expressed under the control of the \(\text{GAL1}\) promoter was constructed and used as a host strain for \textit{in vivo} complementation analyses. YN101 cells grow normally like the wild-type W303–1B strain in SC-Gal but cannot grow in SC-D. When the plasmid-borne Nfs1p precursor with a hexahistidine tag (Nfs1p-h6) was constitutively expressed in YN101 cells, the cell growth in SC-D was restored (section 1 in Fig. 4). In contrast, expression of the hexahistidine-tagged mNfs1p (mNfs1p-h6) could not restore the cell growth in SC-D (Ref. 8, data not shown). These results confirm that the mitochondrial localization of Nfs1p is essential for cell growth. We then examined whether the tagged mutant Nfs1p precursor (GSR-Nfs1p-h6) could affect the complementation ability when expressed in YN101 cells. As shown clearly in section 2 in Fig. 4, GSR-Nfs1p-h6 could not restore cell growth under the wild-type Nfs1p-depleted conditions. We also showed that two other Nfs1p mutants in which either the alternative AUG, corresponding to the second or the third AUG used in hNfs1 had been changed to GCG for alanine, could restore cell growth under the wild-type Nfs1p-depleted conditions (sections 3 and 4 in Fig. 4).

After 40 h of growth in SC-lactate, the \(\text{GAL1}\) promoter-driven expression of the wild-type Nfs1p in YN101 was depressed to a level at which no cross-reactive proteins could be detected by immunoblotting using anti-mouse-Nfs1 antibody (Fig. 5). Under the same growth conditions, constitutively expressed plasmid-borne Nfs1p derivatives could be detected both with the anti-mouse Nfs1 antibody and with the anti-histidine-tag antibody. The GSR-Nfs1p-h6 fusion protein was found to be localized to mitochondria and present in amounts comparable with that of the control Nfs1p-h6 (Fig. 5).

**The Nfs1p Mutant with the RRGSR Sequence Exhibits Nearly Normal Mitochondrial Iron-Sulfur Protein Activities**—We then assayed the enzymatic activities of mitochondrial iron-sulfur proteins in the strain YN101(pTT-GSR-NFS1-h6) to test whether the GSR-Nfs1p functions in mitochondria equivalent to wild-type Nfs1p. The activities of two mitochondrial iron-sulfur cluster-containing enzymes, aconitase and succinate dehydrogenase, were assayed in mitochondrial fractions that contained either Nfs1p-h6 or GSR-Nfs1p-h6 after growth of the cells in the absence of galactose for 40 h. As a control, the activity of a non-iron sulfur protein, malate dehydrogenase, was also assayed. As shown in Fig. 6, the GSR-Nfs1p-h6-containing mitochondria retained the activities of all three enzymes examined, with slightly lower levels than Nfs1p-h6-containing mitochondria. Mitochondria that contained no plasmid-borne Nfs1p exhibited low levels of aconitase and succinate dehydrogenase activity but a similar level of the malate dehydrogenase activity to those containing the plasmid-borne Nfs1p-h6 (Fig. 6). These results indicate that mitochondrial iron-sulfur proteins were assembled in the active holo form in YN101(pTT-GSR-NFS1-h6) in which chromosomal Nfs1p had been depleted but the plasmid-borne GSR-Nfs1p-h6 was expressed. In other words, the mitochondrially localized GSR-Nfs1p-h6 was found to retain the ability to mediate cluster assembly of mitochondrial iron-sulfur proteins, indicating that the mutation in the NLS sequence did not cause any significant dysfunction in iron-sulfur biogenesis in mitochondria.

**Growth Defect of YN101(pTT-GSR-NFS1-h6) in SC-D Was Rescued by Expression of Mature Nfs1p Containing an Intact NLS Sequence**—If the nuclear localization of Nfs1p is essential for cell viability and if the severe growth defect of YN101(pTT-GSR-NFS1-h6) cells in SC-D is caused by the loss of nuclear localization of GSR-Nfs1p-h6, cell growth should be recovered...
when another mutant Nfs1p with an intact NLS sequence is simultaneously expressed outside the mitochondria. To verify this hypothesis, we constructed a series of NFS1 derivatives (Fig. 7A) and subcloned them into the shuttle vector YIpDCE1 (19). Then we integrated each of these NFS1 derivatives into the ade2 locus of the genomic DNA of YN101 (pTT-GSR-NFS1-h6) and YN101 (pTT-GAP). The Ade<sup>+</sup> transformants, in which the proper integration had been confirmed by the PCR method (19), were spotted in a dilution series on either SC-Gal or SC-D (Fig. 7B, C). When the mature Nfs1p (mNfs1p) was simultaneously expressed in YN101 (pTT-GSR-NFS1-h6), cells could grow well in both SC-Gal and SC-D (Fig. 7B, C). In the strain YN101 (pTT-GAP), which contained no NFS1 gene, the growth of cells in the SC-D was not restored when the mNfs1p was expressed (Fig. 7C, C). Interestingly, when the GSR-mNfs1p was simultaneously expressed in YN101 (pTT-GSR-NFS1-h6), cell growth was not recovered under the wild-type Nfs1p-depleted conditions (Fig. 7B, D). Since mNfs1p, which lacks the presequence, was expressed outside the mitochondria, these results indicate that the defective function of GSR-Nfs1p-h6 can be complemented by extramitochondrial mNfs1p, which contains an intact NLS sequence. This means that, in addition to mitochondrial Nfs1p, a fraction of Nfs1p is required outside the mitochondria, most likely in the nucleus, for cells to survive.

NiF proteins possess a conserved cysteine residue that is the critical active site for desulfurase activity (2–4, 7). The corresponding Cys<sup>421</sup> in yeast Nfs1p was also shown to be essential for its iron-sulfur cluster biogenesis activity in mitochondria (9). To examine whether this Cys<sup>421</sup> is also required for the extramitochondrial function of Nfs1p, we constructed an additional plasmid encoding mutated mNfs1p whose Cys<sup>421</sup> was changed to Ala<sup>421</sup> (Ala<sup>421</sup>-mNfs1p). YN101 (pTT-GSR-NFS1-h6) cells transformed with this plasmid could grow on SC-Gal but could not grow on SC-D (Fig. 7B, D), indicating that the desulfurase activity was also important for the function of Nfs1p in the nucleus.

**DISCUSSION**

Nfs1p has mostly been found in mitochondrial matrix in yeast cells (7) and is essential for cell growth (5, 9). A mutant Nfs1p lacking the mitochondrial-targeting presequence could not restore the growth of the wild-type Nfs1p-depleted cells (Ref. 8 and this study). Since cellular iron-sulfur proteins are involved in important metabolic processes such as electron transfer reactions and transcriptional regulation (29–32) and Nfs1p plays a significant role in iron-sulfur cluster assembly in mitochondria (33), it is not surprising that the functional loss of the mitochondrial Nfs1p should cause the loss of cell viability. In this study, we demonstrated that the localization of Nfs1p to the nucleus is also essential for cell viability. Although we failed to detect any endogenous or ectopically expressed Nfs1p in the nucleus directly by Western blotting or immunohistochemistry (Fig. 2), we were able to show that Nfs1p is present in the nucleus using more sensitive techniques (Figs. 4 to 7). Therefore, a small but significant fraction of Nfs1p is localized to the nucleus in vivo and plays an unknown essential role in cell viability. We also showed that the desulfurase activity of
Nfs1p was required for the function of Nfs1p in the nucleus (Fig. 7) as well as in the mitochondria (9).

There are several possible functions for nuclear localized Nfs1p. Nfs1p may be involved in iron-sulfur cluster assembly in the nucleus, as it is in the mitochondrion. A [4Fe-4S] cluster-containing protein called Ntg2p, a yeast homologue of endonuclease III, has been shown to be present in the eukaryotic nucleus (34). However, it is difficult to imagine that other mitochondrial components involved in the iron-sulfur cluster assembly (Isu1p, Isu2p, Nfu1p, Isa1p, Isa2p, Yah1p) (32) also have dual intracellular localization (in both mitochondrion and nucleus) like the Nfs1p protein. In addition, it has been proposed that the pre-assembled iron-sulfur cluster is transported from the mitochondrial matrix to the cytosol by a certain transporter(s) located in the mitochondrial membrane, which is necessary for the maturation of the cytosolic iron-sulfur proteins (8, 32). Therefore, if the extra-mitochondrial Nfs1p is related to the iron-sulfur cluster in the nucleus rather than being involved in maturation, it may function in the repair or maintenance of pre-assembled iron-sulfur clusters of nuclear-localized iron-sulfur proteins such as Ntg2p.

Another possibility is that the nuclear-localized Nfs1p is involved in nucleotide biosynthesis, because the allele of the NFS1 gene was first identified as a suppresser gene named SPL1, mutation of which affected tRNA splicing (5). tRNA splicing occurs on the inner surface of the nuclear membrane (35) and is greatly influenced by specific modification of the ribonucleotides of tRNA. Recent reports on the distinct functions of the E. coli IsoS protein (13–18) may shed some light on the function of nuclear-localized Nfs1p. E. coli IsoS was first shown to possess a cysteine desulfurase activity that is involved in iron-sulfur protein biosynthesis (11) and, subsequently, a sulfur transferase activity by which a sulfur atom is transferred to a uridine to produce a 4-thiouridine in tRNA (13–15). Following this analogy, it is quite possible that nuclear Nfs1p is involved in nucleotide modification as a sulfur donor. Therefore, a mutation in Nfs1p may affect its function in the modification of specific ribonucleotides in the nucleus and on tRNA splicing.

Recently, IsoS proteins found in E. coli and Salmonella species have been reported to be involved in thiamine and nicotinic acid biosyntheses (15, 36). The Bacillus subtilis nifS gene product has also been reported to function in NAD⁺ biosynthesis (37). Since the tRNA splicing process includes an NAD⁺-dependent step in S. cerevisiae (38, 39), the nuclear Nfs1p is likely to be involved in the process via its effect on NAD⁺ biosynthesis. Therefore, nuclear localization of Nfs1p would be essential for cell survival, because correct tRNA splicing is essential for yeast cell growth.

How is the localization of yeast Nfs1p to either the mitochondrion or the nucleus controlled? It has been reported that human NiFS homologues (hNfs1) of different lengths were synthesized from a single transcript by differential translational initiation from alternative AUG codons and that the smaller (presequence-truncated) forms were found in the cytosol and nucleus, whereas the largest form was in mitochondria in a proteolytically processed mature form (10). Yeast Nfs1p also possesses alternative methionine residues at the positions corresponding to the potential alternative translational initiation AUG codons of hNfs1 (Fig. 1A). However, our experimental data (section 3 and 4 in Fig. 4) suggest that the amino-terminal alternative translation does not contribute to localization of yeast Nfs1p to the nucleus. Internal initiation of mRNA translation coupled to a physiological function is rarely observed in eukaryotes (40–42). Moreover, no internal ribosome entry sites have been shown to function in growing yeast (43). Therefore, the dual intracellular localization of the yeast NiFS homologue may be caused by a mechanism distinct from that of the human counterpart, hNfs1. It has not yet been elucidated whether the hNfs1 in the nucleus is essential for cell growth, but we assume that nuclear hNfs1 has some significant function.

Recently, an increasing number of reports demonstrate localization of one protein to different cellular locations in vivo (44–47). It has been proposed that, in some cases, re-localization of a protein to another organelle occurs after the initial localization to one organelle. For example, a single translation product of the yeast fumarase gene, which is distributed between the cytosol and mitochondrial matrix, is targeted to and processed in mitochondria before distribution (45), suggesting that the newly synthesized fumarase precursor can either be fully translocated cotranslationally into the mitochondrial matrix or it can be folded into an import-incompetent state and released by retrograde movement through the translation pore into the cytosol. Dual distribution of Nfs1p may follow a similar mechanism in which binding of the PLP to the mature domain of the precursor might promote folding outside of the mitochondria and prevent further translocation into the mitochondrial matrix. Such Nfs1p molecules can be released into the cytosol and then be re-localized to the nucleus with the aid of its NLS. Alternatively, the dual localization of the Nfs1p

![Graph](image-url)
might be caused by a simple balance between the NLS and mitochondrial precursor sequences after translation in the cytosol.

Studies on the physiological function of the nuclear-localized Nfs1p in yeast cells are now under way in our laboratory. Determination of proteins that interact with Nfs1p in the nucleus may help to elucidate the function of Nfs1p in this location.

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FIG. 7. Complementation of the GSR-Nfs1p-h6 cell viability defect under the wild-type Nfs1p-depleted growth conditions. A, schematic representation of DNA fragments that were integrated into the YN101p(GP-TT-GAP) or YN101p(GP-GSR-NFS1-h6) cells by homologous recombination described by Stearman et al. (19). Thick boxes indicate the various NPS1 derivatives. The mitochondrial localization precursor is shown with a shadow (mt), and the intact NLS (RRRPR) and the mutated (RRGSR) sequences are shown in black and white, respectively. a, no Nfs1p; b, Nfs1p encoded by YIpNFS1; c, mature Nfs1p by YlpNFS1; d, mature Nfs1p with the RRSR sequence by YlpGSNMFS1; e, mature Nfs1p in which Cys231 was changed to Ala231 by YIpA231-mNFS1. Plasmid construction was described under “Experimental Procedures.” Plasmids were linearized by cutting at a unique site in the ade2 gene (indicated as gray thin lines) and integrated into the ade2 locus of YN101p(GP-TT-GSR-NFS1-h6) (B) or YN101p(GP-TT-GAP) (C) cells. Complementation analysis of the transformants was performed by a serial 10-fold dilution of the cells plated onto the uracil-containing SC-Gal (left) and the SC-D (right) plates. Cells were incubated at 30 °C for 3 days.

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