The IscS protein is a pyridoxal phosphate-containing cysteine desulfurase involved in iron-sulfur cluster biosynthesis. In prokaryotes, IscS is also involved in various metabolic functions, including thio-modification of tRNA. By contrast, the eukaryotic ortholog of IscS (Nfs1) has thus far been shown to be functional only in mitochondrial iron-sulfur cluster biogenesis. We demonstrate here that yeast Nfs1p is also required for the post-transcriptional thio-modification of both mitochondrial (mt) and cytoplasmic (cy) tRNAs *in vivo*. Depletion of Nfs1p resulted in an immediate impairment of the 2-thio-modification of 5-carboxymethylaminomethyl-2-thiouridine at the wobble positions of mt-tRNA 

\[ \text{mcm5s2U} \]

and mt-tRNA 

\[ \text{mcm5U} \]

In addition, we observed a severe reduction in the 2-thio-modification of 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U) of cy-tRNA 

\[ \text{cy-tRNA mcm5s2U} \]

although the effect was somewhat delayed compared with that seen in mt-tRNAs. Mass spectrometry analysis revealed an increase in 5-methoxycarbonylmethyluridine concomitant with a decrease in mcm5s2U in cy-tRNAs that were prepared from Nfs1p-depleted cells. These results suggest that Nfs1p is involved in the 2-thio-modification of both 5-carboxymethylaminomethyl-2-thiouridine in mt-tRNAs and mcm5s2U in cy-tRNAs.

NiS is a pyridoxal phosphate-containing cysteine desulfurase that was first described in the biosynthesis of nitrogenase in diazotrophic bacteria such as Azotobacter vinelandii. Subsequently, it was identified as a supplier of the sulfur atom that is incorporated into the iron-sulfur cluster (ISC)1 in nitrogenase (1, 2). IscS is a homolog of NiS that is found in a wide range of organisms, including non-nitrogen-fixing bacteria (3–5) and even eukaryotes (6–8), and it is now known to mobilize a sulfur atom from L-cysteine to a nascent ISC on a scaffold protein such as IscU or NifU (9–12).

In eukaryotes, the IscS homolog Nfs1 (or Nfs1p) is most frequently found in mitochondria (6–8), along with various iron-sulfur proteins that include essential electron carriers of the respiratory cascade. Saccharomyces cerevisiae Nfs1p, which is essential for cell viability, is also located mainly in mitochondria, where it serves as a sulfur supplier in ISC biogenesis (6, 7, 13–16). Nfs1p has also been localized to the nucleus, where it is thought to function in essential processes other than ISC biosynthesis (14).

Recently, Escherichia coli IscS was shown to mobilize a sulfur atom via pyridoxal phosphate-dependent formation of a disulfide intermediate on the enzyme (17). This unique sulfur-mobilizing action of IscS allows it to participate in the biosynthesis of other sulfur-containing cofactors or small molecules, as well as ISC formation. For example, an E. coli IscS deletion mutant showed a significant decrease in the production of nicotinic acid and branched chain amino acids (18). Serial deletions of the E. coli iscS gene region revealed that IscS is involved in amino acid metabolism (19). Furthermore, in E. coli and Salmonella enterica serovar Typhimurium, IscS contributes to thiamine biogenesis by catalyzing the transfer of sulfur in the pathway involved in the formation of thiazole rings (18, 20).

Further evidence of the sulfur-transferring activity of IscS is seen in the thio-modification of nucleotides in tRNA. E. coli IscS transfers a sulfur atom from the substrate cysteine to produce a 4-thiouridine at position 8 of tRNA *in vivo* (21) and *in vitro* (22). This reaction is partly shared with the thiamine biosynthesis pathway described above by cooperating with ThiI (18, 23). E. coli IscS is also involved in 2-thiouridine formation in tRNA *in vitro* (24). In this case, IscS works together with MnmA (24), and the resulting 2-thiouridine is hypermodified to 5-carboxymethylaminomethyl-2-thiouridine via the joint action of two other proteins (MnmE and MnmC) (25). Furthermore, in E. coli and S. enterica, the IscS protein is involved in the biosynthesis of at least five different thio-modified nucleotides in tRNA, suggesting that IscS plays a critical role in thio-modification in bacterial cells (26, 27).

Modified nucleotides are found in various RNAs in virtually all living organisms (28). Although thio-modification has been found in eukaryotic tRNAs, the participation of Nfs1 (or Nfs1p) has yet to be proven. In eukaryotic cells, thio-modified tRNA molecules are found in both mitochondria and the cytoplasm (29–36), whereas Nfs1 is mainly localized in mitochondria. Therefore, the question remains as to whether Nfs1 is involved in sulfur donation during mitochondrial (mt) and/or cytoplasmic (cy) tRNA thio-modification. In this study, we used a yeast conditional mutant strain (in which the expression of Nfs1p was repressed) to investigate the participation of Nfs1p in the thio-modification of both cy- and mt-tRNAs.

**EXPERIMENTAL PROCEDURES**

Yeast Strains and Cell Growth Conditions—S. cerevisiae W303-1B (MATa, ade2-1, his3-11,15, ura3-1, leu2-3,112, trp1-1, can1-100) was used as the wild-type strain, along with its derivative YN101, in which
the Nfs1p gene (NFS1) is expressed under the control of the GAL1 promoter (14). Cells were grown at 30 °C in galactose medium (2% d-galactose, 0.5% casamino acids, and 0.67% yeast nitrogen base without amino acids, plus adequate supplements for selection; BD Biosciences), lactate medium (2% lactate, 0.1% d-glucose, 0.5% casamino acids, and 0.67% yeast nitrogen base without amino acids, plus adequate supplements for selection), or glucose medium (2% d-glucose, 0.5% casamino acids, and 0.67% yeast nitrogen base without amino acids). For sulfate-limited growth, cells cultivated in normal medium were further incubated with medium prepared with yeast nitrogen base lacking ammonium sulfate (BD Biosciences).

### Pulse Labeling of Yeast Cells with l-[^35]S]Cysteine—Yeast cells incubated with the sulfur-lacking medium described above were harvested and further incubated with l-[^35]S]Cysteine (Amersham Biosciences) at a concentration of 10^7 cells/ml/1000 µCi for 30 min at 30 °C. Cells were then harvested and used to prepare the total tRNA.

### Total tRNA Preparation, Followed by Electrophoresis for Detection of the Radiolabeled tRNA Fraction—Total tRNA from yeast cells was extracted with phenol, precipitated with isopropyl alcohol, and washed once with 70% ethanol. tRNAs were applied to 10% polyacrylamide gels containing 8 M urea. Following electrophoresis, the gel was wrapped entirely and exposed to a Fuji Film imaging plate for >30 h. The exposed imaging plate was then subjected to analysis by a Fuji Film BAS-2500 image analyzer to detect radioactivity in the tRNA fractions.

### Detection of Thiomodified Uridine in tRNAs by ([N-Acryloylamino]-phenyl)Mercaric Chloride (APM) Gel Electrophoresis, Followed by Northern Hybridization (APM/Northern)—The presence of thioridine in the tRNA was verified by the retardation of electrophoretic mobility on polyacrylamide gels containing 0.05 mg/ml APM (kindly provided by Naoki Shigi, University of Tokyo) (37) in a procedure originally developed by Igloi (38). Total RNA (0.5 A260 units) was sequestered by PAGE as described above and blotted onto Hybond N+ membranes (Amersham Biosciences). Each tRNA fraction was detected with a specific 32P-labeled probe. The probe oligonucleotides were used: 5'-TGTTGGAATTCTGAGGGTCAAGCC-3' for mt-tRNAUAC, 5'-TGGTTGAATCTGAGGGTCAAGCC-3' for mt-tRNAGU, 5'-CCTGCCATCGGGGTCAAGCC-3' for cy-tRNAAUU, and 5'-CCTGCCATCGGGGTCAAGCC-3' for cy-tRNAAC. Following hybridization, membranes were washed, dried, and then exposed to the imaging plate for 1 h to detect cy-tRNAs and for at least 24 h to detect mt-tRNAs. Radioactivity was detected with the BAS-2500 image analyzer.

### Mass Spectrometry Analysis of RNA Modification in Yeast tRNAs—Total tRNAs (1.5 A260 units in each case) were digested with 10 µg/ml ribonuclease P1 (Seikagaku Kogyo) and 9 units/ml bacterial alkaline phosphatase (Takara Shuzo) at 37 °C for 3 h. The nucleosides were subjected to liquid chromatography/mass spectrometry analysis using an Agilent 1100 liquid chromatography system equipped with a Thermofinnigan LCQ Duo ion-trap mass spectrometer as described previously (26).

### Immunochemical Detection of Yeast Nfs1p—Yeast cells were harvested by centrifugation, and total proteins were extracted by an alkaline SDS method (14). Protein concentrations were determined by the BCA method (Pierce) with bovine serum albumin (Sigma) as a standard. A total of 2 µg of cellular proteins were analyzed immunohistochemically with anti-Nfs1p antibody to examine the expression levels of Nfs1p (14).

### RESULTS

#### Thio-modification of tRNA Is Dependent on the Expression of Yeast Nfs1p—Considering that bacterial IscS proteins participate in the thio-modification of tRNA (18, 21, 22, 24, 26, 27), it is probable that homologous eukaryotic Nfs1 proteins are also involved in the similar post-transcriptional thio-modification of tRNA. To examine this possibility, we first investigated whether the expression of Nfs1p is necessary for the transfer of a sulfur atom from l-cysteine to tRNA in yeast cells. YN101 cells, in which the Nfs1p gene (NFS1) is expressed under the control of the GAL1 promoter, grew normally in galactose medium (designated as YN-G cells), but their growth was inhibited in glucose medium (designated as YN-D cells) (14), in which the expression of NFS1 was repressed (Fig. 1, A and B). YN-G and YN-D cells, together with wild-type cells grown under similar conditions (designated as WT-G and WT-D cells, respectively) and used as controls, were then separately subjected to in vivo pulse labeling experiments with l-[^35]S]Cysteine to see if its radioactive sulfur transfers to tRNAs. As shown in Fig. 1B, total tRNAs from both WT-G and WT-D cells efficiently incorporated the radioactive sulfur of l-[^35]S]Cysteine. In contrast, total tRNAs from YN-D cells were not radiolabeled with sulfur. As predicted, radiolabeling could be induced by the expression of NFS1 following growth in galactose medium (YN-G cells) (Fig. 1B). These results clearly demonstrate that the efficient thio-modification of tRNAs in vivo depends on the presence of NFS1.

#### Nfs1p Depletion Results in a Lack of Thio-modification of Uridine in mt-tRNAs—As yeast Nfs1p is localized mainly in mitochondria (6, 7), we next investigated whether depletion of Nfs1p can affect the thio-modification of mt-tRNAs. The wobble modification in both mt-tRNA[^UAC] and mt-tRNA[^GUG] has recently been found to possess 5-carboxymethylaminomethyl-2-thiouridine-modified uridine. We employed APM-PAGE/Northern analysis using a specific probe, to detect 2-thiouridine modification in each of these two tRNAs (see “Experimental Procedures”) (37). Both mt-tRNAs from WT-G and WT-D cells showed a remarkably retarded migration on the APM gel (Fig. 2, A and B, right panels) compared with the migration on a gel without APM (left panels). However, mt-tRNAs from glucose-grown YN101 cells (YN-D) were not retarded on the APM gel, whereas mt-tRNAs from cells cultured in galactose medium (YN-G) were retarded because of the induction of the 2-thio modifications (Fig. 2, A and B, right panels). We also analyzed a 3-fold excess amount of tRNA prepared from YN-D cells (Fig. 2, A and B, YN-Dx3) to examine whether residual amounts of mt-tRNAs remained to be thio-modified. However, no thiouridine could be detected in both mt-tRNAs. Moreover, we carried out the experiment with YN101 cells under a different Nfs1p-depleted growth condition in which 2% lactate and 0.1% glucose were included instead of 2% glucose (designated as WT-L and YN-L cells) (Fig. 2). Under this non-fermentative growth condition, yeast mitochondria are known to develop well because of the lack of metabolic repression. However, as shown in Fig. 2 (A and B), both mt-tRNAs from YN-L cells displayed a similar absence of thiouridine compared with those from YN-D cells.

These results indicate that the thio-modification of uridine in both mt-tRNA[^UAC] and mt-tRNA[^GUG] requires the presence of Nfs1p. Because both mt-tRNAs are encoded by mitochondrial DNA, the thio-modification of uridine in these mt-tRNAs is most likely an intramitochondrial event that is facilitated by mitochondrial Nfs1p.

#### Deficiency in Thio-modification of Uridine in cy-tRNAs Is Caused by Nfs1p Depletion—We further investigated whether Nfs1p is involved in the 2-thio modification of uridine in cy-tRNAs. The 2-thio modification of cy-tRNA[^UAC] and cy-tRNA[^GUG] at the wobble positions has been reported previously (40). By APM-PAGE/Northern analysis, we found two yeast cy-tRNAs (cy-tRNA[^UAC] and cy-tRNA[^GUG]) from wild-type cells that displayed retarded migration on the APM gel (Fig. 3, right panels), indicating the presence of thiouridine. This was the case regardless of the culture medium. However, in Nfs1p-depleted YN101 cells (YN-L and YN-D), the retarded bands with 2-thiouridine were less intense, and samples also displayed non-retarded bands (Fig. 3). These results demonstrate that Nfs1p depletion affects the 2-thio modification of uridine in these cy-tRNAs in vivo without completely abolishing it.

The most remarkable finding of the results shown in Fig. 3 is that significant fractions of these cy-tRNAs were still thio-modified despite the cells being cultured for 48 h in a medium that results in Nfs1p depletion. This is compared with the
complete absence of 2-thiouridine formation in both mt-
radiography using an imaging analyzer (middle panel). Nfs1p pro-
these cells were immunochemically detected using anti-Nfs1p
sequently not responsible for the delayed impairment of the thio-
modification of mt-tRNAs, Nfs1p is also involved in the thio-
modification of cy-tRNAs at the level of sulfur incorporation into position 2 of mcmm5U to form mcmm5s2U.

**DISCUSSION**

In prokaryotes, IscS-mediated sulfur mobilization is utilized in various processes, including the biosynthesis of ISC, thia-
markedly reduced (data not shown). We performed APM-PAGE/Northern analy-
somehow delayed compared with the immediate defect in the

**Yeast Nfs1p Is Involved in tRNA Modification**

3 Available at www.yeastgenome.org.
thio-modification of mt-tRNAs. One possible explanation is that, compared with the thio-modified mt-tRNAs, thio-modified cy-tRNAs are more stable, and their turnover is quite slow in vivo, despite the fact that we could observe de novo synthesis of thio-modified tRNAs (mostly cy-tRNAs) as early as 30 min after addition of L-[35S]cysteine. We previously showed that a trace amount of the nuclear version of Nfs1p is needed for an as yet unknown essential function besides ISC formation (14). Therefore, it is possible that nuclear Nfs1p might be involved in the thio-modification of cy-tRNA during the process of tRNA maturation in the nucleus. On the other hand, another pathway that incorporates inorganic sulfur compounds such as ammonium sulfate is unlikely, as the presence of excess sulfate in glucose medium did not influence the impairment of the

Fig. 2. Nfs1p-dependent thio-modification of uridine occurs in mt-tRNA_{UUU}^{Lys} and mt-tRNA_{UUG}^{Gln}. Separate amounts (0.05 A_{260} units each) of the total tRNAs prepared from yeast cells were separated by electrophoresis on 8 M urea-containing polyacrylamide gels with (+) and without (−) 120 μM APM, blotted onto the membrane, and subjected to the Northern hybridization analysis with 32P-labeled DNA probes. A and B show the results using the DNA probes specific for mt-tRNA_{UUU}^{Lys} and mt-tRNA_{UUG}^{Gln}, respectively. D3 indicates that a 3-fold increase in the amount of total tRNA (0.15 A_{260} units) from YN-D cells was applied.

Fig. 3. Thio-modification of uridine in cy-tRNA_{UUU}^{Lys2} and cy-tRNA_{UUC}^{Glu3} is also affected by the depletion of Nfs1p. The total tRNAs prepared from cells were separated using urea-containing gel with (+) and without (−) 60 μM APM and then subjected to Northern analysis. A and B show the results with the DNA probes for cy-tRNA_{UUU}^{Lys2} and cy-tRNA_{UUC}^{Glu3}, respectively.

Fig. 4. Impairment of the thio-modification of cy-tRNA_{UUU}^{Lys2} and cy-tRNA_{UUC}^{Glu3} following depletion of Nfs1p is delayed compared with the immediate defect found in mitochondrial thio-modification. YN101 cells were cultured in glucose medium (Nfs1p-depleted conditions) for various incubation times (0, 30, and 48 h). Total tRNAs were prepared from these cells and subjected to APM-PAGE/Northern analysis (Fig. 3) using the probes for mt-tRNAs (A) and cy-tRNAs (B). mt-t(K) and mt-t(Q) indicate mt-tRNA_{UUU}^{Lys} and mt-tRNA_{UUG}^{Gln}, respectively, and cy-t(K) and cy-t(E) indicate tRNA_{UUU}^{Lys2} and cy-tRNA_{UUC}^{Glu3}, respectively.

Fig. 5. Cultivation under sulfate-limiting conditions does not affect the delayed impairment of the thio-modification of cy-tRNA_{UUU}^{Lys2}. YN101 cells were cultured either in galactose medium (G) or glucose medium (D) in the presence of ammonium sulfate for 24 h and then harvested. These harvested cells were cultured for an additional 24 h in fresh galactose or glucose medium under sulfate-containing (+ Sulfate in culture) or sulfate-limiting (− Sulfate in culture) conditions. Note that, for both growth conditions, sulfur-containing amino acids such as cysteine and methionine were included in the medium. Cells were then analyzed by APM-PAGE/Northern analysis using the probe for cy-tRNA_{UUU}^{Lys2} as described in the legend to Fig. 3.
thio-modification of cy-tRNA\textsuperscript{UUU} under the Nfs1p-limiting condition (Fig. 5).

Alternatively, if a certain ISC protein is involved in the thio-modification of cy-tRNAs, and if mitochondrial Nfs1p is responsible for ISC synthesis, Nfs1p depletion may indirectly impair the thio-modification of cy-tRNAs. This would be similar to the case in \textit{E. coli} for the iron-sulfur protein MiaB, which produces an IscS-dependent methylthio-modification of 2-methylthio-N\textsuperscript{6}-isopentanyladenosine at position 37 in \textit{E. coli} tRNA (26). Although it has been reported that \textit{iscS} deletion results in a defect in the methylthio-modification in \textit{E. coli} (44), it is not known whether \textit{E. coli} IscS is directly involved in the methylthio-modification or is just responsible for the biosynthesis of MiaB.

Another possibility is that sulfur atoms found in thionucleotides of cy-tRNAs are generated by Nfs1p-mediated cysteine desulfuration in mitochondria and are then transported outside via an as yet unknown pathway. We observed that the thio-modification of cy-tRNAs seemed to be prolonged in the Nfs1p-depleted cells grown under non-fermentative conditions, where mitochondria were well developed, suggesting that mitochondrial development might further increase such accumulation of any sulfur-containing metabolite. In the case of cytoplasmic iron-sulfur protein maturation, cluster-containing sulfur atoms are delivered via mitochondrial ISC biosynthesis machinery, and mitochondrial glutathione and at least two mitochondrial proteins, Atm1p and Erv1p, have been found to be required in this pathway (7, 45, 46). These mitochondrial factors, including some possible small sulfur-containing non-proteinaceous factors, might also be involved in the sulfur delivery system for cy-tRNAs. In addition, delayed impairment of the thio-modification of cy-tRNAs following Nfs1p depletion might be due to cytoplasmic accumulation of any sulfur-containing intermediate metabolite(s) for a pathway located downstream of the reaction of mitochondrial Nfs1p. Such accumulated metabolites could be used as sulfur donors in the absence of Nfs1p. Further study will be required to test these hypotheses and to identify any additional components required for these thio-modifications.

Bacterial IscS is involved in all thio-modifications of tRNAs, raising the possibility that yeast Nfs1p may also be involved in other thio-modifications of both cy- and mt-tRNAs. Future studies should investigate whether Nfs1p is indispensable in other thio-modifications. Yeast Nfs1p was first identified as a protein involved in tRNA splicing \textit{in vivo} (47), but the relationship between Nfs1p function and tRNA splicing has not yet been elucidated. We hope that the actions of Nfs1p in tRNA thio-modification demonstrated in this study may shed some light on this problem, which we will address in the future.

**FIG. 6.** Liquid chromatography/mass spectrometry nucleoside analysis of total tRNAs prepared from wild-type and Nfs1p-depleted yeast cells. Shown are chromatograms for total tRNAs prepared from WT-D (A) and YN-D (B) cells. Upper panels, chromatograms for modified uridines at a range of m/z 332.5 to 333.5 to detect ionized mcm\textsuperscript{5}s\textsuperscript{2}U. Middle panels, chromatograms at a range of m/z 316.5 to 317.5 to detect ionized mcm\textsuperscript{5}U. Lower panels, chromatograms at a range of m/z 412.5 to 413.5 to detect ionized N\textsuperscript{6}-threonylcarbamoyladenosine (t\textsuperscript{6}A). Relative amounts of each nucleoside were normalized with the content of N\textsuperscript{6}-threonylcarbamoyladenosine, which could be regarded as an internal standard. The chemical structures of nucleosides detected are shown in the chromatograms.
Acknowledgments—We thank Naoki Shigi for providing purified APM and Dr. Takeo Suzuki and Yoshiko Ikeuchi (University of Tokyo) for technical assistance with the liquid chromatography/mass spectrometry analysis.

REFERENCES

1. Zheng, L., and Dean, D. R. (1994) *J. Biol. Chem.* **269**, 18723–18726
2. Zheng, L., White, R. H., Cash, V. L., and Dean, D. R. (1994) *Biochemistry* **33**, 4714–4720
3. Zheng, L., Cash, V. L., Flint, D. H., and Dean, D. R. (1998) *J. Biol. Chem.* **273**, 13264–13272
4. Flint, D. H. (1996) *J. Biol. Chem.* **217**, 10698–106974
5. Jaszczowska, K., and Seidler, A. (2000) *Biochemistry* **39**, 3416–3423
6. Nakai, Y., Yoshihara, Y., Hayashi, H., and Kagamiyama, H. (1998) *FEBS Lett.* **433**, 143–148
7. Kispal, G., Csere, P., Prohl, C., and Lill, D. (1999) *EMBO J.* **18**, 3981–3989
8. Land, T., and Rouault, T. A. (1998) *Mol. Cell* **2**, 867–875
9. Yuvariyan, P., Agar, J. N., Cash, V. L., Johnson, M. K., and Dean, D. R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 599–604
10. Urbina, H. D., Silberg, J. J., Hoff, K. G., and Vickery, L. E. (2001) *J. Biol. Chem.* **276**, 44521–44526
11. Frazzon, J., Fick, J. R., and Dean, D. R. (2002) *Biochem. Soc. Trans.* **30**, 680–685
12. Mühlenhoff, U., and Lill, D. (2000) *Biochim. Biophys. Acta* **1459**, 370–382
13. Li, J., Kogan, M., Knight, S. A., Pain, D., and Dancis, A. (1999) *J. Biol. Chem.* **274**, 33025–33034
14. Nakai, Y., Nakai, M., Hayashi, H., and Kagamiyama, H. (2001) *J. Biol. Chem.* **276**, 8314–8320
15. Strain, J., Lorenz, C. R., Bode, J., Garland, S., Smolen, G. A., Ta, D. T., Vickery, L. E., and Caleot, V. C. (1998) *J. Biol. Chem.* **273**, 31145–31144
16. Lill, R., and Kispal, G. (2000) *Trends Biochem. Sci.* **25**, 352–356
17. Vickery, J. R., Urbina, H., and Vickery, L. E. (2003) *J. Mol. Biol.* **330**, 1049–1059
18. Lauh, C. T., and Kamambati, R. (2000) *J. Biol. Chem.* **275**, 20096–21003
19. Tokumoto, U., and Takahashi, Y. (2001) *J. Biochem. (Tokyo)* **130**, 63–71
20. Skovran, E., and Dumas, D. M. (2000) *J. Bacteriol.* **182**, 3896–3903
21. Kamambati, R., and Lauh, C. T. (2000) *J. Biol. Chem.* **275**, 10727–10730
22. Kamambati, R., and Lauh, C. T. (1999) *Biochemistry* **38**, 16561–16568
23. Mueller, E. G., Palenchar, P. M., and Buck, C. J. (2001) *J. Biol. Chem.* **276**, 33588–33595
24. Kamambati, R., and Lauh, C. T. (2003) *Biochemistry* **42**, 1109–1117
25. Haggervall, T. G., Pomerantsev, S. C., and McElwkey, J. A. (1998) *J. Mol. Biol.* **284**, 33–42
26. Lauh, C. T. (2002) *J. Bacteriol.* **184**, 6820–6829
27. Nilsson, K., Lundgren, H. K., Hagervall, T. G., and Björk, G. R. (2002) *J. Bacteriol.* **184**, 6830–6835
28. Björk, G. R. (1995) in *RNA: Structure, Biosynthesis and Function* (Soll, D., and RajBhandary, U. L., eds) American Society for Microbiology, Washington, D. C.
29. Yasukawa, T., Suzuki, T., Ishii, N., Ueda, T., Ohta, S., and Watanabe, K. (2000) *FEBS Lett.* **467**, 175–178
30. Yasukawa, T., Suzuki, T., Suzuki, T., Ueda, T., Ohta, S., and Watanabe, K. (2000) *J. Biol. Chem.* **275**, 4251–4257
31. Laven, H. M., Cramer, J. H., and Rowend, R. H. (1983) *Biochim. Biophys. Acta* **741**, 1–6
32. Patwardhan, S., and Cherapji, J. D. (1985) *J. Biol. Chem.* **260**, 55–60
33. Grossenbacher, A. M., Stadelmann, B., Heyer, W. D., Thuriaux, P., Kohli, J., Smith, C., Agris, P. F., Kuo, K. C., and Gehrke, C. (1986) *J. Biol. Chem.* **261**, 16351–16355
34. Isel, C., Marquet, R., Keith, G., Ehresmann, C., and Ehresmann, B. (1993) *J. Biol. Chem.* **268**, 25269–25272
35. Ch hed a, G. B., Patr zyc, H. B., Tworek, H. A., and Dutta, S. P. (1999) *Nucleo- sides Nucleotides* **18**, 2155–2173
36. Ashraf, S. S., Sochacha, E., Cain, R., Guenther, R., Malkiewicz, A., and Agris, P. F. (1999) *RNA* **5**, 188–194
37. Shi gi, N., Suzuki, T., Tamakoshi, M., Oshima, T., and Watanabe, K. (2002) *J. Biol. Chem.* **277**, 39128–39135
38. Igloi, G. L. (1988) *Biochemistry* **27**, 3842–3849
39. Kaneko, T., Suzuki, T., Kapushor, S. T., Rubio, M. A., Ghazvini, J., Watanabe, K., Simpson, L., and Suzuki, T. (2003) *EMBO J.* **22**, 657–667
40. Han i, J., and Feldmann, H. (1998) *Nucleic Acids Res.* **26**, 689–696
41. Mihara, H., Kato, S., Lacroix, G. M., Stadtmann, T. C., Kennedy, T. C., Kennedy, R. A., Kurrilhara, T., Takemoto, U., Takakashi, Y., and Esaki, N. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 6679–6683
42. Schwarz, C. J., Dajna, O., Imay, J. A., and Kiley, P. J. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 9009–9014
43. Mühlenhoff, U., Gerber, J., Richhardt, N., and Lill, R. (2003) *EMBO J.* **22**, 4815–4825
44. Peters, P., Björk, G. R., Fontecave, M., and Atta, M. J. (2002) *J. Biol. Chem.* **277**, 13367–13370
45. Lange, H., Kispal, G., Kaut, A., and Lill, R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 1050–1055
46. Sipos, K., Lange, H., Fekete, Z., Ullmann, P., Lill, R., and Kispal, G. (2002) *J. Biol. Chem.* **277**, 26944–26949
47. Kolman, C., and Soll, D. (1993) *J. Bacteriol.* **175**, 1433–1442

*Yeast Nfs1p Is Involved in tRNA Modification*