Structural and Thermodynamic Evidence for a Stabilizing Role of
Nop5p in S-Adenosyl-l-methionine Binding to Fibrillarin*

Mohamed Aittaleb++, Thomas Visone‡, Marcia O. Fenley§, and Hong Li**

*This work was supported by National Institutes of Health Grant
R01 GM66658–01 (to H. L.) and National Science Foundation Grant
CHE-0157961 (to M. O. F.). The costs of publication of this article were
defrayed in part by the payment of page charges. This article must
therefore be hereby marked "advertisement" in accordance with 18
U.S.C. Section 1734 solely to indicate this fact.
‡To whom correspondence should be addressed. Fax: 850-644-7244;
E-mail: hongli@sb.fsu.edu.
§The abbreviations used are: MTase, methyltransferase; snoRNP,
small ribonucleoprotein particle; MJ, Methanococcus jannaschii;
Nop5p, S-adenosyl-l-methionine; AF, Archaeoglobus fulgidus; PF,
Pyrococcus furiosus; ITC, isothermal titration calorimetry; AdaHey,
S-adenosyl-l-homocystein.

In Archaea, fibrillarin and Nop5p form the core complex
of box C/D small ribonucleoprotein particles, which are
responsible for site-specific 2′-hydroxyl methylation of
ribosomal and transfer RNAs. Fibrillarin has a con-
served methyltransferase fold and employs S-adenosyl-
l-methionine (AdoMet) as the cofactor in methyl trans-
fer reactions. Comparison between recently determined
crystal structures of free fibrillarin and fibrillarin-
Nop5p-AdoMet tertiary complex revealed large confor-
mational differences at the cofactor-binding site in
fibrillarin. To identify the structural elements responsible
for these large conformational differences, we re-
framed a crystal structure of Archaeoglobus fulgidus
fibrillarin-Nop5p binary complex at 3.5 Å. This structure
exhibited a pre-formed backbone geometry at the cofac-
tor-binding site similar to that when the cofactor is
bound, suggesting that binding of Nop5p alone to fibril-
larin is sufficient to stabilize the AdoMet-binding
pocket. Calorimetry studies of cofactor binding to fibril-
larin and to fibrillarin-Nop5p binary complex pro-
vided further support for this role of Nop5p. Mutagene-
sis and thermodynamic data showed that a cation-π
bridge formed between Tyr-89 of fibrillarin and Arg-169
of Nop5p, although dispensable for in vitro methylation
activity, could partially account for the enhanced bind-
ing of cofactor to fibrillarin by Nop5p. Finally, assess-
ment of cofactor-binding thermodynamics and catalytic
activities of enzyme mutants identified three additional
fibrillarin residues (Thr-70, Glu-88, and Asp-133) to be
important for cofactor binding and for catalysis.

2′-O-Methylation is one of the most frequent modifications
on specific nucleotides within rRNA and other classes of RNA.
In vertebrates, more than one hundred 2′-O-methyl groups
have been identified in rRNA, most of which occur at highly
conserved locations within functionally important regions (1).
The methyltransferase (MTase)† that is responsible for 2′-O-
methylation of the majority of rRNA nucleotides is the box C/D
small ribonucleoprotein particle (snoRNP) which in addition to
methylyating rRNA also methylates mRNA (2, 3) and small
nuclear RNA (4–7) and processes precursor rRNA into mature
rRNAs.

Box C/D snoRNPs require assembly of box C/D snoRNAs
with a set of nucleolar proteins that include fibrillarin (Nop1p
in yeast), Nop56/58p, and 15.5-kDa proteins (Snu13p in yeast).
In Archaea, homologs of box C/D snoRNPs (box C/D sRNPs)
consist of box C/D sRNAs, fibrillarin, Nop5p (a single homolog
of Nop56/58p), and L7Ae proteins (3, 8). The box C/D RNAs
are responsible for the recognition of methylation target rRNAs via
base pairing of their antisense regions with the target. The s(no)RNP proteins contain the actual methyltransferase activity.
The protein subunit that catalyzes the methyl transfer reactions in s(no)RNP is believed to be fibrillarin. The first
clue that linked the methylation activity of s(no)RNP to fibril-
larin came from the work of Tollervey et al. (9) on yeast, where
the nop1.3 allele, a temperature-sensitive mutant of the nop1
gene coding for yeast fibrillarin, exhibited a strongly inhibited
precursor rRNA methylation phenotype at non-permissive
temperatures. The second evidence came from the crystal
structure of an archaeal fibrillarin homolog from Methanococ-
cus jannaschii (MJ), which revealed the conservation of the
AdoMet-binding fold that is common to all known MTase struc-
tures (10). We recently determined a co-crystal structure of
fibrillarin complexed with Nop5p from Archaeoglobus fulgidus
(AF), which contained a bound AdoMet at the predicted binding
site in fibrillarin (11). This result establishes further the role
of fibrillarin as the methyltransferase.

Similar to the previously known AdoMet-dependent MTases,
fibrillarin contains the same set of conserved motifs that are
important to the binding of AdoMet (see Fig. 1). These include
motifs I–IV at the carboxyl ends of beta strands β1–β4 as
defined by Cheng and Roberts (12) in studying DNA MTases.
Consistent with their important functional roles in catalysis,
three temperature-sensitive mutants of yeast fibrillarin oc-
curred within the conserved AdoMet-binding motifs. For in-
stance, nop1.2, nop1.3, and nop1.7 mutations occurred in motif
III, motif I, and motifs II and IV, respectively (9). Furthermore,
by using an in vitro reconstituted archaeal sRNP enzyme,
Omer et al. (13) demonstrated that substitutions of two amino
acids within motifs I and III of Sulfolobus solfataricus fibrilla-
arin (A85V and P129V) resulted in completely or partially abol-
ished methylation activities, whereas sRNP assembly was un-
affected. These data suggest that fibrillarin has a function
similar to other previously known AdoMet-dependent MTases.

However, recent structural and biochemical evidence sug-
gests that fibrillarin alone binds weakly to AdoMet. There
are currently three known fibrillarin crystal structures, all from
Archaea, MJ (10), AF (11), and Pyrococcus furiosus (PF) (14).
Among these three structures, only that in complex with AF
Nop5p had the cofactor bound at the predicted AdoMet-binding
site. Interestingly, in both structures of fibrillarin without the bound Nop5p (MJ and PP), motif I and the loop connecting β1 and α1 of fibrillarin adopted conformations that would exclude the binding of AdoMet (10, 14). This clearly raised the possibility that Nop5p has a functional role in cofactor binding by modulating the conformation of AdoMet-binding motifs in fibrillarin. However it is also possible that the binding of the cofactor itself induces the conformational change of the cofactor binding pocket in fibrillarin.

In the co-crystal structure of AF fibrillarin-Nop5p complex bound with AdoMet (holocomplex), a number of conserved fibrillarin residues were observed to interact directly with AdoMet. Glu-88 forms two hydrogen bonds with the ribose hydroxyl groups of AdoMet. Thr-70 also forms a hydrogen bond with the carboxyl group of AdoMet. Asp-133 is situated near the positively charged thiomethyl group and thus may facilitate cofactor binding through favorable electrostatic interactions. Finally, Tyr-89 establishes an aromatic stacking interaction with the adenine ring of the cofactor. The opposing side of the phenol ring of Tyr-89 closely packs against a positive Nop5p residue, Arg-169. This structural arrangement between Tyr-89 of fibrillarin and Arg-169 of Nop5p creates a strong cation-π interaction as evident from the large negative electrostatic and van der Waals energies between them ($E_{es} = -5.2$ kcal/mol, $E_{vdw} = -2.8$ kcal/mol) (computed by CAPTURE) (15). Notably, Tyr-89 would be completely exposed to solvent without forming the cation-π interaction. Tyr-89 is well conserved among all known fibrillarin proteins. Arg-169 is also conserved among the Nop family of proteins, suggesting that this cation-π interaction is preserved in all homologous complexes of fibrillarin and Nop5p. The importance of this cation-π bridge formed between fibrillarin and Nop5p may play a critical role in stabilizing the association of the cofactor with fibrillarin.

In this work, we examined the requirement of Nop5p for cofactor binding by combining structural, thermodynamic, and functional analyses. To discern the effect on fibrillarin conformational changes in the cofactor binding pocket, we refined the crystal structure of Nop5p-fibrillarin complex without soaked cofactors at 3.5 Å. We also established isothermal titration calorimetry conditions, which allowed us to directly compare thermodynamic parameters of AdoMet binding to different fibrillarin complexes. By monitoring the changes in heat as the cofactor was titrated into a protein solution, we obtained binding constants and enthalpy changes of AdoMet binding to the Nop5p-fibrillarin complex, to mutant fibrillarin Nop5p complexes, and to fibrillarin. Furthermore, to assess the functional importance of the cofactor interacting residues in both fibrillarin and Nop5p, we compared in vitro methylation activities of the wild-type Nop5p-fibrillarin complex to different fibrillarin complexes. By monitoring the changes in heat as the cofactor was titrated into a protein solution, we obtained binding constants and enthalpy changes of AdoMet binding to the Nop5p-fibrillarin complex, to mutant fibrillarin Nop5p complexes, and to fibrillarin. Furthermore, to assess the functional importance of the cofactor interacting residues in both fibrillarin and Nop5p, we compared in vitro methylation activities of the wild-type Nop5p-fibrillarin complex and its mutants where several AdoMet-binding residues were disrupted. These structural, thermodynamic, and functional studies support the stabilizing role of Nop5p in cofactor binding to fibrillarin and clearly identify residues in fibrillarin that are directly involved in cofactor binding and catalysis.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**—The wild-type Nop5p-fibrillarin protein complex and fibrillarin alone were purified as described previously by Aittaleb et al. (11). All the mutations were performed within co-expressing fibrillarin and Nop5p genes using the QuikChange mutagenesis kit from Stratagene. The mutants were purified in a similar procedure as that for the wild type. All proteins were subjected to gel filtration on a Superdex S200 column (Amersham Biosciences) in a buffer containing 20 mM Tris, pH 8.0, 5% glycerol, 1.0 M NaCl, 5 mM β-mercaptoethanol, and 0.5 mM EDTA. Gel filtration profiles for all mutant fibrillarin-Nop5p complexes were similar to that of the wild-type complex, suggesting no misfolding of the mutant proteins.

**Structure Refinement**—During earlier structure determination of the
fibrillarin-Nop5p complex, we collected a multiple wavelength anomalous diffraction data set from a crystal of fibrillarin-Nop5p complex containing seleno-methionine without soaked cofactors. This data set allowed phase determination. The final structure, however, was refined against the diffraction data set collected from cofactor-soaked fibrillarin-Nop5p crystal because of its higher resolution. To elucidate the structural conformation around the cofactor binding site in the absence of a bound cofactor, we have now refined the coordinates of fibrillarin-Nop5p complex against the data set collected at the selenine K-edge. This data set has the best statistics among the three data sets (11). Refinement was carried out using crystallography NMR software (CNS) (16) by including all 10 selenine atoms and keeping the Bijvoet pairs separated. The statistics of structural refinement are listed in Table I. Structure comparison of the apocomplex with earlier structures is presented in Fig. 2.

Isothermal Titration Calorimetry—Titration experiments were performed by isothermal titration calorimetry (ITC) using a VP-ITC microcalorimeter (Microcal, Inc., Northampton, MA) interfaced with a computer. The titration calorimeter consists of 1.45 ml of sample cell containing a macromolecule solution and a matched thermal reference cell filled with water. AdoMet or S-adenosyl-L-methionine (AdoHcy) was dissolved in the same buffer used for the protein to be titrated. Prior to the experiment, samples were filtered and degassed under vacuum for 10 min in a Thermo Vac system (Microcal). The sample cell was filled with the working buffer (for dilution heat control) or with the protein to be the heat caused by the target RNA on DE81 filter. Wild-type Nop5p-fibrillarin or a mutant was mixed with L7Ae protein prior to caused by the target RNA on DE81 ion exchange filter. Wild-type Nop5p-fibrillarin or a mutant was mixed with L7Ae protein prior to being added to a pre-annealed RNA mixture containing both the AP 5′ box C/D guide RNA (rنا.وس.رنا) and the U6 target RNA (rنا.وس.رنا) to the guide sequence upstream of box D sequences. The radioactivity retained on the DE81 filter at each reaction time point was quantified by scintillation counter. The production of methylation product was plotted in Fig. 5 along with least-square fitted progress curves.

**RESULTS**

**Structural Comparison of AdoMet-binding Sites in Nop5p-free and Nop5p-bound Fibrillarins**—The fibrillarin portion of the AP Nop5p-fibrillarin complex structure (11) was superimposed with those of MJ fibrillarin (10) and of PF fibrillarin (14). The backbone root mean square differences were 1.095 Å (191 Cα atoms) for MJ fibrillarin and 0.934 Å (144 Cα atoms) for PH fibrillarin. The most significant difference within the core structure of the three fibrillarins is in motif I and II where the backbone geometry of motif II loop in the two free fibrillarin structures differ from each other and from the fibrillarin bound with Nop5p and AdoMet. Each free fibrillarin adopted a conformation that would exclude the binding of the cofactor. In both structures, the loops connecting β2 and α2 traversed through where the ribose moiety of AdoMet would lie. Most dramatically, the aromatic residues (Phe-106 in PH fibrillarin and Tyr-106 in MJ fibrillarin) completely swung away from where they could establish the favorable base stacking interactions.

**Table I**

| Model information |  
|-------------------|---|
| Number of monomers | 1 |
| Number of amino acid residues | 445 |
| Number of protein atoms | 3642 |
| Number of waters | 0 |
| Average atomic B factors (Å²) |  
| Main chain | 71.6 |
| Side chain | 74.5 |
| Ramachandran plot |  
| Most favored (%) | 316 |
| Allowed (%) | 83 |
| Generously allowed (%) | 4 |
| Disallowed (%) |  

where $Q$ is the heat content of the solution, $K_b$ is the binding constant, and $M_r$ is the bulk concentration of the protein in volume $V_0$. $X_i$ is the bulk concentration of the ligand, and $n$ is the number of binding sites. During actual fitting, the heat released for each injection increment was used with the correction of displaced volume caused by each injection. Therefore, the enthalpy change ($\Delta H^\circ$) and the binding constant ($K_b$) were directly obtained from the experiments after data processing. The free energy and entropy were subsequently calculated using $\Delta G^\circ = RT\ln K_b$ and $\Delta S^\circ = -\Delta C_p^\circ - \Delta T \Delta F^\circ$, respectively. The resulting binding parameters (binding constant $K_b$, molar Gibbs free energy, $\Delta G^\circ$, molar enthalpy $\Delta H^\circ$, and molar entropy $\Delta S^\circ$) of the proteins with AdoMet and with AdoHcy (D133A mutant only), and the standard deviations are summarized in Table II.
with the cofactor adenine ring and protruded into the solvent region (Fig. 2, B and C). This striking difference in backbone geometry and key binding residues between the two free fibrillarin structures and that bound with cofactor suggested an intrinsic structural flexibility of this region in fibrillarin that could potentially hinder the optimal binding of the cofactor.

The Apocomplex Structure of Nop5p-Fibrillarin Closely Resembles the Holocomplex—To discern whether the observed conformational differences between fibrillarin structures and that bound with cofactor and Nop5p are induced by Nop5p binding or by cofactor binding, we refined the crystal structure of Nop5p-fibrillarin in the absence of soaked AdoMet (apocomplex). The refined apocomplex of Nop5p-fibrillarin was superimposed with the holocomplex reported previously (11). SigmaA-weighted $3F_o - 2F_c$ and $F_o - F_c$ maps were computed by using the observed amplitude and phases resulted from refined coordinates. Both maps clearly showed an absence of a bound cofactor at the predicted AdoMet binding site (Fig. 2A). Fig. 2A also showed that the fibrillarin residues covering the AdoMet binding site overlap well with those in holocomplex (209 Ca atoms, root mean square difference, 0.209 Å). In particular, Tyr-89 in the apocomplex did not deviate from its conformation in the holocomplex where it established a favorable stacking interaction with the cofactor adenine ring. This is in contrast to the large conformation differences of the Tyr-89-equivalent residues in the two free fibrillarin structures. We interpret this result to indicate that binding of Nop5p alone is sufficient to stabilize the structural conformation of fibrillarin for cofactor binding.

Thermodynamic Difference between Cofactor Binding to Nop5p-Fibrillarin Complex and to Fibrillarin Alone—We used isothermal titration calorimetry to carry out a comparative and quantitative study of cofactor binding to fibrillarin alone and to Nop5p-fibrillarin complex (Fig. 3). It can be seen in Table II that the overall Gibbs free energy of AdoMet binding to free fibrillarin was 10.5 μmol and to the Nop5p-fibrillarin complex was 2.7 μmol. Thus AdoMet binds ~4-fold more strongly to Nop5p-fibrillarin than to fibrillarin alone because of more favorable contribution from binding enthalpy but less favorable contribution from entropy. Both processes were enthalpy driven in agreement with the observed polar nature of the AdoMet-binding site. Thus if assuming a similar process of solvent re-arrangement in cofactor binding to either fibrillarin or to Nop5p-fibrillarin complex, the increased binding entropy suggested a less ordered final structure when cofactor bound to fibrillarin alone. This interpretation is again consistent with the structural observation that the Tyr-89-equivalent aromatic residues were unable to establish base stacking interaction in the two free fibrillarin structures (Fig. 2).

To further establish the role of the cation-π formed between Tyr-89 of fibrillarin and Arg-169 of Nop5p in cofactor binding, we titrated the fibrillarin-Nop5p complexes containing either the Y89A or R169A mutation with AdoMet. The Y89A mutant showed clearly impaired binding affinity for AdoMet in terms of its dissociation constant $K_d$ (Table II). There was a significant increase in binding enthalpy (~4.7 kcal/mol versus –11.7 kcal/mol in wild type) and a large gain in the binding entropy $\Delta S^\circ$ term (1.9 kcal/mol versus –4.0 kcal/mol in wild type) when compared with AdoMet binding to the wild-type complex. These thermodynamic results are consistent with a strong role of the phenol ring of Tyr-89 in restricting the bound conformation of AdoMet by maintaining favorable base-stacking interactions with the adenine moiety of the cofactor.

Titration of AdoMet to the R169A mutant revealed a thermodynamic energy compensation, which resulted in no change in the Gibbs free energy, $\Delta G^\circ$. A significant increase in enthalpy (~3 kcal/mol) was balanced by a gain in entropy (~3 kcal/mol). This result may be interpreted as indicating that the decrease in binding potential energy because of the removal of Arg-169 (thus the cation-π interaction) was compensated for by an increase in conformational flexibility of the final cofactor-enzyme complex, assuming the single amino acid mutation has a negligible effect on protein dynamics and on solvent reorganization.

To further demonstrate that AdoMet binds to fibrillarin specifically to Motif I, II, and IV residues, we investigated thermodynamics of AdoMet binding to three Nop5p-fibrillarin mutant complexes. Each of the complexes contains a single mutation at Thr-70, Glu-88, and Asp-133 in fibrillarin, respectively. These three residues were implicated as the cofactor binding residues by the recent crystal structure of the holocomplex (11).

Thr-70 is located at the end of β1 strand (motif I). It forms a hydrogen bond with the carboxyamide group of the methionyl group. Glu-88 is at the end of β2 strand (motif II), and it forms hydrogen bonds with the hydroxyl groups of AdoMet (Fig. 1). These specific interactions are expected to stabilize cofactor binding. Surprisingly, mutations of either Thr-70 or Glu-88 to alanine (T70A or E88A) did not weaken the binding of AdoMet for the enzyme (Table II). T70A had slightly better affinity than that of the wild-type complex. The thermodynamic parameters exhibited enthalpy-entropy compensation similar to that observed for R169A mutant. The increase of binding enthalpy (~2 kcal/mol) was compensated by an increase in entropic term $\Delta S^\circ$ (~2.3 kcal/mol) when compared with the wild-type binding. Given the observed hydrogen bond between Thr-70 and the carboxyamide group of AdoMet, these thermodynamic results of binding are suggestive of conformational flexibilities of cofactor binding to T70A. The thermodynamics of AdoMet binding to E88A exhibited an opposing enthalpy-entropy compensation effect than that observed for T70A. A slight decrease in binding entropy (~1 kcal/mol) was compensated for by a decrease in binding enthalpy (~1.5 kcal/mol), suggesting a complicated effect of Glu-88 mutation on AdoMet binding.
Asp-133 is strictly conserved in fibrillarin and is in proximity of the thiomethyl group of the AdoMet. It appeared that the negatively charged carboxyl group of Asp-133 could be the major stabilizing factor for the positively charged sulfonium ion on AdoMet, as the binding affinity of D133A to the neutral cofactor AdoHcy was improved substantially (14.9 μM) (Table II).

To assess electrostatic contributions to the binding of AdoMet to the Nop5p-fibrillarin complex, we computed electrostatic potentials at the molecular surface of the Nop5p-fibrillarin complex from numerical solutions of the nonlinear Poisson-Boltzmann equation. Fig. 4 displays color-coded electrostatic potential surfaces of Nop5p-fibrillarin in the immediate vicinity of the bound AdoMet. The computed surface potentials clearly showed a charge complementarily at the cofactor binding site in that the carboxyl group of the AdoMet molecule is located within a positively charged pocket, whereas the adenine moiety is within a negatively charged pocket. The computed electrostatic potentials also predicted that the positively charged sulfonium sulfur interacted with the negatively charged Asp-133, as the same negative potential near the sulfonium sulfur in the wild-type protein was absent in the D133A mutant (Fig. 4). This interpretation is in agreement with the reduced binding affinity of AdoMet for D133A (Table II).

Methylation Activities of the Wild-type and the Mutant Nop5p-Fibrillarin Complexes—To fully understand the functional roles of each of the conserved AdoMet-interacting residues, we further carried out in vitro methylation assays using a reconstituted sRNP enzyme developed previously (18) and tested the ability of the mutant enzymes in catalyzing 2′-O-methylation on a target RNA oligomer that was complementary to the guide sequence upstream of box D. The progress curves for methylation reaction catalyzed by the wild-type Nop5p-fibrillarin complex and the mutant are plotted in Fig. 5. Each curve shows an average of at least four duplicated reactions. Fig. 5 clearly shows that whereas the Y89A and R169A mutants maintained some moderate activities, T70A, E88A, and D133A exhibited non-detectable methylation activities in our assays. These results further support an important functional role played by Asp-133, Thr-70, and Glu-88 in catalytic reactions. The abolished activity in D133A and the strict conservation of the Asp-133 residue are consistent with the proposed role of an aspartic residue in catalysis (25). However, having previously demonstrated that it is the COOH domain of Nop5p that binds the guide RNA (11), we could not rule out the possibility that the investigated fibrillarin residues (Thr-70, Glu-88, and Asp-133) are involved in binding the target RNA substrate. Thus, the observed reduction in catalytic activities caused by their mutations could be a result of impaired target substrate binding.

**DISCUSSION**

Understanding the function of ribosomal RNA processing and nucleotide modification requires detailed knowledge of the structure and thermodynamics of the box C/D snoRNP assembly. Archaeal box C/D sRNPs are ribonucleoprotein assemblies that selectively methylates 2′-hydroxyl groups of rRNA and tRNA. The core protein of box C/D sRNPs, fibrillarin, is responsible for the actual methylation reaction by transferring the methyl group from the methyl donor, AdoMet, to the target 2′-OH group on RNA. Fibrillarin forms a tight complex with another core protein, Nop5p, as a dimer of two heterodimers during initial assembly. The potential role of Nop5p in assisting cofactor binding to fibrillarin is investigated in this work by structural, thermodynamic, and in vitro methylation studies. Structural comparison of fibrillarin from different archaeal organisms revealed that the protein backbone and a critical
aromatic residue near the cofactor binding site in free fibrillarin adopt a conformation that is different from that when Nop5p is bound. These alternative conformations in fibrillarin are unfavorable for efficient cofactor binding. Binding thermodynamics from ITC measurement indeed showed a reduced binding affinity of cofactor for fibrillarin in the absence of bound Nop5p. Mutational studies identified a cation-π pair formed between Tyr-89 in fibrillarin and Arg-169 in Nop5p as one important structural element to facilitate the required conformation change at fibrillarin active site for AdoMet binding.

Despite the structural evidence that Thr-70 and Glu-88 bind AdoMet specifically, mutation of these two residues resulted in a slight increase in binding affinities. Such "super binding"
mutants were also observed previously in a solid-phase assay of cofactor interaction with the vaccinia virus cap-dependent 2'-O-methyltransferase, VP39, where two motif IV mutants, although inactive, resulted in stronger binding coefficients of AdoMet (24). In another AdoMet-MTase interaction study by the equilibrium dialysis method, the loss of the 2'-hydroxyl group on AdoMet ribose moiety did not affect the optimal binding of the cofactor to a nucleolar 2'-O-methyltransferase (26). Because of the limitation in these binding assays, no explanatory insight could be provided on the structural and thermodynamic principles responsible for the gain in binding affinities upon removal of the interacting groups. Our analyses on directly measured thermodynamic quantities of cofactor binding to the T70A and E88A mutants offer some explanations on the nature of interactions. The large decrease in binding enthalpy of the cofactor to the E88A mutant suggests that the energetic costs in formation of the specific hydrogen bonds between Glu-88 and the cofactor may be compensated by breakage of hydrogen bonds formed between Glu-88 and solvent molecules or with nearby polar residues. In vitro methylation assays using the wild-type enzyme and those containing site-specific mutations at active sites highlight the functional role of three conserved residues, Thr-70, Glu-88, and Asp-133, in catalysis.

Currently, little is known about the catalytic mechanism of s(no)RNP s. In general, MTases facilitate the methyl transfer reaction by restricting both AdoMet and the methylation target molecule in close proximity, which enables the thiomethyl group of the methionine moiety to be reactive toward polarizable nucleophiles (nitrogen, oxygen, sulfur, or activated carbons) (27). After donating the methyl group, AdoMet is converted into AdoHcy (12). Sequence alignment and superimposition of fibrillarin with other 2'-O-methylation MTases revealed a conserved KDK triad at the site of methyl transfer (28). Substitution of these three residues in the bacterial MTase RrmJ with alanine had deleterious effects on its methylation activity toward the 23 S rRNA substrate (23). This led to the proposal of a general base type of reaction mechanism employed by RrmJ in which a critical lysine residue facilitates nucleophilic attack by deprotonating the 2'-OH of the target RNA (23). In AF fibrillarin, the proposed catalytic triad residues correspond to Lys-42, Asp-133, and Lys-162. Both Lys-42 and Asp-133 are within 3.5 Å of the thiomethyl carbon of the bound AdoMet, which signifies their roles as catalytic residues. Activation of the 2'-hydroxyl could thus be occurring through the similar general base type of mechanism proposed for RrmJ. Asp-133 in fibrillarin could act as the general base by deprotonating the 2'-OH group on the target RNA during catalysis.

Our mutagenesis studies represent the initial efforts in probing the functional role of the potential catalytic residue Asp-133. Although the impaired catalytic activity of D133A is supportive of its role as a catalytic residue, the thermodynamic data on cofactor binding strongly imply its direct involvement in stabilization of cofactor binding. Several structural mechanisms are possible to rationalize the observed stabilization effect of Asp-133 on the bound AdoMet molecule. First, the negatively charged carboxyl side chain may form favorable interaction with the positively charged sulfonium sulfur of the cofactor. Our computed electrostatic potential surfaces of the wild-type and the D133A mutant proteins and the drastically lower binding affinity of the D133A mutant for AdoMet (which contains the sulfonium charge) than from AdoHcy (which lacks the sulfonium charge) are supportive of this mechanism. Second, the carboxylate group of Asp-133 may form a specific interaction with the amino group of AdoMet mediated by a water molecule. In the crystal structure of Nop5p-fibrillarin complex bound with AdoMet, a continuous electron density from the Asp-133 carboxylate group to the amino group was observed (data not shown) that could be attributed to a network of hydrogen bonds mediated by a water molecule, thus supporting this mechanism. Third, Asp-133 may form a salt bridge with the nearby Lys-42 residue, which could restrict the side chain of Lys-42 to an orientation that provides less steric hindrance to the sulfonium methyl group on AdoMet. Evidence supporting this mechanism is found in the binding study with various AdoMet analogs to the nucleolar 2'-O-methyltransferase (26) where substitution of an ethyl group for the sulfonium methyl greatly reduced the binding affinity of the cofactor analog. Although we favor the first mechanism that explains the role of Asp-133 in stabilizing the cofactor, additional studies are required to distinguish these mechanisms.

Previous biochemical studies show that Nop5p is essential in interacting with guide sRNA and in organizing a symmetric protein scaffold for sRNP assembly (18). Together with the thermodynamic and catalytic studies presented here, it is clear that Nop5p has important functional roles both as a scaffold protein in bridging the catalytic subunit to the substrate-guide RNA duplex and as an accessory protein for cofactor binding and catalysis.

Acknowledgments—We thank members of the Li group and Dr. Xiaodong Cheng for helpful discussions.

REFERENCES
1. Maden, B. E. (1990) Prog. Nucleic Acids Res. Mol. Biol. 39, 241–303
2. Cavaille, J., Vitali, P., Bazuyuk, E., Hutenholzer, A., and Bachelierie, J. P. (2001) J. Biol. Chem. 276, 26374–26383
3. Dennis, P. P., Omer, A., and Lowe, T. (2001) Mol. Microbiol. 40, 509–519
4. Tollervey, D., and Kiss, T. (1997) Curr. Opin. Cell. Biol. 9, 337–342
5. Terns, M. P., and Terns, R. M. (2002) Gene Expr. 10, 17–39
6. Maxwell, E. S., and Fournier, M. J. (1995) Annu. Rev. Biochem. 64, 897–934
7. Weinstein, L. B., and Steitz, J. A. (1999) Curr. Opin. Cell Biol. 11, 378–384
8. Omer, A. D., Ziesche, S., Decatur, W. A., Fournier, M. J., and Dennis, P. P. (2003) Mol. Microbiol. 48, 617–629
9. Tollervey, D., Lehtonen, H., Jansen, R., Kern, H., and Hurt, E. C. (1993) Cell 72, 443–457
10. Wang, H., Buisvert, D., Kim, K. K., Kim, R., and Kim, S. H. (2000) EMBO J. 19, 317–323
11. Aittaleb, M., Rashid, R., Chen, Q., Palmer, J. R., Daniels, C. J., and Li, H.
S-Adenosyl-L-Methionine Binding to Fibrillarin

(2003) Nat. Struct. Biol. 10, 256–263
12. Cheng, X., and Roberts, R. J. (2001) Nucleic Acids Res. 29, 3784–3795
13. Omer, A. D., Ziesche, S., Ebhardt, H., and Dennis, P. P. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5289–5294
14. Deng, L., Starostina, N. G., Liu, Z. J., Rose, J. P., Terns, R. M., Terns, M. P., and Wang, B. C. (2004) Biochem. Biophys. Res. Commun. 315, 726–732
15. Gallivan, J. P., and Dougherty, D. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9459–9464
16. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M.; Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
17. Wiseman, T., Williston, S., Brandts, J. F., and Lin, L. N. (1989) Anal. Biochem. 179, 131–137
18. Rashid, R., Attaleh, M., Chen, Q., Spiegel, K., Demeler, B., and Li, H. (2003) J. Mol. Biol. 333, 295–306
19. Boschitsch, A. H., and Fenley, M. O. (2004) J. Comput. Chem. 25, 905–955
20. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
21. Sitkoff, D., Sharp, K. A., and Honig, B. (1984) J. Phys. Chem. 88, 1978–1988
22. Sanner, M. F., Olson, A. J., and Spehner, J. C. (1996) Biopolymers 38, 305–320
23. Hager, J., Staker, B. L., Bugl, H., and Jakob, U. (2002) J. Biol. Chem. 277, 41978–41986
24. Shi, X., Yao, P., Jose, T., and Gerashon, P. (1996) RNA (N. Y.) 2, 88–101
25. Feder, M., Pas, J., Wyrwicz, L. S., and Bujnicki, J. M. (2003) Gene 302, 129–138
26. Segal, D. M., and Eichler, D. C. (1989) Arch. Biochem. Biophys. 275, 334–343
27. Walsh, C. (1979) Enzymatic Reaction Mechanisms, W. H. Freeman and Company, San Francisco
28. Maravic, G., Feder, M., Pengor, S., Flogel, M., and Bujnicki, J. M. (2003) J. Mol. Biol. 332, 99–109
Structural and Thermodynamic Evidence for a Stabilizing Role of Nop5p in S-Adenosyl-L-methionine Binding to Fibrillarin
Mohamed Aittaleb, Thomas Visone, Marcia O. Fenley and Hong Li

J. Biol. Chem. 2004, 279:41822-41829.
doi: 10.1074/jbc.M406209200 originally published online July 30, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406209200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 27 references, 5 of which can be accessed free at
http://www.jbc.org/content/279/40/41822.full.html#ref-list-1