Dbp9p, a Member of the DEAD Box Protein Family, Exhibits DNA Helicase Activity*

Received for publication, January 9, 2004, and in revised form, March 1, 2004
Published, JBC Papers in Press, March 17, 2004, DOI 10.1074/jbc.M400231200

Takashi Kikuma, Masaya Ohtsu, Takahiko Utsugi, Shoko Koga, Kohji Okuhara, Toshikiko Eki‡, Fumihiro Fujimori, and Yasufumi Murakami§

From the Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, Noda, Chiba 278-8510, Japan and the Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan

The yeast Dbp9p is a member of the DEAD box family of RNA helicases, which are thought to be involved in RNA metabolism. Dbp9p seems to function in ribosomal RNA biogenesis, but it has not been biochemically characterized. To analyze the enzymatic characteristics of the protein, we expressed a recombinant Dbp9p in Escherichia coli and purified it to homogeneity. The purified protein exhibited RNA unwinding and binding activity in the absence of NTP, and this activity was abolished by a mutation in the RNA-binding domain. We then characterized the ATPase activity of Dbp9p with respect to cofactor specificity; the activity was found to be severely inhibited by yeast total RNA and moderately inhibited by poly(U), poly(A), and poly(C) but to be stimulated by yeast genomic DNA and salmon sperm DNA. In addition, Dbp9p exhibited DNA-DNA and DNA-RNA helicase activity in the presence of ATP. These results indicate that Dbp9p has biochemical characteristics unique among DEAD box proteins.

RNA helicases are enzymes that unwind double-stranded RNA molecules in an energy-dependent manner through the hydrolysis of NTP. These proteins are widely distributed among a variety of organisms ranging from viruses and prokaryotes to mammals. RNA helicases are associated with virtually all biological processes requiring RNA, including transcription, splicing, RNA transport, ribosome biogenesis, RNA editing, translation, and RNA decay (1). The largest family of RNA helicases is the DEAD box protein family.

The DEAD box proteins have seven to eight distinctive motifs. The DEAD is derived from the amino acid sequence of motif II, the Walker B motif (2, 3). In vitro analyses of DEAD box proteins such as the translation initiation factor eIF-4A and the human nuclear protein p68 have demonstrated that these proteins possess RNA-dependent ATPase activity and are capable of melting short RNA duplex structures in an ATP-dependent manner (3–5). For example, eIF-4-A, an archetypical member of the DEAD box protein family, is capable of unwinding partial duplex RNA in a bidirectional manner and acting on RNA or DNA-RNA, but not on the DNA duplex (4, 6). Extensive mutational analyses of the conserved regions of DEAD box proteins have demonstrated that these regions are important to ATP binding, ATP hydrolysis, RNA binding, RNA unwinding, and coupling of these different activities. In addition to these typical DEAD box RNA helicases, some DEAD box proteins have recently been shown to have peculiar characteristics. Hepatitis C virus NS3 drives the unwinding activity with all ribo- and deoxyribo-NTPs (6). Moreover, CsdA, an Escherichia coli DEAD box protein, unwinds double-stranded RNA in the absence of NTP (7). These reports suggest that the DEAD box family includes proteins with a wide variety of biochemical activities.

The yeast Saccharomyces cerevisiae contains over 20 different DEAD box proteins, many of which are essential to viability. Combined with biochemical analyses, yeast genetic analyses have revealed the functions of many DEAD box proteins. For example, the DEAD box proteins Prp5p and Prp28p are involved in pre-messenger RNA splicing (8–10), whereas Ded1p, which has ATP-dependent RNA helicase activity, is necessary for translation initiation (11–13). Tif1p and Tif2p also participate in translation initiation (14). However, it is ribosome biogenesis that DEAD box proteins are most often associated with, because 13 DEAD box proteins have been implicated in ribosome biogenesis (1). Ribosomes are one of the largest and most complex macromolecular assemblies (reviewed in Ref. 15). DBP9 encodes an essential nuclear DEAD box protein. Genetic depletion of Dbp9p results in a deficit of 60 S ribosomal subunits and in the appearance of half-mer polysomes. In vitro depletion of Dbp9p results in reduced synthesis of the 27 S precursor to the mature 25 S and 5.8 S ribosomal RNA, which terminally leads to a net deficit in 60 S ribosomal subunits (16). These results strongly suggest that Dbp9p participates in ribosome biogenesis.

Dbp9p is classified as a DEAD box protein based on sequence homology (17). It has never, however, been shown experimentally to unwind RNA. In this study, therefore, we purified and characterized Dbp9p, which was found to display several specific characteristics: it unwinds RNA in the absence of NTP, its ATPase activity is inhibited by RNA but stimulated by DNA, and it exhibits DNA-DNA and DNA-RNA helicase activity. Our work suggests that the DEAD box family proteins vary widely with respect to substrate specificity.

MATERIALS AND METHODS

Construction of Plasmids—The pBluescript-Dbp9 vector containing an N-terminal FLAG tag was a kind gift from A. Shiratori (17). The open reading frame of Dbp9 was subcloned into an expression vector pET32b by KpnI/XhoI sites (pET32-Dbp9). The expression clones were transformed to Escherichia coli (AD494). The Dbp9 mutated forms were

*This work was supported by grants-in-aid from the New Energy Development Organization (NEDO), the Organization for Pharmaceutical Safety and Research, and the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This paper is available online at http://www.jbc.org

‡ To whom correspondence should be addressed: Dept. of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan. Tel.: 81-4-7121-1501 (ext. 4408); Fax: 81-4-7122-1360; E-mail: yasufumi@rs.noda.tus.ac.jp

§ To whom correspondence should be addressed: Dept. of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278-8510, Japan. Tel.: 81-4-7124-1501 (ext. 4408); Fax: 81-4-7122-1360; E-mail: yasufumi@rs.noda.tus.ac.jp

20692 This paper is available online at http://www.jbc.org
Expression and Purification of Recombinant Dbp9p—Plasmids containing *E. coli* were grown in 2× YT medium to *A_{660nm} = 0.4 at 30 °C. The expression of recombinant protein was induced by 1 mM isopropyl-1-thio-β-D-galactopyranoside at 30 °C for 2.5 h. The cells were harvested by centrifugation, washed with sonication buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 0.5 mM NaCl), and sonicated in the same buffer. The lysate was clarified by centrifugation at 12,000 rpm for 20 min at 4 °C. The lysate was dialyzed against buffer containing 20 mM potassium phosphate, pH 7.5, 0.5 mM MgCl₂, and 0.5 mM potassium phosphate, pH 7.5, 20 mM KCl, and 0.5 mM MgCl₂ and then loaded onto a 9-ml Probond nickel-chelating resin (Invitrogen). The lysate was then washed with 10-column volumes of the loading buffer (50 mM imidazole, 20 mM phosphate buffer, pH 7.5, 0.5 mM KCl, 0.5 mM MgCl₂). Step elutions were carried out in 3-column volumes by increasing the imidazole to 100 and then 500 mM. Dbp9p was mostly eluted in the 500-mM elution, as verified by SDS-PAGE. The elution was loaded on a 150-ml Sephacryl S 400 (Amersham Biosciences) gel-filtration column equilibrated in the same buffer. The proteins were eluted in 200 ml of the same buffer. Forty 5-ml fractions were collected and analyzed. Two fractions mostly containing Dbp9p were pooled and dialyzed against 20 mM potassium phosphate, pH 7.5, 20 mM KCl, and 10% glycerol. The protein was then loaded on a phosphocellulose cation exchange column equilibrated in the same buffer and eluted with a gradient of 0.02–1.0 M KCl. Fractions were analyzed for ATPase activity. Peak ATPase activity was found to correlate with the peak of Dbp9p. 

ATPase Assay—The measurement of hydrolysis of [γ-32P]ATP was carried out in a reaction mixture containing 50 µM ATP, 20 mM Hepes-KOH (pH 7.6), 2 mM DTT, 2% PEG8000, 3 mM MgCl₂, 0.5 mM (18.5 kBq) of [γ-32P]ATP, and various amounts of purified Dbp9p samples. The mixture was incubated at 37 °C for 1 h. Aliquots of each reaction were spotted on a thin layer chromatography plate. ATP and free phosphate were separated by thin-layer chromatography in 0.5 mM LiCl, 1 M formic acid. The sheets were dried and exposed on x-ray film. When needed, Pₐ on the TLC plate was quantitated by counting in a liquid scintillation counter.

RNA Unwinding Assay—Preparation of substrate S1.S1 substrate for the RNA-unwinding and RNA binding assay was prepared following a previously described method (18). S1 was 81 bp T7 RNA polymerase transcript from plasmid Bluescript II KS (Stratagene) digested with HindIII. These transcripts were synthesized in the presence of [α-32P]GTP as described previously (19). We next purified S1 using a QIAquick nucleotide removal kit (Qiagen, Valencia, CA) and a MicroSpin G25 column (Amersham Biosciences).

RNA Unwinding and Binding Assays—RNA substrate mixtures (20 µl) containing 20 mM Hepes-KOH (pH 7.6), 3 mM MgCl₂, 100 mM KCl, 2 mM DTT, and 25, 50, or 100 ng of Dbp9p or 100 ng of mutated Dbp9p (R414I, R414T, R414K) were incubated at 37 °C for 30 min. Reactions were primed by the addition of 5 µl of a mixture containing 0.1 mM Tris-HCl, pH 7.4, 20 mM EDTA, 0.5% SDS, 0.1% Nonidet P-40, 0.1% xylene cyanol, 50% glycerol, and 0.2 mg/ml proteinase K. As controls, we prepared a sample with an RNA substrate that was heat-denatured by incubating for 5 min at 99 °C and without the addition of proteinase K. Aliquots of each sample were loaded onto an 8% SDS-polyacrylamide gel and electrophoresed at 100 V. The gel was packed into a hybridization bag and exposed on an imaging plate (Fuji).

Characterization of Dbp9p

**Fig. 1.** Purification of a recombinant Dbp9p protein. A, expression and purification of recombinant Dbp9p. Dbp9p was overproduced in *E. coli* and purified as described under “Materials and Methods.” Samples were analyzed by SDS-PAGE in a 7.5% gel, and the gel was stained with Coomassie Brilliant Blue. The position of the recombinant Dbp9p fragment is indicated on the right. M, protein size markers; Extract, total cell extract from isopropyl-1-thio-β-D-galactopyranoside-induced cells. B, elution fraction of final purification by phosphocellulose and the ATPase activity of each fraction. Aliquots (15 µl) of the indicated gradient fractions were assayed for ATPase activity using the conditions described under “Materials and Methods.” The ATPase assay was performed with [γ-32P]ATP.

The RNA binding assay was carried out by the same method except that the RNA unwinding buffer lacked proteinase K. The mixture was loaded onto 4% nondenaturing polyacrylamide gel and separated by electrophoresis.

Cofactors for ATPase—Poly(A), poly(U), and poly(C) were from Amersham Pharmacia Biotech. Heat-denatured yeast total DNA refers to yeast total DNA that was heated at 100 °C for 10 min. DNaseI-treated yeast total DNA was prepared by digestion with 1260 units of DNasel...
at 25 °C for 1 h. pUC19 linear DNA was prepared by EcoRI (TAKARA) digestion. We added 10-, 100-, or 1000-ng nucleotides to ATPase reaction mixtures containing 50 ng of Dbp9p and then stirred. Measurement of ATP hydrolysis was carried out as described in ATPase assay.

DNA Helicase, DNA-RNA Helicase Assay—This assay is based on Tuteja et al. (20). The DNA substrate used in the helicase assay consists of 32P-labeled complementary oligonucleotides annealed to M13mp19 phage ssDNA to create a partial duplex. The DNA probe sequence is 5’-H11032-TTT TTT TTT TTT TT GTT TTC CCA GTC ACG AC TTT TTT TTT TTT TTT TTT-T. The T-3’ RNA probe sequence is 5’-H11032-UUU UUU UUU UUU UU GTT TTC CCA GTC ACG AC UUU UUU UUU UUU UU-3’. Twenty-five nanograms of these DNAs or RNAs was 5’-end-labeled by T4 polynucleotide and 0.925 MBq [32P]-32P in a T4 polynucleotide kinase buffer (50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 5 mM DTT). Free [32P]-32P-32P was removed using a MicroSpin G-25 column (Amersham Pharmacia Biotech). This labeled oligomer was annealed to M13mp19 ssDNA (1 μg) in 40 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 50 mM NaCl, and 1 mM DTT.

Forty-nine microliters of 32P-labeled oligomer mixture and 5 μl of 0.2 μg/μl DNA were mixed in a solution containing 40 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 50 mM NaCl, and 1 mM DTT. The mixture was heated at 95 °C for 3 min and allowed to anneal at 65 °C for 20 min followed by slow cooling to room temperature for 40 min. The substrates were purified using a MicroSpin S-400 HR column (Amersham Biosciences). The standard reaction mixture (15 μl) consisted of 20 mM Tris-HCl (pH 8.5), 8 mM DTT, 1 mM MgCl2, 1 mM ATP, 30 mM KCl, 4% sucrose 80 μg/μl bovine serum albumin (Sigma), 5 μl of 32P-labeled helicase substrate and Dbp9p. The reaction mixture was incubated at 37 °C for 30 min and stopped by the addition of 10 μl of a helicase reaction stop buffer (0.3% SDS, 10 mM EDTA, 5% glycerol, and 0.03% bromphenol blue). After further incubation at 37 °C for 5 min, the substrate and product were separated by electrophoresis on a 12% native polyacrylamide gel (mini gel size, 8 × 10 cm) in TBE buffer (89...
mM Tris borate (pH 8.2) and 2 mM EDTA (pH 8.0)). After electrophoresis, the gel was exposed to an imaging plate for 2 h and the bands were detected by a BAS2000 bioimaging analyzer (Fuji). The helicase activity using the DNA-RNA substrate was also determined as described above but in the presence of RNasein.

RESULTS

Expression and Purification of Recombinant Dbp9p—Dbp9p seems to function in RNA metabolism, especially in ribosome assembly, based on its sequence motifs and the phenotypes of the dbp9 mutants (16). To characterize Dbp9p biochemically, we constructed a plasmid for expressing Dbp9p with a FLAG-, a His-, a Trx-, and an S tag in E. coli. The expression of the recombinant protein was induced by growing the E. coli with 1 mM isopropyl-1-thio-D-galactopyranoside at 30 °C for 2.5 h. Large-scale preparations of the recombinant protein were purified with anti-His tag affinity chromatography (Probond) and gel-filtration column chromatography (Sephacryl S400). This partially purified Dbp9p was purified further through phosphocellulose cation exchange column chromatography (Fig. 1A). During the purification, fractions were simultaneously assayed for ATPase activity. In the course of phosphocellulose column chromatography, the elution profile was monitored by an ATPase assay. The peak fraction of the ATPase consisted of a homogeneous preparation of a protein with an apparent molecular mass of 80 kDa (Fig. 1B), which is in good agreement with the predicted molecular mass of Dbp9p with all the tags described above. Identification of the purified protein was also verified by an anti-FLAG tag antibody (data not shown).

The above purification procedure was applied to E. coli strains expressing the Dbp9p mutants with the replacement of Arg-414, which is a conserved amino acid within the RNA binding motif by isoleucin, threonine, and lysine (named Dbp9p(R414A), Dbp9p(R414T), and Dbp9p (R414K), respectively.

![Fig. 5. RNA inhibits the ATPase activity of Dbp9p. The ATPase assay was carried out. We added yeast total RNA (A) or poly(U) (■), poly(A) (▼), poly(C) (▲) into the mixtures. The y-axis shows the ATP activity of each additive relative to that by no addition.](image)

![Fig. 6. DNA stimulates Dbp9p ATPase activity. ATPase activity was measured through P_i radioactivity. The y-axis shows the ATP activity of each additive relative to that with no addition. A, Dbp9p ATPase activity with yeast genome (○) or salmon testis DNA (■). Both of the DNAs stimulated Dbp9p ATPase activity. B, Dbp9p ATPase activity with DNaseI-treated yeast genome (○) or heat-denatured yeast genome (■). The DNaseI-treated yeast genome did not stimulate Dbp9p ATPase activity. The heat-denatured yeast genome stimulated Dbp9p ATPase activity. C, Dbp9p ATPase activity with linear pUC19 (○) or circular pUC19 (■). Linear pUC19 was EcoRI-cut pUC19. Both DNAs stimulated Dbp9p ATPase activity slightly.](image)
This Arg residue of mammalian eIF-4A has been reported to be critical in both ATP hydrolysis and RNA binding activities. Substitution of the same Arg residue of the yeast eIF-4A gene, TIF1, to Thr or Ile impaired the function of this elongation factor (22, 23). As expected, the Dbp9p mutant proteins purified by gel filtration chromatography did not show ATPase activity (Fig. 2).

Dbp9p Shifts a Secondary Structure of RNA through Unwinding Activity—The presence of both the DEAD box motif and ATPase activity in Dbp9p leads to the prediction that Dbp9p might exhibit RNA unwinding activity. To determine whether Dbp9p can unwind RNA, we measured its RNA unwinding activity using a folded RNA substrate. We prepared S1 RNA, which was obtained by transcribing pBluescript with T7 RNA polymerase. The structure of the S1 RNA used is illustrated in Fig. 3A. The input S1 shows two forms on the gel: a fast-migrating unfolded RNA and a slow-migrating folded RNA. Incubation of S1 RNA with Dbp9p decreased the latter form as the amount of input Dbp9p increased (Fig. 3B). Interestingly, this reaction occurred in the absence of NTP. However, the mutant forms of Dbp9p did not change the migration pattern of the input RNA. These results indicate that Dbp9p has NTP-independent RNA unwinding activity and that the motif VI of Dbp9p is necessary for this unwinding activity.

To confirm the direct interaction between Dbp9p and S1 RNA, we carried out a gel shift assay. Preincubation of Dbp9p with S1 RNA gave rise to a shifted band of the RNA (Fig. 4). The amount of shifted RNA increased along with the amounts of input Dbp9p.

Dbp9p Has DNA-dependent ATPase Activity—As described above, we demonstrated that Dbp9p has RNA unwinding activity. A common property of numerous NTPases/helicases is their activation and even the dependence of their ATPase activity on RNA, DNA, or homopolymeric polynucleotides. The E. coli DEAD box protein CsdA, which has been shown to be able to unwind RNA duplex in the absence of ATP, has not been characterized in terms of the cofactor dependence of ATPase (7). We, therefore, had much interest in the specificity of Dbp9p.
Characterization of Dbp9p  

ATPase activity. Dbp9p was incubated with $^{32}$P[ATP], and the formation of $^{32}$P was measured using thin-layer chromatography in the presence of a variety of nucleotides. The ATPase activity of Dbp9p was reduced up to one-fifth by the addition of yeast total RNA and was slightly reduced by poly(U), poly(A), and poly(C) (Fig. 5A). In contrast, the addition of increasing amounts of yeast genome DNA or salmon sperm DNA increased the amount of ATP hydrolyzed up to 8–12 fold, showing that the Dbp9p ATPase activity is stimulated by DNA (Fig. 6A). Stimulation of the ATPase activity by the addition of purified circular pUC19 DNA and EcoRI-treated linear pUC19 DNA up to 1.7- and 2.5-fold, respectively, was also observed, indicating that DNA ends are not required for this stimulation. To confirm that this stimulation was caused by DNA and not by contaminated proteins, we carried out the same ATPase analysis using yeast genome DNA treated with DNaseI or heat-treated DNA. Heat-treated DNA stimulated ATPase activity, whereas DNase-treated DNA did not (Fig. 6B). These results indicate that DNA itself, not any contaminated protein, activates the ATPase activity of Dbp9p.

Dbp9p Has DNA Helicase Activity—The ATPase activity of Dbp9p was found to be at least partially inhibited by RNA and stimulated by DNA. This result was different from our expectation based on the amino acid sequence and phenotypic analysis. Dbp9p was therefore tested for its ability to function as a DNA helicase. We used a partial duplex substrate having a 17-bp annealed portion and hanging tails of 14 nucleotides at both the 5'- and 3'-ends, and the 5'-ends were labeled with $^{32}$P. Incubation of $^{32}$P-labeled forked DNA with Dbp9p resulted in the reduction of $^{32}$P DNA-M13ssDNA and an increase in $^{32}$P DNA according to the amount of Dbp9p, although incubation with mutant Dbp9ps (R414I, R414T, and R414K) failed to unwind this substrate (Fig. 7).

We also performed a helicase assay with DNA-RNA substrate, synthesizing 45-nt RNA annealed with single-stranded circular M13 DNA. Reduction of $^{32}$P RNA-M13ssDNA was observed according to the amount of Dbp9p, meaning that Dbp9p has DNA-RNA helicase activity. Dbp9ps with site-directed mutation, however, did not show the helicase activity (Figs. 8 and 9).

We then investigated whether this DNA helicase activity requires ATP hydrolysis. We incubated Dbp9p with $^{32}$P DNA-M13ssDNA as a substrate in the presence of absence of ATP. In a reaction without ATP, there was no strand displacement by Dbp9p (Fig. 9A). Also, no dissociation of $^{32}$P RNA-M13ssDNA by Dbp9p was observed in the absence of ATP (Fig. 9B). These results indicate that the DNA-DNA and DNA-RNA helicase activities of Dbp9p require ATP.

**DISCUSSION**

The DBP9 gene encodes a DEAD box protein and is required for 27 S ribosomal RNA processing. Proteins containing the conserved DEAD box protein motifs are assumed to hydrolyze ATP and unwind RNA, but a direct biochemical demonstration of helicase activity remains essential. Because there has been no biochemical analysis of Dbp9p, we extensively characterized its biochemical activity in the present study.

Some DEAD box proteins need the interaction with other proteins to exert their ATPase or helicase activity (4). Here we show that Dbp9p possesses an unwinding activity on S1 ssRNA without ATP. This result indicates that Dbp9p has ATPase-independent RNA helix-stabilizing activity and apparently does not need to interact with other proteins to display RNA unwinding activity like that of *E. coli* CsdA (7), although Dbp9p seems to interact with Dbp6p functionally in vivo (16). In the RNA unwinding assays, there must be several artificial conditions because most of the DEAD box proteins identified thus far do not show any substrate specificity. Therefore, one could speculate that the unwinding reaction mediated by Dbp9p would be nonenzymatic. However, we think this possibility is excluded by the experiments with the mutated proteins, which could not hydrolyze ATP. The mutated proteins were unable to unwind the S1 RNA substrate.

Dbp9p hydrolyzes ATP to produce free phosphate (Fig. 2B). The R414I, R414T, and R414K mutations, which have a conserved Arg residue in the RNA binding motif replaced with Ile, Thr, and Lys, respectively, impaired the ATPase activity of Dbp9p. This Dbp9p ATPase activity was inhibited by one-third of the wild-type Dbp9p was added to the mixtures. Samples were subjected to electrophoresis on 12% SDS acrylamide gel, and the bands were detected by autoradiography. H.D., heat-denatured; N.C., negative control.
activity of S. cerevisiae Mer3p, a DEAD box protein, is stimulated by DNA (26). But there are few other DEAD box proteins whose ATPase activity is known to be stimulated by DNA.

The Dbp9p was able to displace the 17-nt fragment that had been annealed to the M13mp18 single-stranded circular DNA in a reaction containing ATP. Some DEAD box RNA helicases, such as RNA helicase A and human GleBP, have DNA-DNA and DNA-RNA helicase activities (23, 27). Some viral helicases, such as NPHII and HCV NS3, also have DNA-DNA and RNA-RNA helicase activities (6, 28, 29). In the case of DEAD box proteins, DP103 and AtDRH1 show dsRNA unwinding and DNA-RNA helicase activities (30, 31), but no DEAD box protein has been shown to have DNA helicase activity. The DNA helicase activity of Dbp9p was found to be abolished by an amino acid substitution in its RNA-binding domain (Fig. 7). To our knowledge, this is the first study to show that a DEAD box protein has DNA helicase activity. It is not known whether the unwinding of the DNA-DNA duplex by the recombinant Dbp9p is significant in terms of its physiological function. Although the hypothesis that Dbp9p could act as a DNA helicase in addition to RNA helicase is attractive, experimental data in support of such a possibility are lacking.

NOH61 is a human ortholog of Dbp9p. Although it has not been determined whether NOH61 has helicase activity, the nucleolar localization and the participation in ribosome biogenesis are common to both proteins (32). The addition of ds and ssDNA activated the ATPase activity of NOH61 3.9- and 1.6-fold, respectively. The biochemical characteristic that ATPase activity is stimulated by the addition of DNA could also be applied to NOH61. But NOH61 cannot complement the defect of dbp9 mutation. In addition, NOH61 has a different substrate than that of Dbp9p in ribosome biogenesis. It is therefore speculated that NOH61 plays a role in the later stages of the processing that leads 65 S preribosomal particles to mature to 60 S ribosomal subunits, whereas Dbp9p seems to act at a very early stage in the formation of the 60 S equivalent. These differences might be based on the difference between the biochemical characteristics of these two orthologous proteins: the ATPase activity of NOH61 is severely inhibited by RNA but that of NOH61 is not.

Acknowledgments—We sincerely thank Dr. Hiroaki Katagi for critical reading and helpful comments on the manuscript. We thank Wataru Gunji for the gift of the yeast total RNA and DNA.

REFERENCES
1. De la Cruz, J., Kressler, D., and Linder, P. (1999) Trends Biochem. Sci. 24, 192–198
2. Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Nielsen, P. J., Nishi, K., Schnier, J., and Slonimski, P. P. (1989) Nature 347, 121–122
3. Tanner, N. K., and Linder, P. (2001) Mol. Cell 8, 251–262
4. Rosen, F., Edery, I., Mevoritch, K., Dever, T. E., Merrick, W. C., and Sonenberg, N. (1990) Mol. Cell. Biol. 10, 1134–1144
5. Huang, Y., and Liu, Z. R. (2002) J. Biol. Chem. 277, 12810–12815
6. Du, M. X., Johnson, R. B., Sun, X. L., Stauchke, R. A., Colacino, J., and Wang, Q. M. (2002) Biochem. J. 363, 147–155
7. Jones, P. G., Mitra, M., Kim, Y., Jiang, W., and Iwase, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 76–80
8. Dalbadie-McFarland, G., and Abelson, J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4236–4240
9. O’Day, C. L., Dalbadie-McFarland, G., and Abelson, J. (1996) J. Biol. Chem. 271, 33261–33267
10. Strauss, E. J., and Guthrie, C. A. (1991) Genes Dev. 5, 629–641
11. Iost, I., Dreyfus, M., and Linder, P. (1999) J. Biol. Chem. 274, 17677–17683
12. Chuang, R. Y., Weaver, P. L., Liu, Z., and Chang, T. H. (1997) Science 275, 1468–1471
13. Liu, H. Y., Nesky, B. S., and Walworth, N. C. (2002) J. Biol. Chem. 277, 2637–2643
14. Blum, S., Schmid, S. R., Pause, A., Buser, P., Linder, P., Sonenberg, N., and Trachsel, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7664–7668
15. Leary, B. J., and Huang, S. (2001) FEBS Lett. 509, 145–150
16. Daugeron, M. C., Kressler, D., and Linder, P. (2001) J. Biol. Chem. 276, 2637–2643
17. Shiratori, A., Shibata, T., Ariasa, M., Hanaoka, F., Murakami, Y., and Eki, T. (1999) Yeast 15, 219–253
18. Flores-Rozas, H., and Hurwitz, J. (1993) J. Biol. Chem. 268, 21372–21383
19. Valdez, B. C. (2000) Eur. J. Biochem. 267, 6395–6402
20. Tuteja, N., Rahman, K., Tuteja, R., Ochsen, A., Skopar, D., and Falaschi, A. (1992) Nucleic Acids Res. 20, 5329–5337
21. Oh, J. Y., and Kim, J. J. (1999) Nucleic Acids Res. 27, 2753–2759
22. Pause, A., and Sonenberg, N. (1992) EMBO J. 11, 2643–2646
23. Schmid, S. R., and Linder, P. (1991) Mol. Cell. Biol. 11, 3463–3471
24. Liang, L., Diehl-Jones, W., and Lasko, P. (1994) Development 120, 1201–1211
25. You, L. R., Chen, C. M., Yeh, T. S., Tsai, Y. T., Mai, R. T., Lin, C. H., and Lee, Y. H. (1999) J. Virol. 73, 2841–2853
26. Nakagawa, T., Flores-Rozas, H., and Kolodner, R. D. (2001) J. Biol. Chem. 276, 31487–31493
27. Costa, M., Ocher, A., Staub, A., and Falaschi, A. (1999) Nucleic Acids Res. 27, 817–821
28. Bayliss, C. D., and Smith, G. L. (1996) J. Virol. 70, 794–800
29. Tai, C. L., Chi, W. K., Chen, D. S., and Hwang, L. H. (1996) J. Virol. 70, 8477–8484
30. Yan, X., Mouillet, J. F., Ou, Q., and Sadovsky, Y. (2003) Mol. Cell. Biol. 23, 414–423
31. Okazaki, M., Mesiti, T., and Iwabuchi, M. (1998) Nucleic Acids Res. 26, 2638–2643
32. Zirres, R. F., Elbrachter, J., Kneissel, S., and Schmidt-Zachmann, M. S. (2000) Mol. Biol. Cell 11, 1153–1167

Fig. 9. Dbp9p DNA and DNA-RNA helicase activities are ATP-dependent. Helicase assay with 1 mM ATP (+ATP) or without ATP (−ATP). The experimental procedures are the same as described for Figs. 7 and 8. A, DNA-DNA substrate. B, DNA-RNA substrate. These bands were detected by autoradiography.
Dbp9p, a Member of the DEAD Box Protein Family, Exhibits DNA Helicase Activity
Takashi Kikuma, Masaya Ohtsu, Takahiko Utsugi, Shoko Koga, Kohji Okuhara,
Toshihiko Eki, Fumihiro Fujimori and Yasufumi Murakami

J. Biol. Chem. 2004, 279:20692-20698.
doi: 10.1074/jbc.M400231200 originally published online March 17, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M400231200

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 32 references, 20 of which can be accessed free at
http://www.jbc.org/content/279/20/20692.full.html#ref-list-1