BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

pubs.acs.org/biochemistry

Duplex Destabilization by Four Ribosomal DEAD-Box Proteins

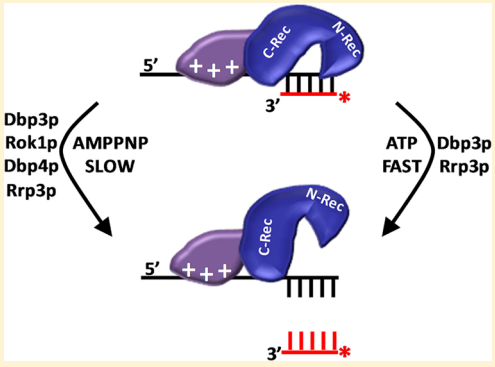
Ivelitza Garcia,[†] Michael J. Albring,[†] and Olke C. Uhlenbeck*[,‡]

[†]Department of Chemistry, Allegheny College, Meadville, Pennsylvania 16335, United States

[‡]Department of Molecular Biosciences, Northwestern University, Evanston, Illinois 60208, United States

Supporting Information

ABSTRACT: DEAD-box proteins are believed to participate in the folding of RNA by destabilizing RNA secondary or tertiary structures. Although these proteins bind and hydrolyze ATP, the mechanism by which nucleotide hydrolysis is coupled to helix destabilization may vary among different DEAD-box proteins. To investigate their abilities to disrupt helices and couple ATP hydrolysis to unwinding, we assayed the *Saccharomyces cerevisiae* ribosomal DEAD-box proteins, Dbp3p, Dbp4p, Rok1p, and Rrp3p utilizing a series of RNA substrates containing a short duplex and either a 5′ or 3′ single-stranded region. All four proteins unwound a 10 bp helix in vitro in the presence of ATP; however, significant dissociation of longer helices was not observed. While Dbp3p did not require a single-stranded extension to disrupt a helix, the unwinding activities of Dbp4p, Rok1p, and Rrp3p were substantially stimulated by either a 5′ or 3′ single-stranded extension. Interestingly, these proteins showed a clear length dependency with 3′ extensions that was not observed with 5′ extensions, suggesting that they bind substrates with a preferred orientation. In the presence of AMPPNP or ADP, all four proteins displayed displacement activity suggesting that nucleotide binding is sufficient to facilitate duplex disruption. Further enhancement of the strand displacement rate in the presence of ATP was observed for only Dbp3p and Rrp3p.



DEAD-box proteins make up a large subclass of helicase superfamily 2 (SF2) and are present in all organisms and are essential components of many cellular pathways that involve the synthesis and maturation of RNA.[1,2] DEAD-box proteins are defined by 9−12 conserved motifs[3,4] located within two covalently linked RecA-like domains, which comprise the protein's catalytic core.[5,6] The catalytic core forms an ATP binding pocket and an RNA binding site connected by a network of interdomain interactions.[2] This family of proteins can undergo a cycle of RNA and ATP binding, ATP hydrolysis, and protein conformational changes that, in some cases, result in RNA strand displacement in vitro.[1,7,8] DEAD-box proteins also contain auxiliary domains thought to be involved in RNA binding or in the recruitment of accessory factors.[9,10] These ubiquitous proteins are usually part of multiprotein complexes[11−13] and, in certain cases, genetically[14,15] or biochemically[16−18] linked to distinct steps in a given pathway.[14,15,19,20] For example, of the 25 *Saccharomyces cerevisiae* DEAD-box proteins, 14 function at various discrete steps in the ribosome biogenesis pathway. The four *S. cerevisiae* DEAD-box proteins (Dbp3p, Dbp4p, Rok1p, and Rrp3p) chosen for this study are essential components of this pathway, but their precise functions have yet to be elucidated.[15,20] Dbp3p stimulates the rate of pre-rRNA cleavage by the RNP endonuclease RNase MRP, conceivably by increasing access to the cleavage site.[16,19,21] Rrp3p ensures the fidelity of early pre-rRNA cleavage events possibly by facilitating an RNA structural rearrangement or the formation of a specific protein complex.[19,22] Dbp4p and Rok1p appear to be essential for the assembly and/or disassembly of multiprotein snoRNP complexes.[19] Depletion of Dbp4p yields a dramatic accumulation of the C/D-box snoRNA U14,[23] whereas Rok1p is genetically linked to the snoRNAs snR10 and snR30 as well as protein Rrp5.[24,25] Thus, while each protein appears to be involved with one or more RNA molecules, its precise site of action is unclear, and it is unknown whether they even act to modify RNA structure.

Previous experiments have established that Dbp3p, Dbp4p, Rok1p, and Rrp3p act as monomers and catalyze ATP hydrolysis in the presence of RNA.[26−28] Our previous study showed that the rate of ATP hydrolysis of all four proteins is enhanced by rRNA.[28] At saturating rRNA levels, ATP turnover rates ranged from 13 to 170 min^{-1} and ATP binding affinities ranged from 0.24 to 2.3 mM, which are consistent with the values of other members of this family of proteins.[2] When a variety of structured and unstructured RNA substrates were compared, none of the four proteins showed significant RNA sequence specificity. The four proteins each showed a characteristic site size for maximal ATPase activity, ranging from 10 to 40 single-stranded residues. Interestingly, all four proteins contain a basic amino acid region within their C-terminal domains (CTDs). Prior studies have suggested that a basic CTD binds RNA nonspecifically through electrostatic interactions in other DEAD-box proteins.[28]

Received: August 30, 2012
Revised: November 15, 2012
Published: November 15, 2012

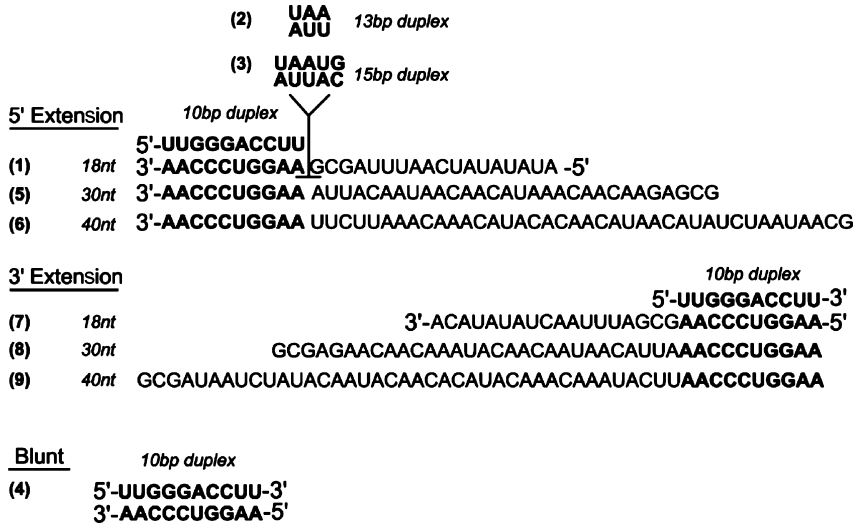


Figure 1. RNA bisubstrates consisting of a 10 bp RNA duplex and 0, 18, 30, and 40 nt single-stranded RNA extensions (substrates 1 and 4−7). Bold residues indicate the portion of the strands that are complementary to the common 5′-^{32}P-labeled 10-mer. Substrates 2 and 3 have elongated duplex regions of 13 and 15 bp, respectively.

The proposed cellular role of many ribosomal processing DEAD-box proteins centers on mediating RNA duplex rearrangements to alter pre-rRNA−snoRNA interactions or RNA−protein interactions. To date, the duplex unwinding activity of only one *S. cerevisiae* DEAD-box protein involved in ribosome biogenesis, Has1p, has been characterized.[29] Similar to previously studied DEAD-box proteins,[30,31] Has1p requires a single-stranded extension 5′ or 3′ of the duplex to initiate unwinding.[29] This protein unwinds RNA duplexes more efficiently with 3′ extensions than with 5′ extensions of the same length. Even though these characteristics are consistent with other SF2 proteins, strand displacement properties can vary widely.[2,32] It is, therefore, unclear whether the *S. cerevisiae* ribosomal DEAD-box proteins possess common catalytic properties, despite their diverse cellular roles. The goal of this study is to examine the RNA unwinding capacities of Dbp3p, Dbp4p, Rok1p, and Rrp3p with various nucleotides to address the following questions. Can these *S. cerevisiae* ribosomal DEAD-box proteins act as helicases? How efficient is unwinding? How do these proteins couple hydrolysis and RNA duplex disruption? While this in vitro approach has the obvious disadvantages of not studying the function of the protein with physiological RNA substrates or with accessory proteins, it permits the establishment of the intrinsic unwinding capabilities for each enzyme with respect to ATPase activity. Relating hydrolysis and unwinding may reveal important clues about their functional roles. Such an analysis also will permit comparisons with other well-characterized DEAD-box proteins with which similar assays have been performed and, thereby, identify potential similarities or differences in the mechanisms of this family of proteins.[32,33]

EXPERIMENTAL PROCEDURES

Materials. All nucleotides (ATPs, dATP, ADP, and AMPPNP) were purchased from Sigma-Aldrich. Where indicated, AMPPNP and ADP were treated with hexokinase and glucose to remove trace amounts of contaminating ATP as previously described.[34] Treated nucleotides were extracted with phenol and chloroform to remove the hexokinase protein. The purity of each nucleotide was determined by analytical high-performance liquid chromatography (HPLC).[35] Designed RNA helicase substrates were ordered from IDT (Integrated DNA Technologies, Inc.). *S. cerevisiae* rRNAs (18S and 25S) were isolated from midlog *S. cerevisiae* EJ101 cells utilizing an improved SDS−urea extraction method.[28] Dbp3p, Dbp4p, Rok1p, and Rrp3p were expressed and purified from *Escherichia coli* BL21-CodonPlus(DE3)-RIL cells (Stratagene) as previously described.[28] Proteins were purified under high-salt conditions with a strong ion exchange column to ensure the level of ATP contamination was negligible as judged by the absorbance at 260 nm. Each protein was concentrated into a storage buffer [50 mM Hepes (pH 7.5), 500 mM NaCl, 2 mM DTT, 0.1 mM EDTA, and 55% glycerol] and stored at −80 or −20 °C. Protein concentrations were determined by both Bradford and Lowry assays.

Bimolecular Substrate Annealing. The 10-mer strand was radiolabeled at the 5′ end using T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP. Bimolecular RNA substrates were formed by annealing a long single-stranded RNA (termed the bottom strand) to the complementary 10-mer (the top strand) to produce a 10 bp duplex. Annealing reaction mixtures contained 0.40 μM bottom strand, 0.35 μM ^{32}P-labeled top strand, 50 mM Hepes (pH 7.5), and 50 mM KCl in a total volume of 10 μL. After incubation at 95 °C for 1 min, the reaction mixture was annealed for 3 min at 65 °C and slowly cooled to room temperature within 15 min. Bimolecular substrates were kept at room temperature and used immediately. Because a higher concentration of RNA bisubstrate was required to monitor steady-state ATP hydrolysis, the annealing protocol described above was scaled up to 400 μM bottom strand and 400 μM top strand. Both annealing protocols yielded 75−85% annealed RNA bisubstrate.

ATPase Assay. The rate of ATP hydrolysis was measured using the previously described coupled spectroscopic assay, on microtiter plates.[9,28] In determining k_{max} and $K_{1/2(RNA)}$, we obtained hydrolysis rate curves in 50 mM Hepes (pH 7.5) [50 mM Mes (pH 6.5) for Rok1p], 25 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 200 μM NADH, 1 mM phosphoenolpyruvate, 13−26 μg/mL lactate dehydrogenase, 23 μg/mL pyruvate kinase, 6

mM ATP-Mg^{2+}, and 12 RNA concentrations that span from $0.1K_{1/2(RNA)}$ to approximately $4K_{1/2(RNA)}$. Protein concentrations for ATPase assays were identical to those used in previous experiments [Dbp3p (60 nM), Dbp4p (100 nM), Rok1p (250 nM), and Rrp3p (250 nM)].[28] Rates were determined from a linear fit of Abs_{338} versus time. RNA kinetic parameters, k_{max} and $K_{1/2}$, were determined from a Michaelis−Menten fit of the rate versus RNA concentrations as in previous studies.[28] All reported values are the average of at least three independent data sets and reported with the indicated standard deviation.

Displacement Assay. Top strand displacement reaction mixtures contained 50 mM Hepes (pH 7.5) [50 mM Mes (pH 6.5) for Rok1p], 50 mM KCl, 1 mM DTT, 0.1% Tween 20, 0.1 mg/mL BSA, 5 mM $MgCl_2$, 5% glycerol, 10 nM bisubstrate, and 600 nM DEAD-box protein. All time courses and end point reactions were performed in 30 μL reaction mixtures at 30 °C and initiated by the simultaneous addition of 6 mM ATP:Mg^{2+} and 750 nM nonradiolabeled 10-mer trap to prevent reannealing of the radiolabeled 10-mer. Reactions were terminated by the addition of 10 μL of quench solution [20 mM Hepes (pH 7.5), 20% glycerol, 40 mM EDTA, and 0.4% SDS]. Strand displacement was visualized by loading 20 μL of the quench reaction mixtures onto a nondenaturing 14% acrylamide−bisacrylamide (29:1) gel with dimensions of 390 mm × 210 mm × 1 mm in 0.3 M Tris-borate and 0.66 mM EDTA. Polyacrylamide gels were run at 25 °C for 2 h at 200 V and subsequently dried and quantified with a phosphorimager. The fraction of RNA substrate unwound was calculated by measuring the intensity of the resolved bands. All reactions followed simple single-exponential decay. All reported values are the averages of at least three independent determinations and reported with their corresponding standard error.

RESULTS

RNA Bimolecular Substrate Design and Initial Strand Displacement Characterization of Four DEAD-Box Proteins. Several factors were considered while designing substrates for the purpose of determining the effects of helix stability and single-strand extension on the duplex disruption activity of four DEAD-box proteins. The nine substrates utilized to study strand displacement are shown in Figure 1. Substrate 1 and substrates 4−9 contain the same symmetric 10 bp duplex terminating with two AU pairs on either end. In high-ionic strength buffers, a 10 nM solution of the 10 bp duplex has a calculated ΔG of −17.4 kcal/mol at 25 °C.[36] Its calculated dissociation rate is 0.001 min^{-1} ($t_{1/2}$ = 11 h), assuming a diffusion-limited association rate constant (10^8 M^{-1} s^{-1}).[36] Thus, the RNA helix is kinetically stable during the time course of our unwinding experiments. However, if 2 bp were disrupted from either terminus or if a single internal base pair were disrupted, the dissociation rate of the remaining helix would be calculated to increase to either 0.019 min^{-1} ($t_{1/2}$ = 36 min) or 3.3 min^{-1} ($t_{1/2}$ = 12.6 s), respectively.[36] Thus, the designed bisubstrates permit sensitive detection of helix disruption. In addition to the perfect duplex [substrate 4 (Figure 1)], substrates were constructed with 18-, 30-, or 40-nucleotide (nt) single-stranded extensions on either the 5′ terminus [substrates 1, 5, and 6 (Figure 1)] or the 3′ terminus [substrates 7−9 (Figure 1)]. The extensions contain few G residues and, thus, are expected to have little stable secondary structure. Various extension lengths were chosen because the four DEAD-box proteins are known to have different site size requirements for their RNA-dependent ATPase activity.[28] Finally, two variants of substrate 1 containing a 13 bp helix (substrate 2) or a 15 bp helix (substrate 3) were designed to test whether the enzymes can disrupt longer, more stable helices.

For the kinetic analysis of each DEAD-box protein, top strand displacement assays were performed under conditions where the protein concentration exceeded the concentration of the RNA substrate and in the presence of an excess of nonradiolabeled 10-mer top strand to ensure that only single displacement events were observed. Figure 2A illustrates the

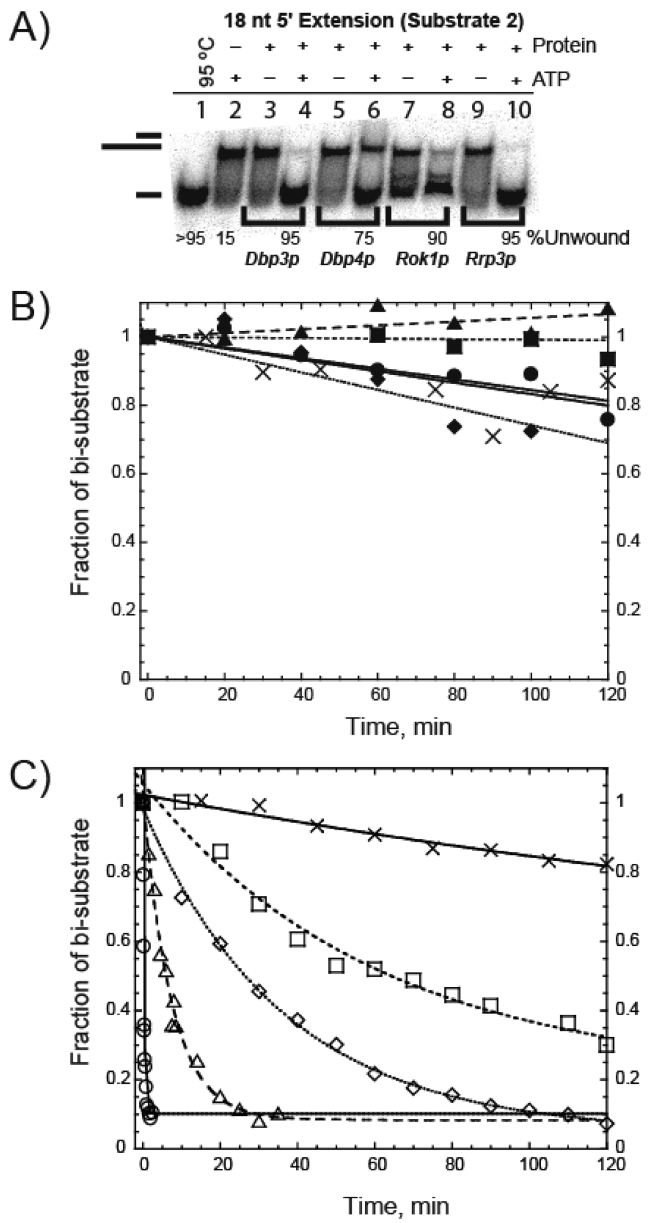


Figure 2. Helix disruption of substrate 1 by four DEAD-box proteins. (A) Nondenaturing gel of end point assays after a 2 h incubation at 30 °C. Heat denaturation and reannealing of substrate 1 in the presence of trap RNA (lane 1). Displacement of duplex with no protein (lane 2) or 600 nM Dbp3p, Dbp4p, Rok1p, and Rrp3p in the presence (lanes 4, 6, 8, and 10, respectively) and absence of ATP (lanes 3, 5, 7, and 9, respectively). Time course of duplex unwinding in the absence of ATP (B) and in the presence of 6 mM ATP:Mg^{2+} (C) with no protein (×) or 600 nM Dbp3p (circles), Dbp4p (squares), Rok1p (diamonds), and Rrp3p (triangles).

dx.doi.org/10.1021/bi301172s | Biochemistry 2012, 51, 10109−10118

disruption of substrate 1 by each of the four proteins at 600 nM after a 2 h incubation at 30 °C analyzed on a nondenaturing gel. On the basis of ATPase assays,[28] this protein concentration should be near or at saturation with substrate 1 for Dbp3p, Dbp4p, and Rrp3p but be subsaturating for Rok1p. When the substrate was heated to 95 °C to dissociate the duplex, the presence of the nonradiolabeled 10-mer prevented reannealing of the radiolabeled top strand (Figure 2A, lane 1). Representative time courses of dissociation of substrate 1 from quantitative native gels are shown in panels B and C of Figure 2. The measured spontaneous dissociation rate of substrate 1 was 0.0017 ± 0.0001 min^{-1}, which agrees closely with the calculated value (cross-mark symbol in Figure 2B) and corresponds to less than 20% displacement of substrate 1 after 2 h. In the presence of 600 nM Dbp3p, Dbp4p, Rok1p, or Rrp3p and in the absence of ATP (lane 3, 5, 7, or 9, respectively, of panels A and B of Figure 2), the average dissociation rates were similar to the measured spontaneous rate (Figure 2B). In the presence of both ATP and protein, the extent of helix unwinding increases dramatically (Figure 2A,C, lanes 4, 6, 8, and 10). Experiments with varying protein concentrations reveal that 600 nM for each DEAD-box protein was sufficient for complete helix disruption (Figure S1 of the Supporting Information). The time course for each protein in the presence of a saturating level of ATP follows single-exponential decay with rates ranging from 0.012 ± 0.005 to 3.6 ± 0.1 min^{-1} (Figure 2C). Dbp3p is the fastest DEAD-box protein (k_{obs} = 3.6 ± 0.1 min^{-1}), while Rrp3p has a moderate rate (k_{obs} = 0.17 ± 0.03 min^{-1}). Dbp4p (k_{obs} = 0.012 ± 0.005 min^{-1}) and Rok1p (k_{obs} = 0.021 ± 0.002 min^{-1}) are the slowest proteins, but both are significantly faster (6- and 11-fold, respectively) than the measured spontaneous dissociation rate of substrate 1. These end point and time course assays clearly showed that displacement activity requires ATP and that the four yeast ribosomal DEAD-box proteins have distinct rates of duplex displacement when assayed under identical conditions.

Single-Stranded Extension and Duplex Length Requirements for Helix Displacement. The helix length of a bisubstrate can greatly decrease the efficiency of helix disruption by DEAD-box proteins because additional base pairs would need to be disrupted to initiate spontaneous strand separation.[32,37] Several DEAD-box proteins have been shown to displace duplexes of up to 14 bp in length; thus, the four selected proteins were assayed with substrates 2 and 3, which contain an 18 nt 5′ extension and either a 13 or 15 bp duplex, respectively (Figure 1).[31,32,38] Figure 3 compares the effect of duplex length on the displacement activity with substrates 1−3 in the presence and absence of 600 nM protein. In the absence of protein, displacement rates of substrates 2 and 3 are slower than that of substrate 1 as expected because of their increased stability. When the proteins were added in the presence of ATP, the observed increases in the rate of duplex melting were negligible (~4-fold) compared to those without ATP and protein. These results demonstrate that Dbp3p, Dbp4p, Rok1p, and Rrp3p exhibit displacement activity only with 10 bp duplexes.

An increasing number of DEAD-box proteins have demonstrated the ability to dissociate an RNA duplex in the absence of a single-stranded extension.[30,39,40] To examine this behavior for Dbp3p, Dbp4p, Rok1p, and Rrp3p, a duplex RNA with no extensions was utilized to monitor strand dissociation [substrate 4 (Figure 1)]. As seen in Figure 4, Dbp3p was able to displace the perfect 10 bp helix at 1.4 ± 0.2 min^{-1}, which is

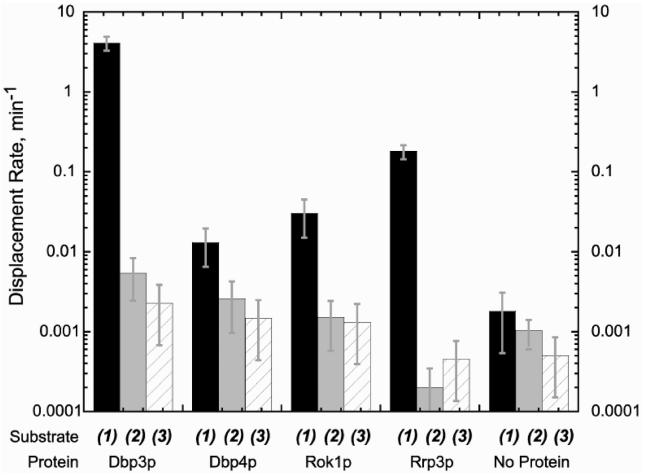


Figure 3. Effects of duplex length on displacement for Dbp3p, Dbp4p, Rok1p, and Rrp3p. The black, gray, and partially shaded bars illustrate data for substrates 1 (10 bp), 2 (13 bp), and 3 (13 bp), respectively. Experiments were performed at 30 °C in 10 nM bisubstrate, 750 nM cold 10-mer (trap), 6 mM ATP:Mg^{2+}, and 600 nM DEAD-box protein. The observed displacement rates for substrate 2 and 3 are too slow to accurately measure.

slightly lower than the rate observed in the presence of a 5′ single-stranded extension. The observation that Dbp3p does not require a single strand for efficient separation of a 10 bp helix suggests that its DEAD-box core binds to the helix with sufficient stability to facilitate dissociation. In contrast, Dbp4p, Rok1p, and Rrp3p disrupt the perfect duplex with measured rates of 0.002 ± 0.0005, 0.003 ± 0.0003, and 0.004 ± 0.0002 min^{-1}, respectively. These observed rates are similar (≤2-fold) to the strand displacement rate of substrate 1 in the absence of ATP (0.0017 ± 0.0003 min^{-1}). This lack of displacement activity may result from the inability to achieve full protein saturation.[30] The analysis of the perfect duplex suggests that, for three of the four proteins, the single-stranded region has an important role in the binding of the DEAD-box core to a duplex RNA.

Effects of Extension Strand Length and Orientation on Duplex Dissociation. Many well-characterized SF1 and SF2 helicases require a single-stranded extension to promote duplex displacement.[7,31,33,39,41] Previous experiments have demonstrated that the single-stranded segment of a bisubstrate can facilitate the loading of the catalytic core onto the RNA duplex initiating displacement and that the length of this strand can affect the efficiency of helix separation.[7,30,31,33,39] To determine the effects of extension length and orientation on displacement, substrates were constructed with an 18, 30, or 40 nt single-stranded region on either the 5′ terminus [substrate 1, 5, or 6, respectively (Figure 1)] or the 3′ terminus [substrate 7, 8, or 9, respectively (Figure 1)]. Figure 4 compares the displacement activity for the four proteins in the presence of substrates 1 and 4−9. Although their absolute rates vary, all four proteins were insensitive to the length of the 5′ extension. Interestingly, the four proteins responded very differently to RNAs with 3′ extensions. Because Dbp3p could efficiently unwind a perfect duplex, it was not surprising that its unwinding rate was unresponsive to the length of the 3′ extension. In contrast, the observed rates for Dbp4p, Rok1p, and Rrp3p increased as the length of the 3′ extension increased. In each case, a 40 nt 3′ extension (substrate 9) was needed to match the rate observed for the 5′ extensions. This asymmetric

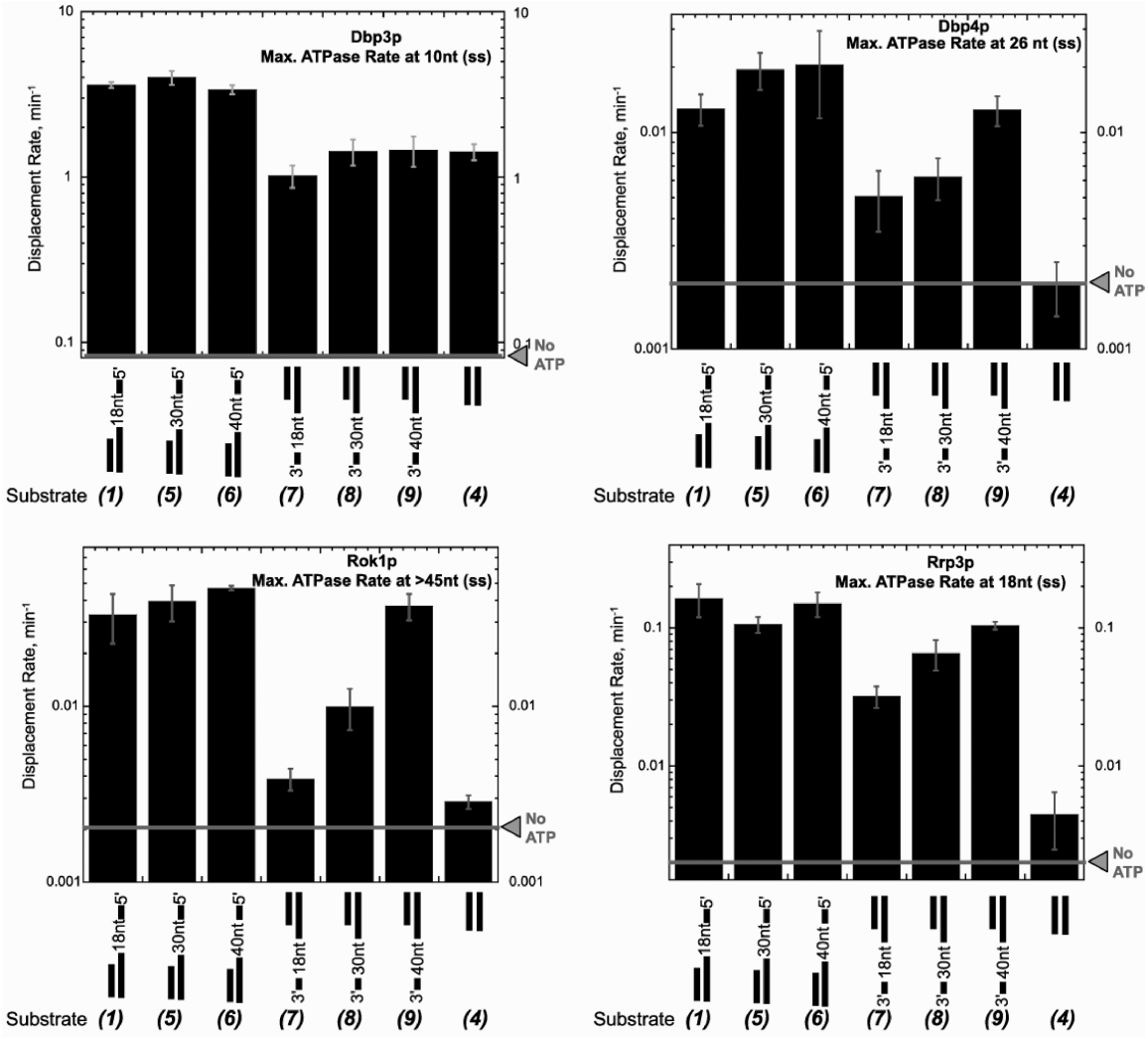


Figure 4. Effects of extension length and orientation on the displacement for Dbp3p, Dbp4p, Rok1p, and Rrp3p. The gray line indicates the average unwinding rate in the absence of ATP and/or protein. Experiments were performed at 30 °C in 10 nM bisubstrate, 750 nM cold 10-mer (trap), 6 mM ATP:Mg^{2+}, and 600 nM DEAD-box protein. Minimal ssRNA lengths were previously determined.[28]

Table 1. Steady-State ATPase Kinetic Parameters[a]

		Dbp3p		Dbp4p		Rok1p		Rrp3p	
RNA	substrate	k_{max} (min^{-1})	$K_{1/2}$ (nM)	k_{max} (min^{-1})	$K_{1/2}$ (nM)	k_{max} (min^{-1})	$K_{1/2}$ (nM)	k_{max} (min^{-1})	$K_{1/2}$ (nM)
5′ Extended Duplex									
18 nt	1	185 ± 30	125 ± 39	81 ± 2	110 ± 40	11 ± 3	300 ± 75	17 ± 1	1230 ± 400
30 nt	5	199 ± 11	158 ± 23	70 ± 3	90 ± 9	13 ± 5	500 ± 29	19 ± 9	920 ± 98
40 nt	6	200 ± 19	90 ± 9	67 ± 2	112 ± 29	11 ± 5	210 ± 58	21 ± 5	1080 ± 280
3′ Extended Duplex									
18 nt	7	189 ± 22	224 ± 50	82 ± 9	210 ± 47	7 ± 3	290 ± 14	20 ± 9	645 ± 67
30 nt	8	198 ± 18	187 ± 17	89 ± 5	191 ± 16	15 ± 8	550 ± 63	17 ± 2	1240 ± 350
40 nt	9	204 ± 32	87 ± 20	81 ± 9	150 ± 27	15 ± 7	650 ± 70	21 ± 4	1100 ± 300
10 bp duplex	4	29 ± 9	11200 ± 2000	95 ± 7	3800 ± 1000	6 ± 2	1700 ± 200	19 ± 8	612 ± 91
Minimal ssRNAs									
13 nt	–	181 ± 28	3100 ± 900	–	–	–	–	–	–
25 nt	–	–	–	62 ± 5	800 ± 300	–	–	–	–
45[b] nt	–	–	–	–	–	5 ± 2[b]	>3000[b]	-	-
16 nt	–	–	–	–	–	–	–	16 ± 4	5000 ± 3000

[a]All reactions were performed in 50 mM Hepes (pH 7.5) [Mes (pH 6.5) for Rok1p], 5 mM $MgCl_2$, and 1 mM DTT at a saturating ATP concentration (6 mM). [b]Reactions that could not achieve saturation. All values represent the average of at least three independent data sets. Minimal ssRNAs substrates were determined previously. [28]

dx.doi.org/10.1021/bi301172s | *Biochemistry* 2012, 51, 10109−10118

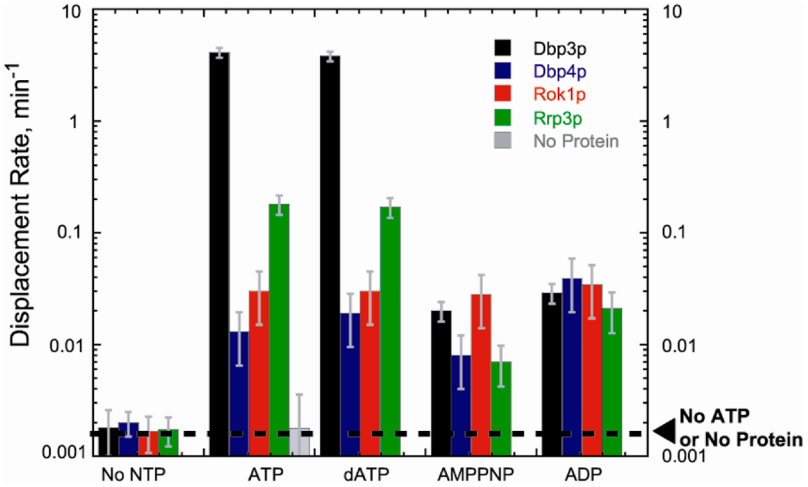


Figure 5. Nucleotide dependence on RNA duplex separation. The black dashed line indicates the average dissociation rate in the absence of protein or the absence of ATP. Experiments were performed at 30 °C in 10 nM substrate 2, 750 nM cold 10-mer (trap), 6 mM nucleotide:Mg^{2+}, and 600 nM DEAD-box protein.

length dependence on the single-strand extensions may reflect how the DEAD-box catalytic core can position itself with respect to the helical substrate (see Discussion).

The steady-state ATPase activity of all four proteins was determined for each extension length under saturating ATP conditions because the various displacement rates may be influenced partially by the site size requirement as well as the architecture of the bisubstrate. Table 1 summarizes the results for substrates 1 and 4−9 (Figure 1) as well as for minimal single-stranded RNAs (ssRNAs) determined previously.[28] All RNAs listed were capable of stimulating ATP hydrolysis for each DEAD-box protein to different extents. The ATP turnover rates for the selected proteins, in the presence of substrate 1 and 5−9, were comparable to the rates obtained with the corresponding minimal single-stranded RNAs. As seen in Table 1, Rrp3p's k_{max} with a 16 nt ssRNA substrate (16 ± 4 min^{-1}) was comparable to the k_{max} values of the 5′ and 3′ extended substrates (1 and 5−9), which ranged from 17 to 21 min^{-1}; thus, the critical length requirement for the 3′ extensions is not a direct result of altered ATP hydrolysis. Likewise, the blunt substrate (substrate 4) stimulated the ATPase activity to an extent similar to that of minimal ssRNAs for Dbp4p, Rok1p, and Rrp3p. The rate of Dbp3p-mediated hydrolysis decreased 6-fold with the blunt substrate, yet it is efficiently melted. Interestingly, fast duplex displacement correlated to rapid hydrolysis, while slow ATP turnover corresponded to slow duplex dissociation. The overall strand separation rates were directly proportional to hydrolysis rates instead of inversely proportional, as seen in a few bacterial DEAD-box proteins.[30,32,33,42,43] The measured apparent binding affinities [$K_{1/2(RNA)}$] of the 5′ and 3′ extended substrates (1 and 5−9) were substantially tighter than those of the minimal ssRNAs and the perfect duplex (substrate 4). For example, Dbp3p has $K_{1/2(RNA)}$ values of 3100 ± 900 nM for the 13 nt ssRNA and 125 ± 39 nM for substrate 1. These data indicate that addition of a duplex to the single-stranded region increases the binding energy for all four of the proteins irrespective of their single-stranded site size requirements.

Role of Nucleotide Binding and Hydrolysis during RNA Duplex Displacement. Recent studies have shown that duplex separation by DEAD-box proteins is connected to a particular step within the hydrolysis cycle.[2,44,45] For some DEAD-box proteins, unwinding can be facilitated by nucleotide binding and subsequent hydrolysis recycles the enzyme.[40,45,46] The bacterial DEAD-box protein DbpA, in contrast, requires ATP hydrolysis to initiate duplex disruption.[43] The rate of duplex separation for substrate 1 in the presence of various nucleotides was determined for the four DEAD-box proteins. As seen in Figure 5, the measured displacement rates in the presence of dATP were identical to the rates observed in the presence of ATP, similar to other DEAD-box proteins.[7] However, the effects on duplex separation with ADP and AMPPNP differed substantially for the four proteins (Figure 5). The activities of Dbp3p and Rrp3p decreased 180- and 24-fold, respectively, when ATP was replaced with AMPPNP or ADP. In contrast, the rate of duplex separation for Dbp4p and Rok1p in the presence of AMPPNP or ADP was similar to that observed with ATP (Figure 5). For all four proteins, the rates in the presence of AMPPNP or ADP are approximately 10-fold faster than the rates measured in the absence of nucleotide. While the activities of several DEAD-box proteins in the presence of AMPPNP or ADP were found to be the result of trace amounts of ATP contamination in the nucleotide preparation,[7,40,45] our experiments show that this is not the case for the four proteins in this study. Both AMPPNP and ADP were treated with hexokinase, and the absence of ATP in each nucleotide was confirmed by analytical HPLC (Figure S2 of the Supporting Information).[35] Indeed, pretreatment with hexokinase did not substantially decrease the displacement rates (Table 2). These results indicate that ADP or AMPPNP binding is sufficient to slowly displace the duplex for all four proteins, yet the rapid duplex displacement observed for Dbp3p and Rrp3p requires ATP hydrolysis.

■ DISCUSSION

All four of the selected yeast DEAD-box proteins were found to disrupt a 10 bp helix in the presence of ATP at a rate significantly faster than the spontaneous dissociation rate, thereby formally classifying them as helicases. However, none of the proteins were able to significantly disrupt a slightly longer 13 bp helix. This inability to disrupt a longer helix is a common characteristic of most DEAD-box proteins,[31,38,47] and it distinguishes them from DNA helicases, which share many of the conserved elements yet are able to unwind long duplexes in

Table 2. Kinetic Parameters with Hexokinase-Treated Nucleotides[a]

protein	nucleotide	k_{dis} (×100 min^{-1})
Dbp3	none	0.17 ± 0.03
	AMPPNP	0.7 ± 0.1
	ADP	1.0 ± 0.4
Dbp4	none	0.18 ± 0.05
	AMPPNP	0.8 ± 0.2
	ADP	1.2 ± 0.3
Rok1	none	0.16 ± 0.06
	AMPPNP	1.1 ± 0.5
	ADP	1.0 ± 0.4
Rrp3	none	0.17 ± 0.05
	AMPPNP	3 ± 2
	ADP	4 ± 1

[a]All reactions were performed at 30 °C in 50 mM Hepes (pH 7.5) [50 mM Mes (pH 6.5) for Rok1p], 50 mM KCl, 1 mM DTT, 0.1% Tween 20, 0.1 mg/mL BSA, 5 mM $MgCl_2$, 5% glycerol, 10 nM substrate 1, 750 nM cold 10-mer (trap), 6 mM nucleotide:Mg^{2+}, and 600 nM DEAD-box protein.

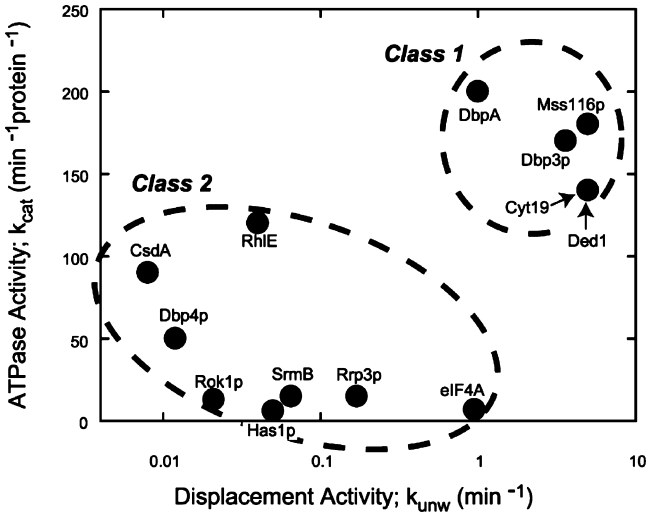


Figure 6. Comparison of ATPase and displacement activity of Has1p,[29] RhlE,[32] CsdA,[32] SrmB,[32] eIF4A,[29,38] Mss116p,[51] Cyt19,[51] SrmB,[51] Ded1,[51] and DbpA.[50] Most proteins listed reflect dissociation rates of a 9−11 nt duplex substrate except in the cases of Has1p[29] (16 nt) and CsdA (14 nt).[32]

a highly processive fashion.[48,49] This difference presumably reflects the paucity of longer helices in folded RNA structures. Thus, DEAD-box proteins do not need to ensure that multiple rounds of ATP hydrolysis and helix disruption occur per binding event. Disruption of 1 or 2 bp may be all that is needed for these proteins to carry out their function.

The observed helix unwinding rates of the four DEAD-box proteins span 2 orders of magnitude, ranging from the fast Dbp3p (3.6 min^{-1}) and the intermediate Rrp3p (0.1 min^{-1}) to the very slow Dbp4p (0.012 min^{-1}) and Rok1p (0.021 min^{-1}). These rates are lower than the corresponding rates of ATP hydrolysis obtained with the same substrates (7−200 min^{-1}), consistent with the idea that the enzymes can undergo multiple "futile" rounds of binding, ATP hydrolysis, and release of the substrate without catalyzing helix disruption.[4] Nevertheless, the duplex disruption rates for the four proteins roughly correlate with the ATPase rates at saturating substrate concentrations. For example, Dbp3p can rapidly disrupt a helix (3.6 min^{-1}) and hydrolyze ATP (185 min^{-1}), while Dpb4p shows slower duplex disruption (0.012 min^{-1}) and ATPase (81 min^{-1}) activities. A similar comparison of nine additional DEAD-box proteins maintained the weak correlation, and thus, two potential classes of DEAD-box proteins emerge. The first, "fast", class of proteins (DbpA,[50] Mss116p,[51] Dbp3p, Cyt19,[51] and Ded1[51]) hydrolyze ATP at a rate of >130 min^{-1} and unwind duplexes at a rate of >1 min^{-1}. For the "slow" class of proteins (Rok1p, Dbp4p, Rrp3p, Has1p,[29] SrmB,[32] CsdA,[32] RhlE,[32] and eIF4A[38]), both activities are considerably lower (Figure 6). It is unclear whether this classification is meaningful. Because each DEAD-box protein functions on quite different substrates in distinct pathways, the varying intrinsic properties of each protein simply may reflect the kinetics of these pathways. Alternatively, the various measured rates may indicate the ability of these proteins to function on what are likely to be nonphysiological substrates. Finally, it is known that the ATPase and helicase activities of DEAD-box proteins can be altered dramatically by the presence of accessory proteins.[52−58] In this regard, it is interesting that with the exception of Ded1,[51] all the members of the fast class either are transiently associated or do not participate in large RNP processing complexes.[21,59−61] In contrast, at least five members of the slow class (Dbp4p, Has1p, Rok1p, eIF4A, and SrmB) have been genetically or biochemically identified to bind specific accessory proteins (Dbp8p, Esf2p, Rrp5p, Gar1p, eIF4B, eIF-iso4F, MLN51, eIF4H, eIF4F, L4, and L24).[25,53,54,56,62−64] Thus, their relatively weak activity when measured independently does not reflect their catalytic capacity.

Length Dependency of Displacement Activity. Most well-characterized DEAD-box proteins, such as eIf4A,[31] Ded1,[30] SrmB,[32] CsdA,[32] Mss116p,[40] and Has1,[29] require a single-stranded region 5′ or 3′ of a duplex for efficient base pair disruption.[2] Only a few DEAD-box proteins, such as RhlE,[32] are able to unwind perfect RNA duplexes as well as substrates containing 3′ or 5′ extensions. Among the four proteins in this study, Rrp3p, Dbp4p, and Rok1p require single-stranded extensions, while Dbp3p does not. Interestingly, all three proteins that require an extension show an asymmetric length dependence on the single-strand regions. Varying the length of the 5′ extension from 18 to 40 nt has no effect on the rate, while the helicase activity increases as the 3′ extension increases in length from 18 to 40 nt. Rok1p, the protein with the largest single-stranded site size requirement for hydrolysis,[28] shows the strongest dependence on 3′ extension length. In contrast, the helicase activity of Rrp3p, which has the smallest site size, shows an only modest dependence on 3′ extension length. Although such asymmetry in the length dependence of 3′ and 5′ extensions on helicase rate has not been examined in many other cases, similar data have been reported for eIF4A and *E. coli* DbpA.[31,65] In addition, several other DEAD-box proteins show quite different activities when 3′ and 5′ extensions are compared.[29,30]

A simple model to account for the orientation preference is to assume that the proteins do not bind 3′ and 5′ extended helicase substrates in the same way. Because the cocrystal structures of six DEAD-box proteins with single-stranded RNA show the N-Rec domain binding to the 3′ terminus of an oligonucleotide and the C-Rec domain binding to the 5′ terminus,[66−70] it is reasonable to expect that the catalytic cores of Dbp4p, Rok1p, and Rrp3p interact with helicase substrates in a similar fashion. This orientation places the basic C-terminal

"tails" for these proteins adjacent to the 5′ extensions where electrostatic interactions could enhance substrate binding as well as permit the catalytic domains to disrupt an RNA duplex.[27,71,72] Thus, to facilitate duplex disruption in the presences of a 3′ extension, the single-stranded region would have to wrap around the duplex to bind to the basic C-terminal tail. As a result, a longer 3′ extension is needed to promote optimal helicase activity in our experiments. Such a looping or wraparound model also was suggested to explain the catalytic properties of *E. coli* DbpA in which helicase substrates contained a hairpin either 3′ or 5′ to the duplex.[65]

Dependency of ATP Hydrolysis in Duplex Unwinding. While the four yeast DEAD-box proteins were unable to unwind a short model duplex in the absence of nucleotides, all four showed a similar slow rate (0.01−0.04 min^{-1}) of helix disruption in the presence of ADP, a product of ATP hydrolysis, or AMPPNP, a nonhydrolyzable binding analogue. These rates do not reflect trace amounts of contaminating ATP present in the preparation of ADP or AMPPNP, because this impurity was carefully removed with hexokinase. Thus, these results indicate that a complete cycle of ATP binding, hydrolysis, and product release is not necessary for slow, single-turnover, helix disruption. While this observation is not common in the biochemical analysis of DEAD-box proteins, similar results have been reported in few cases. Proteolysis and fluorescent experiments have established a protein conformational change upon addition of AMPPNP or ADP.[46,73−75] YxiN can destabilize a short RNA duplex in the presence of AMPPNP. Similarly, DDX1 can facilitate duplex unwinding in the presence of ADP,[76] whereas Ded1 utilizes ADP to modulate duplex annealing and unwinding activity depending on the nature of the helicase substrate.[77] Because the ADP-dependent rate reported in this study is only 6−10-fold faster than the spontaneous rate of helix dissociation, it is possible that the helicase assays used for other DEAD-box proteins were not optimal for the detection of such low activity. Thus, slow helix disruption by ADP may be a property of all DEAD-box proteins.

In the presence of ATP, two of the proteins (Rrp3p and Dbp3p) showed enhanced helix disruption rates (10−200-fold) compared to the AMPPNP unwinding activity. Rok1p and Dbp4p, in contrast, showed no measurable enhancement. The faster helicase rates, in the presence of ATP, suggest that Rrp3p and Dbp3p function in RNA remodeling as is generally proposed for this class of proteins.[1] However, the absence of a hydrolysis-dependent rate for Rok1p or Dbp4p makes assigning a function for these proteins difficult. The observed slow activity exceeds the doubling time of yeast, making it unlikely to be relevant to the ribosomal assembly pathways. This may reflect the fact that the assay used a nonphysiological substrate or that an accessory protein was omitted. Alternatively, because the unwinding rate with ATP matches the rate with ADP, these proteins may not function in RNA remodeling.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary methods and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: o-uhlenbeck@northwestern.edu. Telephone: (847) 491-5139. Fax: (847) 491-5444.

Funding

This work was funded by National Institutes of Health Grant R01-GM60268-08 (to O.C.U.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Diana Brautigam for a careful reading of the manuscript.

■ ABBREVIATIONS

rRNA, ribosomal RNA; snoRNA, small nucleolar RNA; snoRNP, small nucleolar ribonucleoprotein; CTD, C-terminal domain; NTD, N-terminal domain; N-Rec, N-terminal RecA-like; C-Rec, C-terminal RecA-like; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADH, nicotinamide adenine dinucleotide; SDS, sodium dodecyl sulfate; RRM, RNA recognition motif; mer, oligomer; nt, nucleotide.

■ REFERENCES

(1) Rocak, S., and Linder, P. (2004) DEAD-box proteins: The driving forces behind RNA metabolism. *Nat. Rev. Mol. Cell Biol. 5*, 232−241.

(2) Cordin, O., Banroques, J., Tanner, N. K., and Linder, P. (2006) The DEAD-box protein family of RNA helicases. *Gene 367*, 17−37.

(3) Gorbalenya, A. E., and Koonin, E. V. (1993) Helicases: Amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol. 3*, 419−429.

(4) Linder, P., and Jankowsky, E. (2011) From unwinding to clamping: The DEAD box RNA helicase family. *Nat. Rev. Mol. Cell Biol. 12*, 505−516.

(5) Story, R. M., and Steitz, T. A. (1992) Structure of the recA protein−ADP complex. *Nature 355*, 374−376.

(6) Fairman-Williams, M. E., Guenther, U. P., and Jankowsky, E. (2010) SF1 and SF2 helicases: Family matters. *Curr. Opin. Struct. Biol. 20*, 313−324.

(7) Diges, C. M., and Uhlenbeck, O. C. (2001) *Escherichia coli* DbpA is an RNA helicase that requires hairpin 92 of 23S rRNA. *EMBO J. 20*, 5503−5512.

(8) Bjornson, K. P., Amaratunga, M., Moore, K. J., and Lohman, T. M. (1994) Single-turnover kinetics of helicase-catalyzed DNA unwinding monitored continuously by fluorescence energy transfer. *Biochemistry 33*, 14306−14316.

(9) Karginov, F. V., Caruthers, J. M., Hu, Y., McKay, D. B., and Uhlenbeck, O. C. (2005) YxiN is a modular protein combining a DEx(D/H) core and a specific RNA-binding domain. *J. Biol. Chem. 280*, 35499−35505.

(10) Schwer, B. (2001) A new twist on RNA helicases: DExH/D box proteins as RNPases. *Nat. Struct. Biol. 8*, 113−116.

(11) Staley, J. P., and Guthrie, C. (1998) Mechanical devices of the spliceosome: Motors, clocks, springs, and things. *Cell 92*, 315−326.

(12) Krogan, N. J., Peng, W. T., Cagney, G., Robinson, M. D., Haw, R., Zhong, G., Guo, X., Zhang, X., Canadien, V., Richards, D. P., Beattie, B. K., Lalev, A., Zhang, W., Davierwala, A. P., Mnaimneh, S., Starostine, A., Tikuisis, A. P., Grigull, J., Datta, N., Bray, J. E., Hughes, T. R., Emili, A., and Greenblatt, J. F. (2004) High-definition macromolecular composition of yeast RNA-processing complexes. *Mol. Cell 13*, 225−239.

(13) Silverman, E., Edwalds-Gilbert, G., and Lin, R. J. (2003) DExD/H-box proteins and their partners: Helping RNA helicases unwind. *Gene 312*, 1−16.

(14) de la Cruz, J., Kressler, D., and Linder, P. (1999) Unwinding RNA in *Saccharomyces cerevisiae*: DEAD-box proteins and related families. *Trends Biochem. Sci. 24*, 192−198.
(15) Kressler, D., Linder, P., and de la Cruz, J. (1999) Protein trans-acting factors involved in ribosome biogenesis. *Mol. Cell. Biol. 19*, 7897−7912.
(16) Bernstein, K. A., Granneman, S., Lee, A. V., Manickam, S., and Baserga, S. J. (2006) Comprehensive mutational analysis of yeast DEXD/H box RNA helicases involved in large ribosomal subunit biogenesis. *Mol. Cell. Biol. 26*, 1195−1208.
(17) Granneman, S., Bernstein, K. A., Bleichert, F., and Baserga, S. J. (2006) Comprehensive mutational analysis of yeast DEXD/H box RNA helicases required for small ribosomal subunit synthesis. *Mol. Cell. Biol. 26*, 1183−1194.
(18) Tsu, C. A., and Uhlenbeck, O. C. (1998) Kinetic analysis of the RNA-dependent adenosine triphosphatase activity of DbpA, an *Escherichia coli* DEAD protein specific for 23S ribosomal RNA. *Biochemistry 37*, 16989−16996.
(19) Venema, J., and Tollervey, D. (1999) Ribosome synthesis in *Saccharomyces cerevisiae*. *Annu. Rev. Genet. 33*, 261−311.
(20) Tollervey, D. (1996) Trans-acting factors in ribosome synthesis. *Exp. Cell Res. 229*, 226−232.
(21) Weaver, P. L., Sun, C., and Chang, T. (1997) Dbp3p, a putative RNA helicase in *Saccharomyces cerevisiae* is required for efficient pre-rRNA processing predominantly at site A3. *Mol. Cell. Biol. 17*, 1354−1365.
(22) O'Day, C., Chavanikamannil, F., and Abelson, J. (1996) 18S rRNA processing requires the RNA helicase-like protein Rrp3. *Nucleic Acids Res. 24*, 3201−3207.
(23) Kos, M., and Tollervey, D. (2005) The Putative RNA Helicase Dbp4p Is Required for Release of the U14 snoRNA from Preribosomes in *Saccharomyces cerevisiae*. *Mol. Cell 20*, 53−64.
(24) Oh, J., and Kim, J. (1999) ATP hydrolysis activity of the DEAD box protein Rok1p is required for *in vivo* ROK1 function. *Nucleic Acids Res. 27*, 2753−2759.
(25) Vos, H. R., Bax, R., Faber, A. W., Vos, J. C., and Raue, H. A. (2004) U3 snoRNP and Rrp5p associate independently with *Saccharomyces cerevisiae* 35S pre-rRNA, but Rrp5p is essential for association of Rok1p. *Nucleic Acids Res. 32*, 5827−5833.
(26) Talavera, M. A., Matthews, E. E., Eliason, W. K., Sagi, I., Wang, J., Henn, A., and de la Cruz, E. M. (2006) Hydrodynamic characterization of the DEAD-box RNA helicase DbpA. *J. Mol. Biol. 355*, 697−707.
(27) Grohman, J. K., Del Campo, M., Bhaskaran, H., Tijerina, P., Lambowitz, A. M., and Russell, R. (2007) Probing the mechanisms of DEAD-box proteins as general RNA chaperones: The C-terminal domain of CYT-19 mediates general recognition of RNA. *Biochemistry 46*, 3013−3022.
(28) Garcia, I., and Uhlenbeck, O. C. (2008) Differential RNA-dependent ATPase activities of four rRNA processing yeast DEAD-box proteins. *Biochemistry 47*, 12562−12573.
(29) Rocak, S., Emery, B., Tanner, N. K., and Linder, P. (2005) Characterization of the ATPase and unwinding activities of the yeast DEAD-box protein Has1p and the analysis of the roles of the conserved motifs. *Nucleic Acids Res. 33*, 999−1009.
(30) Yang, Q., and Jankowsky, E. (2006) The DEAD-box protein Ded1 unwinds RNA duplexes by a mode distinct from translocating helicases. *Nat. Struct. Mol. Biol. 13*, 981−986.
(31) Rogers, G. W., Jr., Lima, W. F., and Merrick, W. C. (2001) Further characterization of the helicase activity of eIF4A. Substrate specificity. *J. Biol. Chem. 276*, 12598−12608.
(32) Bizebard, T., Ferlenghi, I., Iost, I., and Dreyfus, M. (2004) Studies on three *E. coli* DEAD-box helicases point to an unwinding mechanism different from that of model DNA helicases. *Biochemistry 43*, 7857−7866.
(33) Yang, Q., Del Campo, M., Lambowitz, A. M., and Jankowsky, E. (2007) DEAD-box proteins unwind duplexes by local strand separation. *Mol. Cell 28*, 253−263.
(34) Del Campo, M., Tijerina, P., Bhaskaran, H., Mohr, S., Yang, Q., Jankowsky, E., Russell, R., and Lambowitz, A. M. (2007) Do DEAD-box proteins promote group II intron splicing without unwinding RNA? *Mol. Cell 28*, 159−166.
(35) Stocchi, V., Cucchiarini, L., Canestrari, F., Piacentini, M. P., and Fornaini, G. (1987) A very fast ion-pair reversed-phase HPLC method for the separation of the most significant nucleotides and their degradation products in human red blood cells. *Anal. Biochem. 167*, 181−190.
(36) Xia, T., SantaLucia, J., Jr., Burkard, M. E., Kierzek, R., Schroeder, S. J., Jiao, X., Cox, C., and Turner, D. H. (1998) Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs. *Biochemistry 37*, 14719−14735.
(37) Rossler, O. G., Straka, A., and Stahl, H. (2001) Rearrangement of structured RNA via branch migration structures catalysed by the highly related DEAD-box proteins p68 and p72. *Nucleic Acids Res. 29*, 2088−2096.
(38) Rogers, G. W., Jr., Richter, N. J., and Merrick, W. C. (1999) Biochemical and kinetic characterization of the RNA helicase activity of eukaryotic initiation factor 4A. *J. Biol. Chem. 274*, 12236−12244.
(39) Iost, I., Dreyfus, M., and Linder, P. (1999) Ded1p, a DEAD-box protein required for translation initiation in *Saccharomyces cerevisiae*, is an RNA helicase. *J. Biol. Chem. 274*, 17677−17683.
(40) Halls, C., Mohr, S., Del Campo, M., Yang, Q., Jankowsky, E., and Lambowitz, A. M. (2007) Involvement of DEAD-box proteins in group I and group II intron splicing. Biochemical characterization of Mss116p, ATP hydrolysis-dependent and -independent mechanisms, and general RNA chaperone activity. *J. Mol. Biol. 365*, 835−855.
(41) Delagoutte, E., and von Hippel, P. H. (2002) Helicase mechanisms and the coupling of helicases within macromolecular machines. Part I: Structures and properties of isolated helicases. *Q. Rev. Biophys. 35*, 431−748.
(42) Kossen, K., Karginov, F. V., and Uhlenbeck, O. C. (2002) The carboxy-terminal domain of the DExDH protein YxiN is sufficient to confer specificity for 23S rRNA. *J. Mol. Biol. 324*, 625−636.
(43) Henn, A., Cao, W., Licciardello, N., Heitkamp, S. E., Hackney, D. D., and de la Cruz, E. M. (2010) Pathway of ATP utilization and duplex rRNA unwinding by the DEAD-box helicase, DbpA. *Proc. Natl. Acad. Sci. U.S.A. 107*, 4046−4050.
(44) Chen, Y., Potratz, J. P., Tijerina, P., Del Campo, M., Lambowitz, A. M., and Russell, R. (2008) DEAD-box proteins can completely separate an RNA duplex using a single ATP. *Proc. Natl. Acad. Sci. U.S.A. 105*, 20203−20208.
(45) Liu, F., Putnam, A., and Jankowsky, E. (2008) ATP hydrolysis is required for DEAD-box protein recycling but not for duplex unwinding. *Proc. Natl. Acad. Sci. U.S.A. 105*, 20209−20214.
(46) Aregger, R., and Klostermeier, D. (2009) The DEAD box helicase YxiN maintains a closed conformation during ATP hydrolysis. *Biochemistry 48*, 10679−10681.
(47) Jarmoskaite, I., and Russell, R. (2011) DEAD-box proteins as RNA helicases and chaperones. *Wiley Interdiscip. Rev.: RNA 2*, 135−152.
(48) Caruthers, J. M., and McKay, D. B. (2002) Helicase structure and mechanism. *Curr. Opin. Struct. Biol. 12*, 123−133.
(49) Tuteja, N., and Tuteja, R. (2004) Unraveling DNA helicases. Motif, structure, mechanism and function. *Eur. J. Biochem. 271*, 1849−1863.
(50) Elles, L. M., and Uhlenbeck, O. C. (2008) Mutation of the arginine finger in the active site of *Escherichia coli* DbpA abolishes ATPase and helicase activity and confers a dominant slow growth phenotype. *Nucleic Acids Res. 36*, 41−50.
(51) Del Campo, M., Mohr, S., Jiang, Y., Jia, H., Jankowsky, E., and Lambowitz, A. M. (2009) Unwinding by local strand separation is critical for the function of DEAD-box proteins as RNA chaperones. *J. Mol. Biol. 389*, 674−693.
(52) Maeder, C., Kutach, A. K., and Guthrie, C. (2009) ATP-dependent unwinding of U4/U6 snRNAs by the Brr2 helicase requires the C terminus of Prp8. *Nat. Struct. Mol. Biol. 16*, 42−48.

(53) Bi, X., and Goss, D. J. (2000) Wheat germ poly(A)-binding protein increases the ATPase and the RNA helicase activity of translation initiation factors eIF4A, eIF4B, and eIF-iso4F. *J. Biol. Chem. 275*, 17740−17746.
(54) Rogers, G. W., Jr., Richter, N. J., Lima, W. F., and Merrick, W. C. (2001) Modulation of the helicase activity of eIF4A by eIF4B, eIF4H, and eIF4F. *J. Biol. Chem. 276*, 30914−30922.
(55) Granneman, S., Lin, C., Champion, E. A., Nandineni, M. R., Zorca, C., and Baserga, S. J. (2006) The nucleolar protein Esf2 interacts directly with the DExD/H box RNA helicase, Dbp8, to stimulate ATP hydrolysis. *Nucleic Acids Res. 34*, 3189−3199.
(56) Ozes, A. R., Feoktistova, K., Avanzino, B. C., and Fraser, C. S. (2011) Duplex unwinding and ATPase activities of the DEAD-box helicase eIF4A are coupled by eIF4G and eIF4B. *J. Mol. Biol. 412*, 674−687.
(57) Vanzo, N. F., Li, Y. S., Py, B., Blum, E., Higgins, C. F., Raynal, L. C., Krisch, H. M., and Carpousis, A. J. (1998) Ribonuclease E organizes the protein interactions in the *Escherichia coli* RNA degradosome. *Genes Dev. 12*, 2770−2781.
(58) Noble, C. G., and Song, H. (2007) MLN51 stimulates the RNA-helicase activity of eIF4AIII. *PLoS One 2*, e303.
(59) Shajani, Z., Sykes, M. T., and Williamson, J. R. (2011) Assembly of bacterial ribosomes. *Annu. Rev. Biochem. 80*, 501−526.
(60) Mohr, S., Stryker, J. M., and Lambowitz, A. M. (2002) A DEAD-box protein functions as an ATP-dependent RNA chaperone in group I intron splicing. *Cell 109*, 769−779.
(61) Huang, H. R., Rowe, C. E., Mohr, S., Jiang, Y., Lambowitz, A. M., and Perlman, P. S. (2005) The splicing of yeast mitochondrial group I and group II introns requires a DEAD-box protein with RNA chaperone function. *Proc. Natl. Acad. Sci. U.S.A. 102*, 163−168.
(62) Hoang, T., Peng, W. T., Vanrobays, E., Krogan, N., Hiley, S., Beyer, A. L., Osheim, Y. N., Greenblatt, J., Hughes, T. R., and Lafontaine, D. L. (2005) Esf2p, a U3-associated factor required for small-subunit processome assembly and compaction. *Mol. Cell. Biol. 25*, 5523−5534.
(63) Venema, J., and Tollervey, D. (1996) RRP5 is required for formation of both 18S and 5.8S rRNA in yeast. *EMBO J. 15*, 5701−5714.
(64) Trubetskoy, D., Proux, F., Allemand, F., Dreyfus, M., and Iost, I. (2009) SrmB, a DEAD-box RNA helicase involved in ribosome assembly in *E. coli*, is specifically targeted to 23S rRNA in vivo. *Nucleic Acids Res. 37*, 6540−6549.
(65) Diges, C. M., and Uhlenbeck, O. C. (2005) *Escherichia coli* DbpA is a 3′ → 5′ RNA helicase. *Biochemistry 44*, 7903−7911.
(66) Sengoku, T., Nureki, O., Nakamura, A., Kobayashi, S., and Yokoyama, S. (2006) Structural basis for RNA unwinding by the DEAD-box protein *Drosophila* Vasa. *Cell 125*, 287−300.
(67) Collins, R., Karlberg, T., Lehtio, L., Schutz, P., van den Berg, S., Dahlgren, L. G., Hammarstrom, M., Weigelt, J., and Schuler, H. (2009) The DEXD/H-box RNA helicase DDX19 is regulated by an α-helical switch. *J. Biol. Chem. 284*, 10296−10300.
(68) von Moeller, H., Basquin, C., and Conti, E. (2009) The mRNA export protein DBP5 binds RNA and the cytoplasmic nucleoporin NUP214 in a mutually exclusive manner. *Nat. Struct. Mol. Biol. 16*, 247−254.
(69) Del Campo, M., and Lambowitz, A. M. (2009) Structure of the yeast DEAD box protein Mss116p reveals two wedges that crimp RNA. *Mol. Cell 35*, 598−609.
(70) Andersen, C. B., Ballut, L., Johansen, J. S., Chamieh, H., Nielsen, K. H., Oliveira, C. L., Pedersen, J. S., Seraphin, B., Le Hir, H., and Andersen, G. R. (2006) Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA. *Science 313*, 1968−1972.
(71) Mohr, G., Del Campo, M., Mohr, S., Yang, Q., Jia, H., Jankowsky, E., and Lambowitz, A. M. (2008) Function of the C-terminal domain of the DEAD-box protein Mss116p analyzed *in vivo* and *in vitro*. *J. Mol. Biol. 375*, 1344−1364.
(72) Mallam, A. L., Jarmoskaite, I., Tijerina, P., Del Campo, M., Seifert, S., Guo, L., Russell, R., and Lambowitz, A. M. (2011) Solution structures of DEAD-box RNA chaperones reveal conformational changes and nucleic acid tethering by a basic tail. *Proc. Natl. Acad. Sci. U.S.A. 108*, 12254−12259.
(73) Henn, A., Shi, S. P., Zarivach, R., Ben-Zeev, E., and Sagi, I. (2002) The RNA helicase DbpA exhibits a markedly different conformation in the ADP-bound state when compared with the ATP- or RNA-bound states. *J. Biol. Chem. 277*, 46559−46565.
(74) Theissen, B., Karow, A. R., Kohler, J., Gubaev, A., and Klostermeier, D. (2008) Cooperative binding of ATP and RNA induces a closed conformation in a DEAD box RNA helicase. *Proc. Natl. Acad. Sci. U.S.A. 105*, 548−553.
(75) Lorsch, J. R., and Herschlag, D. (1998) The DEAD box protein eIF4A. 2. A cycle of nucleotide and RNA-dependent conformational changes. *Biochemistry 37*, 2194−2206.
(76) Li, L., Monckton, E. A., and Godbout, R. (2008) A role for DEAD box 1 at DNA double-strand breaks. *Mol. Cell. Biol. 28*, 6413−6425.
(77) Yang, Q., and Jankowsky, E. (2005) ATP- and ADP-dependent modulation of RNA unwinding and strand annealing activities by the DEAD-box protein DED1. *Biochemistry 44*, 13591−13601.