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Characterization of a DEAD box ATPase/RNA helicase protein of *Arabidopsis thaliana*

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ABSTRACT

We have isolated cDNAs encoding a novel member of the DEAD box RNA helicase family from *Arabidopsis*. The protein, named AtDRH1, is composed of 619 amino acids and the central portion has high similarity with the helicase core region of a prototypic RNA helicase, the human nuclear protein p68. The N- and C-terminal regions are considerably diverged from the animal and yeast p68 homologs at the amino acid sequence level, but like the p68 subfamily members, an RGG box-like domain is present near the C-terminus. RNA blot analysis showed that the AtDRH1 transcript accumulates at a high level and almost equally in every part of the *Arabidopsis* plant. The purified, recombinant AtDRH1 was capable of unwinding double-stranded RNA in the presence of ATP or dATP and of hydrolyzing ATP. The ATPase activity was stimulated by some single-stranded RNAs and DNAs, including poly(A) and poly(dT), but not by poly(dA). The ability of the polynucleotides to stimulate the ATPase activity was largely consistent with their affinity for AtDRH1. These results show that AtDRH1 is a novel type of ATP/dATP-dependent RNA helicase and polynucleotide-dependent ATPase.

INTRODUCTION

Structural modulation of RNA is fundamental to proper execution of a large number of intracellular processes, including mRNA maturation, ribosome assembly and translation and often involves a group of proteins, designated RNA helicases, that can unwind RNA:RNA and/or RNA:DNA duplexes (1,2). A large number of (putative) RNA helicases have been identified so far and they have been grouped into three families based on their amino acid sequences, i.e. DEAD, DEA/IH and DECH box families (1,3).

DEAD box proteins have been found in all the prokaryotes and eukaryotes examined thus far (2,3). They share a central core region with seven conserved motifs that are spaced similarly, while the N- and C-terminal regions of the core differ in both sequence and length among family members (4). DEAD box proteins are assumed to be ATP-dependent RNA helicases, based on the *in vitro* RNA helicase activity of the mouse translation initiation factor eIF-4A (5), the human nuclear protein p68 (6), *Xenopus* An3 (7) and *Drosophila* vasa (8). Mutational analyses of eIF-4A have suggested that its RNA unwinding activity depends upon the hydrolysis of ATP, while some *Escherichia coli* DEAD box proteins can destabilize duplex RNA without ATP hydrolysis (4,9,10).

The human p68 protein was first detected by its immunological cross-reaction with the SV40 large T antigen (11). It has been shown *in vitro* that p68 has an ATP-dependent RNA helicase activity (6) and an RNA-dependent ATPase activity (12). p68-related proteins (or genes) have been found in human (p68 and p72), mouse (p68), *Drosophila* (RM62), fission yeast (*dbp2*), budding yeast (*DBP2*) and tobacco (DB10) and they compose a p68 subfamily (13–17). The human p68 protein has been shown to undergo dramatic change in nuclear location during the cell cycle (12). The yeast genes *DBP2* and *dbp2* have been shown to be essential for normal growth (14,18). However, biological function of p68 subfamily proteins is still unclear.

Recently, we have identified a novel transcriptional activation domain containing the GCB (GBF-conserved box) motif in the wheat bZIP factor HALF-1 (19). In the course of searching for proteins interacting with the GCB motif by two-hybrid screening, we isolated a cDNA clone encoding an *Arabidopsis* p68 homolog, termed AtDRH1. Here, we show that AtDRH1 can unwind RNA in a dATP- as well as an ATP-dependent manner and that several single-stranded nucleic acids, including not only RNA species but DNA species such as poly(dT), can stimulate the ATPase activity of AtDRH1. Further, we note a correlation between the affinity of polynucleotides for AtDRH1 and their ability to stimulate the ATPase activity. Thus, the *Arabidopsis* p68 AtDRH1 possesses ATP/dATP-dependent RNA helicase and polynucleotide-dependent ATPase activities.

MATERIALS AND METHODS

cDNA cloning of AtDRH1

All the recombinant DNA techniques used were according to Sambrook *et al.* (20), unless otherwise specified. The *Eco*RI insert of pG4DD-E3 (19) was recloned into the *Eco*RI site of pGBT9 (Clontech) to create pGB-E3, which directs expression of the fusion protein of the GAL4 DNA binding domain (amino acids 1−147) and the E3 domain of HALF-1 (amino acids 203−256) under the control of the alcohol dehydrogenase I promoter. The yeast strain HF7c [MAT**a**, *ura3-52*, *his3-200*,

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lys2-801, *ade2-101*, *trp1-901*, *leu2-3*, *112*, *gal4-542*, *gal80-538*, *LYS2*::*GAL1-HIS3*, *URA3*::(*GAL4* 17mers)$_3$-*CYC1-lacZ*] carrying pGB-E3 was transformed with an *Arabidopsis* cDNA library (Clontech) as described by Rose *et al.* (21) and incubated at 30°C for 5 days. A primary yeast two-hybrid screening of 3×10^6 clones led to the isolation of 99 His^+ colonies and their β-galactosidase activities were measured. pGB-E3-dependent induction of β-galactosidase activity was confirmed with the yeast strain SFY526 [MAT**a**, *ura3-52*, *his3-200*, *lys2-801*, *ade2-101*, *trp1-901*, *leu2-3*, *112*, *can*^{r} *gal4-542*, *gal80-538*, *URA3*::*GAL1-lacZ*] and a positive clone, termed cA173, was selected for further analysis. An *Arabidopsis* cDNA library in λgt11 (Clontech) was screened with the cDNA insert of cA173 as a probe to isolate λ173-1, whose insert was further used to isolate λ173-15. All three clones were subcloned into pBluescript SK+ (pBSK-cA173, -173-1 and -173-15 respectively) and sequenced by the dideoxy method. pBSK-173full, carrying the whole coding region for AtDRH1, was generated by using restriction enzyme sites, *Bsg*I and *Bgl*II, which were located in the overlapping regions between cA173 and λ173-1 and between λ173-1 and λ173-15, respectively.

Overexpression and purification of His-AtDRH1

The *Eco*RI insert of pBSK-173full was recloned into pET-28a (Novagen) to generate pET-AtDRH1. The His-tagged AtDRH1 protein (His-AtDRH1) was expressed in BL21(DE3)pLysS transformed with pET-AtDRH1 and purified as described previously (19). The bacterial pellet was lysed in 0.1 M Tris−HCl (pH 7.5), 0.6 M NaCl, 14 mM β-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride (PMSF) by sonication and insoluble materials were removed by centrifugation. The supernatant was loaded onto a HiTrap Chelating column (Pharmacia) chelated with Ni^{2+}. After washing sequentially with 0.1 M Tris−HCl (pH 7.5), 0.6 M NaCl and 10 mM imidazole and with the same buffer containing 40 mM imidazole, His-AtDRH1 was eluted with 50 mM Tris−HCl (pH 7.5), 50 mM NaCl, 14 mM β-mercaptoethanol, 200 mM imidazole and 50% glycerol. The purified protein was stored at −20°C.

RNA blot analysis

RNA was extracted from 7-day-old seedlings cultured in a modified Murashige–Skoog medium (22) using TRIZOL LS reagents (Gibco BRL) according to the supplier's recommendations. RNA blotting was performed essentially as described by Sambrook *et al.* (20). Briefly, 10 µg of the total RNA was fractionated on a formaldehyde-containing 1% agarose gel, stained with ethidium bromide, photographed and transferred onto the Hybond-N filter (Amersham). In some experiments, 20 µg of RNA were denatured with 1.1 M glyoxal and electrophoresed. The DNA probe was prepared by labeling the *Eco*RI insert of cA173 using the Megaprime labeling kit (Amersham).

Partially double-stranded RNA

A synthetic double-stranded DNA fragment containing a promoter sequence for T7 RNA polymerase was prepared by annealing 5′ phosphorylated oligonucleotides, 5′-pAGCTTAAATACGACTCACTATAGGGCGAGAGGATCC-3′ and 5′-pGGATCCTCTCGCCCTATAGTGAGTCGTATTTA-3′, and inserting the duplex between the *Hin*dIII and *Sma*I sites of pUC19 to create pUC-T7p. Similarly, two oligonucleotides, 5′-GATCCGTATTCTATAGTGTCACCTAAATG-3′ and 5′-AATTCATTTAGGTGACACTATAGAATACG-3′, were phosphorylated, annealed and inserted between the *Bam*HI and *Eco*RI sites of pUC19 to create pUC-SP6p, which carries a promoter for SP6 RNA polymerase. pUC-T7p and pUC-SP6p were linearized with *Eco*RI and *Hin*dIII respectively and transcribed with T7 or SP6 RNA polymerase (TaKaRa) according to the supplier's instruction. When labeling, 20 µM [α-^{32}P]UTP (100 Ci/mmol) was included in the reaction. After removal of free nucleotides by passing twice through a ProbeQuant G-50 Micro Column (Pharmacia), transcripts from pUC-T7p and -SP6p were mixed in 10 mM Tris−HCl (pH 7.5) and 100 mM NaCl and incubated sequentially at 80°C for 10 min and at 45°C for 3 h to obtain partially double-stranded RNA.

RNA unwinding assay

The RNA unwinding assay was carried out by the methods of Hirling *et al.* (6) and Lee and Hurwitz (23) with slight modifications. Partially double-stranded RNA (0.1 pmol) was mixed with 20 ng (0.25 pmol) of His-AtDRH1 in 20 µl of helicase/ATPase buffer [30 mM Tris−HCl (pH 7.5), 8 mM $MgCl_2$, 100 mM NaCl, 15 mM DTT, 30 µg/ml BSA, 0.5 U/µl RNasin (TaKaRa)] in the presence or absence of 2 mM (d)NTP. After incubation at 30°C for 30 min, reactions were stopped by adding 5 µl of 2% SDS, 0.1 M EDTA (pH 8.0), 0.1% Nonidet P-40 and 10% Ficoll 400. An aliquot (15 µl) of each reaction was loaded onto a 0.1% SDS−15% polyacrylamide (29:1) gel, electrophoresed at 20 mA with 0.5× TBE containing 0.1% SDS, then autoradiographed.

ATPase assay

ATPase activity was measured by an activated charcoal method (12). Briefly, His-AtDRH1 (40 ng) was incubated in 40 µl of helicase/ATPase buffer containing 0.1 or 0.2 mM [γ-^{32}P]ATP (1 Ci/mmol) in the presence or absence of 30 ng/µl synthetic polynucleotides (Pharmacia) at 30°C for 10−60 min. An aliquot (5 µl) was removed at appropriate intervals and added into 200 µl of a solution containing 50 mM HCl, 5 mM H_3PO_4 and 7% activated charcoal. After the charcoal was precipitated by centrifugation to remove unreacted ATP, 10 µl of the supernatant was subjected to Cerencov counting to quantitate released [^{32}P]phosphate.

Electrophoretic mobility shift assay (EMSA)

The RNA binding reaction was carried out in helicase/ATPase buffer containing 0.1 pmol of ^{32}P-labeled RNA and various amounts of His-AtDRH1 in a final volume of 20 µl. Where indicated, 150 ng of a polynucleotide was added as a competitor. The mixture was incubated at 30°C for 60 min, then stopped by adding 5 µl of 0.1 M Tris−HCl (pH 7.5) and 50% glycerol. An aliquot (15 µl) of each reaction was loaded onto a 5% polyacrylamide (29:1) gel containing 5% glycerol and electrophoresed at 20 mA with 0.5× TBE. The RNA−protein complexes were visualized by autoradiography.

RESULTS

Cloning of the AtDRH1 cDNA

Yeast two-hybrid screening of an *Arabidopsis* cDNA library with an activation domain (E3) of HALF-1 as bait led to the isolation

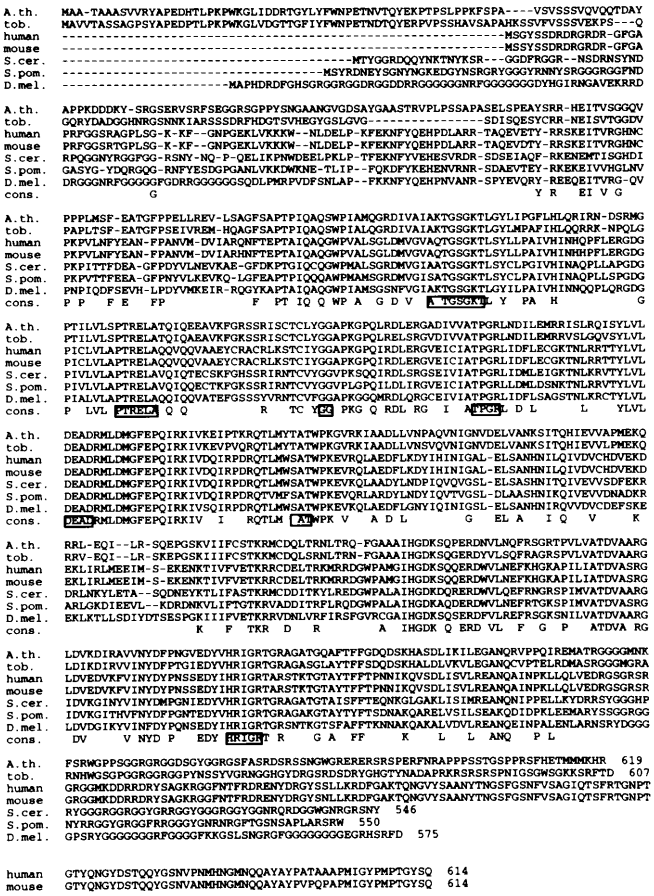

Figure 1. Alignment of the deduced amino acid sequences of *A.thaliana* AtDRH1 (A.th.), tobacco DB10 (tob.), human and mouse p68, *Saccharomyces cerevisiae* (S.cer.) and *Schizosaccharomyces pombe* (S.pom.) Dbp2p and *D.melanogaster* RM62 (D.mel.). Amino acids identical among at least six proteins are shown in the consensus line (cons.), in which seven conserved helicase motifs are boxed. The amino acid length of each protein is indicated at the end.

of a clone termed cA173. The cDNA insert of cA173 was ~0.7 kb in length and had an open reading frame (ORF) encoding a polypeptide highly similar to the N-terminal portion of tobacco DB10 (15). Since cA173 was thought to be derived from the 5′-part of the mRNA, further screening of a λgt11 library was performed and, consequently, two cDNA clones, λ173-1 and λ173-15, were isolated. The nucleotide sequence assembled from the three cDNAs revealed an ORF for a protein of 619 amino acids (Fig. 1). Southern and northern blot analyses indicated that a single species of mRNA was produced from a single copy gene (Fig. 2 and not shown).

The deduced protein had high similarity with p68-type DEAD box proteins, including tobacco DB10 (Fig. 1). This *Arabidopsis* protein was named AtDRH1 (*Arabidopsis thaliana* DEAD box RNA helicase 1), since it was capable of unwinding RNA (detailed later).

As shown in Figure 1, seven highly conserved amino acid motifs characterizing the DEAD box proteins are all present in the middle region of AtDRH1 (amino acids 133–532). In the SAT (Ser-Ala-Thr) motif, however, the first Ser residue was changed for Thr in AtDRH1. The resulting sequence Thr-Ala-Thr is the same as the consensus motif located at the corresponding position in the DEA/IH family proteins (3). The C-terminal region (amino

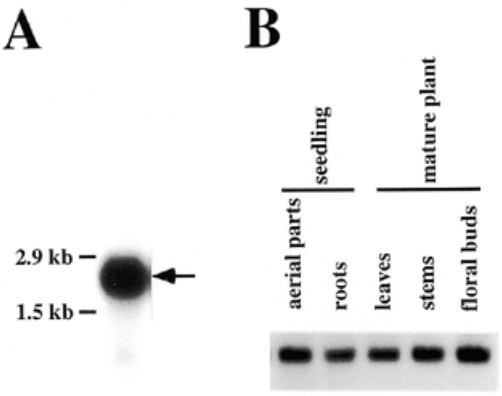

Figure 2. Expression of the AtDRH1 mRNA. (**A**) Northern hybridization of total RNA extracted from *Arabidopsis* seedlings cultured in a modified Murashige–Skoog medium. Ten micrograms of total RNA were fractionated on a 2.1 M formaldehyde–1% agarose gel and hybridization was performed using the cA173 insert as a probe. A single strongly hybridizable transcript of ~2.3 kb in length was detected. Positions of rRNAs are indicated at the left (2.9 and 1.5 kb). (**B**) Total RNAs (20 µg) prepared from aerial parts and roots of seedlings and leaves, stems and floral buds of mature plants were denatured with 1.1 M glyoxal and electrophoresed on a 1% agarose gel.

acids 538–609) is rich in Gly, Arg and Ser (45 residues out of 72). Interruption of Gly-richness by Arg or aromatic residues such as Tyr and Phe and the presence of multiple RGG motifs suggest that this region is a variant of the RGG box, a motif involved in RNA binding (24). The N-terminal region (amino acids 1–67) has very high similarity to DB10 (~90% similarity). This N-terminal portion seems to be specific for plant p68-type proteins, because animal and yeast p68-type DEAD box proteins lack the corresponding region.

Expression of AtDRH1 mRNA

RNA blot analysis gave a single, strongly hybridizing band of a transcript of ~2.3 kb in length in the total RNA extracted from 7-day-old *Arabidopsis* seedlings cultured in a liquid medium (Fig. 2A). The expression level was hardly affected by ABA, NaCl and cold treatments (data not shown). The same size of mRNA was detected in the total RNA from roots and aerial parts of seedlings and the leaves, stems and floral buds of mature plants (Fig. 2B). This is in contrast to the observation that in p68 and p72, two transcripts are differently expressed in different tissues (16,17). Since almost the same amount of the mRNA relative to the total RNA was detected in all the tissues examined, expression of the AtDRH1 mRNA is constitutive and the AtDRH1 protein seems to play a role in a basic activity of cells.

RNA unwinding activity of AtDRH1

Only a few members of the DEAD box family have been characterized at the protein level. To determine the characteristics of AtDRH1, a His-tagged protein (His-AtDRH1) was expressed in *E.coli* and affinity purified (Fig. 3). First, an RNA unwinding activity was analyzed using a partially double-stranded RNA as substrate (Fig. 4). The substrate RNA was stable at 30 °C during the reaction (Fig. 4B, lane 1) and heat denaturation produced a faster migrating, single-stranded RNA (Fig. 4B, lane 2). This single-stranded RNA was also generated when the substrate was incubated with His-AtDRH1 in the presence of ATP or dATP (Fig. 4B, lanes 5

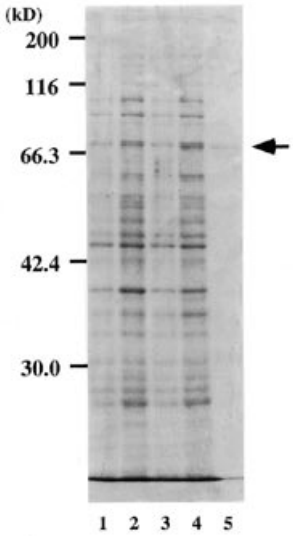

Figure 3. Purification of the His-tagged AtDRH1 protein. Bacterially expressed His-AtDRH1 was purified using a Ni^{2+}–Sepharose resin. Total proteins from *E.coli* BL21(DE3)pLysS carrying pET-28b (lanes 1 and 2) or pET-AtDRH1 (lanes 3 and 4) before (lanes 1 and 3) or after (lanes 2 and 4) 2-h induction with IPTG and purified His-AtDRH1 (lane 5) were separated by SDS−10% PAGE and visualized by Coomassie blue staining. An arrow indicates the position of His-AtDRH1. Positions of molecular mass markers are shown at the left.

and 9), indicating that His-AtDRH1 is capable of unwinding duplex RNA. Such strand displacement by His-AtDRH1 was not detected in the absence of (d)NTPs (Fig. 4B, lane 3) or with any (d)NTP other than (d)ATP (Fig. 4B, lanes 4, 6−8, 10 and 11). Therefore, AtDRH1 is an ATP/dATP-dependent RNA helicase. A divalent cation is required for the unwinding activity because the reaction was blocked by adding EDTA (Fig. 4B, lane 12).

ATPase activity of AtDRH1

Most but not all of the DEAD box proteins have an ATPase activity. To examine the ATPase activity of AtDRH1, [γ-^{32}P]ATP was incubated together with His-AtDRH1 and release of [^{32}P]phosphate was measured by an activated charcoal method (12). As shown in Figure 5A, His-AtDRH1 exhibited an ATPase activity, which was stimulated ~8-fold by including poly(A) in the reaction (Fig. 5B). In contrast, no stimulation was observed with poly(dA) (Fig. 5).

We further examined the effect of other commercially available polynucleotides on the ATPase activity. As shown in Table 1, all the polynucleotides tested, besides poly(dA), stimulated the ATPase activity of His-AtDRH1. A high level of stimulation was observed with poly(A) and also with poly(dT). Poly(U) and poly(dC) stimulated the ATPase activity moderately. The effect of poly(C) was low. These results indicate that ATPase activity can be stimulated by both RNA and DNA species and, therefore, AtDRH1 is also a polynucleotide-dependent ATPase.

Nucleic acid binding activity of AtDRH1

To determine whether stimulation of the ATPase activity of AtDRH1 by polynucleotides is related to its ability to bind to the corresponding polynucleotides, EMSA was performed with the ^{32}P-labeled, partially double-stranded RNA which had been used in the RNA unwinding assay (see Fig. 4A). As shown in Figure 6,

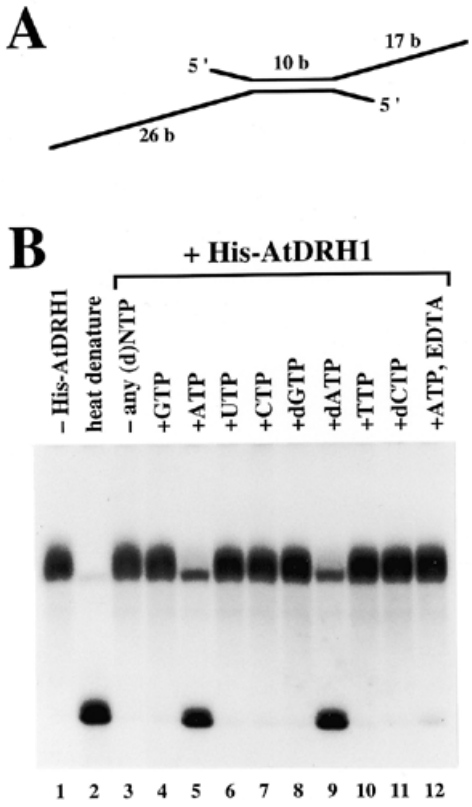

Figure 4. RNA helicase activity of AtDRH1. (**A**) Schematic representation of the partially double-stranded RNA substrate. The RNA substrate contains a 10-bp duplex region, 17- and 26-nt-long single-strand regions at the 3′-ends and 5-nt-long single-strand regions at the 5′-ends. The top strand was radiolabeled (^{32}P). (**B**) RNA unwinding activity of AtDRH1. The RNA substrate was incubated with His-AtDRH1 in the presence or absence of various (d)NTPs (lanes 4−12). The products of the reaction were separated by SDS−15% PAGE and the radiolabeled strand was visualized by autoradiography. Lane 1, the substrate RNA incubated under the same condition without AtDRH1; lane 2, the substrate incubated for 10 min at 90°C; lane 3, reaction in the absence of (d)NTP; lane 12, reaction in the presence of 25 mM EDTA.

when an increasing amount of His-AtDRH1 was incubated with the labeled RNA probe, slowly migrating bands appeared, indicating the formation of AtDRH1−RNA complexes (lanes 2−4). The formation of these complexes was effectively inhibited by an excess amount of unlabeled poly(A), poly(U) and poly(dT) (lanes 5, 6 and 9), slightly by poly(dC) (lane 10) and little, if any, by poly(C) and poly(dA) (lanes 7 and 8). Such binding preference was nearly consistent with the effect of each polynucleotide on stimulation of the ATPase activity (Table 1). This suggests that the ATPase activity of AtDRH1 depends upon its binding to a polynucleotide.

DISCUSSION

We have isolated *Arabidopsis* cDNAs encoding AtDRH1, a new member of the DEAD box protein family. Amino acid sequence similarity of the central helicase core region, together with the presence of RGG repeats near the C-terminus, indicated that AtDRH1 belongs to the p68 subfamily. Biochemical analyses with purified, recombinant AtDRH1 revealed that it can function as an ATP/dATP-dependent RNA helicase and a polynucleotide-dependent ATPase.

Table 1. Stimulation of the ATPase activity of AtDRH1 by polynucleotides

Polynucleotide added	ATP hydrolysis (c.p.m.)[a]	Relative ATP hydrolysis (%)[b]
None	722	17
Poly(A)	4235	100
Poly(U)	2537	60
Poly(C)	1837	43
Poly(dA)	1118	26
Poly(dT)	3862	91
Poly(dC)	3275	77

[a]ATP hydrolysis was measured as release of free [^{32}P]phosphate. The reaction was carried out at 30°C for 20 min in the presence of 0.2 mM [γ-^{32}P]ATP (1 Ci/mmol). Background counts due to spontaneous ATP hydrolysis, obtained from the reaction without His-AtDRH1, had been deducted. Counts presented correspond to an aliquot (0.5 µl) of the reaction containing 0.5 ng His-AtDRH1.
[b]The ATPase activity in the reaction containing poly(A) was set at 100%.

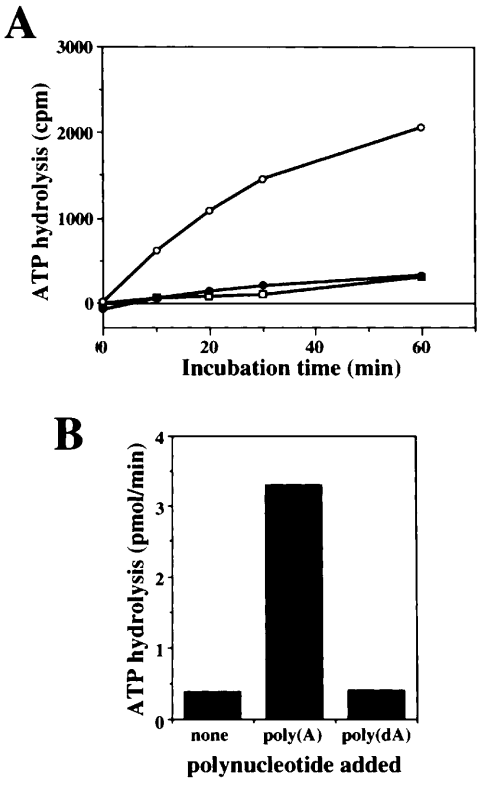

Figure 5. ATPase activity of AtDRH1. (**A**) Time course of the ATP hydrolysis. His-AtDRH1 was incubated with 0.1 mM [γ-^{32}P]ATP (1 Ci/mmol). An aliquot was removed from the reaction mixture at the time indicated and added to a 7% activated charcoal solution. ATP hydrolysis was measured as release of free [^{32}P]phosphate. Background counts due to spontaneous ATP hydrolysis had been deducted. Reactions were performed in the presence of poly(A) (open circles) or poly(dA) (closed circles) or in the absence of polynucleotides (open squares). (**B**) The rate of ATP hydrolysis. The initial rate of ATP hydrolysis was calculated based on the results shown in (A).

Whereas a large number of DEAD box proteins have been identified, only a few have been demonstrated to have RNA helicase activity *in vitro*, e.g. human p68, mouse eIF-4A, *Xenopus* An3 and Xp54, *Drosophila* vasa and *E.coli* DbpA and CsdA/DeaD (5–10,25,26). In analyses of some DEAD proteins, the failure to detect helicase activity might be ascribed to the lack of other helper components or a suitable RNA substrate in the reaction, as discussed for the case of p72, a p68-related human protein (16). Actually, eIF-4A requires another factor, eIF-4B, to fulfil the RNA helicase activity (27). Our observation that the purified AtDRH1 exhibited a strong RNA helicase activity indicates that this protein can unwind duplex RNA by itself.

The activity of AtDRH1 in binding to partially duplex RNA was clearly shown by EMSA (Fig. 6). The binding was effectively inhibited by some poly(deoxy)ribonucleotides such as poly(dT) and poly(A). This may suggest that AtDRH1 binds to partially duplex RNA and polynucleotide competitors at the same, or a closely located, site on the molecule. Since the AtDRH1–RNA interaction occurred in the absence of ATP and since the ATPase activity was stimulated more effectively by polynucleotides with higher affinity for AtDRH1, irrespective of whether they have a duplex region, the ATPase activity appears to depend upon the binding of a single-stranded region of polynucleotides. Taking into account that (d)ATP is required for the helicase activity of AtDRH1, it is supposed that the unwinding reaction would proceed in the order of RNA binding, ATP hydrolysis and release of the destabilized molecule. It has been shown that RNA helicase A consumes ATP at the step of RNA release (23) and that *E.coli* DEAD box proteins, DbpA and DeaD, have RNA-dependent ATPase activity but can destabilize duplex RNA without ATP (9,10). Further work is needed to determine whether ATP hydrolysis is required at the step where AtDRH1 unwinds duplex RNA or where single-stranded, unwound molecules are released from the protein, or both.

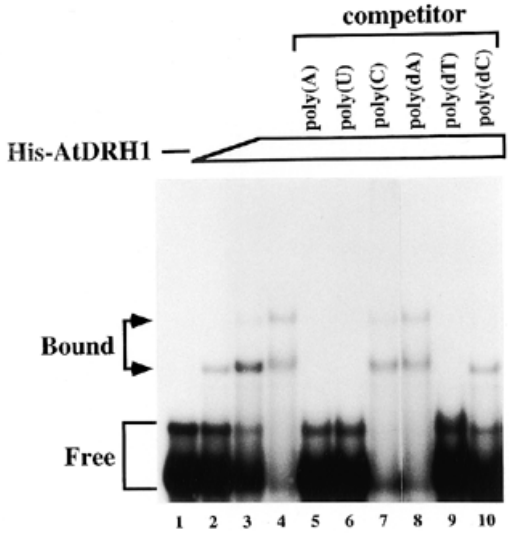

Figure 6. Nucleic acid binding of AtDRH1. The partially double-stranded RNA (see Fig. 4A) was incubated alone (lane 1) or together with 5, 10 or 20 ng His-AtDRH1 (lanes 2–4). Two slowly migrating complexes were detected at higher concentrations of His-AtDRH1 (lanes 3 and 4), which might be due to binding of His-AtDRH1 to both ends of the probe. Lanes 5–10, synthetic polynucleotides, indicated above each lane, were included in the reaction containing 20 ng His-AtDRH1.

The involvement of the RGG box in non-sequence-specific RNA binding has been demonstrated for the hnRNP U protein, nucleolin and Epstein-Barr nuclear antigen 1 (24,28,29). Recently, the RGG repeat-containing GYR domain of a spinach DEAD box protein, PRH75, has also been shown to be capable of binding to RNA (30). Therefore, AtDRH1 is likely to interact with RNA through the C-terminal region containing RGG repeats. However, it is unclear whether a preference for some nucleic acids that AtDRH1 exhibited can be ascribed solely to the C-terminal region. eIF-4A, almost composed of the helicase core region, still has a weak ATP-dependent, RNA binding activity even in the absence of its RNA binding partner eIF-4B (4). Some PRH75-derived deletion mutants lacking the GYR domain still retain a weak RNA binding activity (30). In these cases, the HRIGRXXR motif in the helicase core region has been implicated in binding to RNA (30,31). Therefore, the helicase core region of AtDRH1 may also be involved in establishing specific RNA−protein interaction *in vivo*.

p68-type DEAD box proteins have been found in a variety of eukaryotes, including yeast, *Drosophila*, vertebrates and plants. However, the biological role of this conserved protein family has not yet been elucidated. Human p68 has been supposed to be involved in nucleolar assembly, from the observation of its transient association with the nucleoli at the time when prenucleolar bodies are condensing after mitosis (14). In *E.coli*, p68 can stabilize inefficiently translated mRNAs like DeaD and SrmB (32). Yeast genes *DBP2* and *dbp2* are essential in normal growth and a *DBP2* null mutation can be rescued by p68 (14,18). Recently, Dbp2p has been identified by two-hybrid screening with Upf1p as bait (33). Since *UPF1* is a gene required for nonsense-mediated mRNA decay (34), it is possible that *DBP2* is involved in degradation of specific RNAs. Thus, p68-type DEAD box proteins may be multifunctional or their function may not have been conserved during evolution.

In relation to these presumptions, the N- and C-terminal regions of these family members are considerably diverged at the sequence level (Fig. 1). These regions seem to be important in determining RNA substrates, which must be closely related to the biological function of the respective proteins. Plant proteins, AtDRH1 and DB10, have a unique conserved region in the N-terminus. Only the human and mouse p68 proteins have an IQ domain near the C-terminus, which is known to be the overlapping site for calmodulin binding and protein kinase C phosphorylation (35). Yeast and *Drosophila* proteins have extra RGG repeats near the N-terminus. Unlike p68 and p72, which have two transcripts each expressed tissue-specifically in a different manner (16,17), a single species of AtDRH1 mRNA was detected abundantly and constitutively in all the tissues examined (Fig. 2B), irrespective of culture conditions, suggesting that AtDRH1 plays a role in a basic activity of plant cells. Biochemical analyses done in this work will be a help in future studies searching for *in vivo* substrates, which will lead to elucidation of the biological function of the p68-type plant DEAD box protein AtDRH1.

As described earlier, the AtDRH1 cDNA clone was obtained during a two-hybrid screening performed with the intention of finding proteins interacting with the transcriptional activation domain of HALF-1. Thus, when considering a possible function of AtDRH1, we cannot ignore a recent interesting report showing that RNA helicase A, a DEAH box protein with RGG motifs near the C-terminus, mediates the functional interaction between the transcriptional coactivator CBP and RNA polymerase II (36).

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