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Cloning and characterization of a DEAD box RNA helicase from the viable seedlings of aged mung bean

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Abstract

Seeds stored under adverse conditions will reduce the viability of germination as a result of induced aging. We have established a procedure to induce accelerated aging for studying the process of aging in mung bean (*Vigna radiata*) seeds at the molecular level. A full-length cDNA was isolated from acceleratedly aged mung bean seedlings. The cDNA, *VrRH1* (*Vigna radiata* RNA helicase 1), contains an open reading frame of 2139 bp encoding a protein of 713 amino acids. *VrRH1* has seven highly conserved motifs including the DEAD box as in the case of other plant RNA helicases. *VrRH1* was sub-cloned into an expression vector pET-28b (+), over-expressed in *Escherichia coli* BL 21 and purified by a Ni^{2+}-agarose column. The expressed protein showed double-stranded RNA unwinding and ATPase activities. Either ATP or dATP is required for the unwinding activity, indicating that *VrRH1* is an ATP/dATP-dependent RNA helicase. Northern blot analysis showed the presence of mRNAs hybridized with a full-length cDNA fragment of *VrRH1* (V*rRH* transcripts) in mung bean seeds that were imbibed for 16 to 32 h after accelerated aging treatment. The amount of these mRNAs reached a maximum in 24 h imbibed seeds after the treatment. The accumulation of *VrRH* transcripts was shown to lead to the appearance of 25S and 18S rRNAs in the imbibed aging mung bean seeds. The results suggest that *VrRH1* may play a role in the viability of mung bean seeds.

Abbreviations: AGI, *Arabidopsis thaliana* Genome Institute; An3, animal clone 3; AtDRH1, *Arabidopsis thaliana* DEAD box RNA helicase 1; CsdA, cold shock DEAD-box protein A of *E. coli*; DbpA, DEAD-box protein A of *E. coli*; PPVCI, plum pox virus cylindrical inclusion; PRH75, plant RNA helicase 75; VrRH1, *Vigna radiata* RNA helicase 1; *VrRH1*, VrRH1 cDNA; Xp54, *Xenopus* particles 54

Introduction

RNA helicases catalyze the local unwinding of complex RNA structures which play an important role in biological systems (Schmid and Linder, 1992; Fuller-Pace, 1994; de la Cruz *et al.*, 1999). Large numbers of putative helicases have been identified since the nuclear protein p68 was shown to have high sequence homology to the eukaryotic translation initiation factor eIF-4A, an established RNA helicase (Ford *et al.*, 1988). Helicases, including DNA and RNA helicases, are grouped into two major superfamilies (superfamilies I and II) based on the occurrence of seven conserved motifs (Gorbalenya and Koonin, 1993). Most RNA helicases belong to superfamily II and can be further classified into families (DEAD-box proteins, DEAH-box proteins and DExH-box proteins) on the basis of consensus sequences in the motif II of the seven conserved motifs (de la Cruz *et al.*, 1999). The putative RNA helicases of superfamily II found in a wide variety of organisms including bacteria, viruses, yeasts, plants, and mammals are involved in very

diverse biological functions, such as pre-mRNA splicing, rRNA processing, and mRNA export, translation and decay. Although they are all considered to be putative RNA helicases, only a few of them have been shown to exhibit ATP-dependent RNA helicase activity (Okanami *et al.*, 1998; Iost *et al.*, 1999). In contrast to the helicase-core region, which contains the conserved motifs, most RNA helicases have very different N- and/or C-terminal extensions that might confer substrate specificity and/or define sequences required for interaction with other protein and subcellular localization (Wang and Guthrie, 1998; Aubourg *et al.*, 1999).

The numerous genomic sequences and expressed sequence tags identified by the *Arabidopsis thaliana* Genome Institute (AGI) have allowed a systematic and functional study of the DEAD-box RNA helicase family. Sequence comparisons associated with expression study led to the characterization of 28 novel *A. thaliana* DEAD-box RNA helicases forming a family of 32 members, named AtRH (Aubourg *et al.*, 1999). With the availability of the complete sequence of the *Saccharomyces cerevisiae* genome, the entire family of putative RNA helicases in this eukaryotic model organism has been described. Based on sequence comparisons, the yeast genome contains 27 DEAD-box proteins and 13 DexH proteins (Linder *et al.*, 2000). A number of plant DEAD-box RNA helicases have been described with tobacco (Owttrim *et al.*, 1992; Itadani *et al.*, 1994; Owttrim *et al.*, 1994; Brander and Kuhlemeier, 1995), *A. thaliana* (Metz *et al.*, 1992; Lorkovic *et al.*, 1997; Okanami *et al.*, 1998; Gagliardi *et al.*, 1999) and spinach (Lorkovic *et al.*, 1997). The *Arabidopsis* DEAD box AtDRH1 protein was shown to exhibit ATP/dATP-dependent RNA helicase activity (Okanami *et al.*, 1998).

Embryo viability and germinability of seeds decrease during storage, particularly under adverse conditions. The two most important environmental factors influencing seed longevity are ambient temperature and seed moisture (Osborne, 1980; Buchvarov and Gantcheff, 1984; Puntarulo and Boveris, 1990). To date, mechanisms underlying the loss of viability and vigor are not fully understood. By comparing the differential gene expressions between aged seeds and non-aged seeds it may be possible to identify genes associated with aging of seeds. We used a mRNA differential display technique to isolate differentially expressed cDNAs from acceleratedly aged versus non-aged seedlings. Here we report the isolation and characterization of a cDNA named *VrRH1* encoding RNA helicase from 12 h imbibed seeds that were aged for 9 days at 50 °C and 45% relative humidity.

Materials and methods

Preparation of mung bean seeds and seedlings aged in an accelerated manner

Mung bean seeds (*Vigna radiata* VC 3890 sel. 5) in closed bottles were put in a 50 °C/45% relative humidity (RH) incubator. The mung bean samples were taken at various time intervals and examined for their germination rate and water content. 50 °C and 45% RH were chosen for preparation of mung bean seeds aged in an accelerated fashion because the seeds treated under these conditions exhibited a sigmoidal pattern of germination with the passage of time comparable to the theoretical natural aging curve (Buchvarov and Gantcheff, 1984), and because the water content of these seeds remained essentially the same (11.5%) for up to 15 days (data not shown). The acceleratedly aged seeds under these conditions for 9 days showed 37% germination rate (untreated seeds had a 98–99% germination rate).

The duration in days of aging is abbreviated as 'd', and the duration of imbibition in h as 'h'. Thus seeds aged for 9 days followed by imbibition for 12 h are designated 9-d-12-h. When 9-day aged seeds were imbibed for 12 h, these 9-d-12-h seedlings could be roughly divided into three groups: Group 1, axis $\geq$0.5 cm (9-d-12-h seedlings with axis $\geq$0.5 cm); Group 2, axis <0.5 cm (9-d-12-h seedlings with axis <0.5 cm); and Group 3, without emergence of axis (9-d-12-h seedlings without axis). We found that only Group 1 seedlings could continue to grow and develop into mature plants (viable), whereas both Groups 2 and 3 were non-viable. Since we failed to isolate differentially expressed cDNA fragments from 9-day aged seeds versus control seeds, Group 1 seedlings were used to isolate differentially expressed cDNAs against non-aged seedlings.

cDNA cloning of a full-length VrRH1

To generate differentially expressed cDNAs from the 9-day aged seeds, total RNAs from 9-day aged and non-aged seeds both of which imbibed in water for 12 h were extracted by the hot phenol method (Shirzadegan *et al.*, 1991). The total RNAs were purified by an oligo-dT column and 1 μg of poly(A)$^+$ RNA was used for reversed transcription (Superscript

II RT, BRL) using random hexamers as primers. Differentially expressed cDNA fragments were amplified by PCR with arbitrary but defined sequences as primers (Sokolov and Prockop, 1994). The following 8 primers were individually used in each PCR reaction: P1, AGGCTTCCATGGAAAATGGG; P2, CGTGGCATCGCGTGGAGCGAAGC; P3, GGCAGCTGCGGTTCAAAC; P4, GCCAGAAGCACTCCAACCCG; P5, GTCAAGCCGCTGTGGGACAG; P6, TTTAACATTCATATCCATT; P7, TCTGCTACAAACCCTGCAAG; P8, GCTGTGATCTCTGCATGTGC. As a result, a 1.2 kb DNA fragment was obtained with the P7 primer. Two terminal-specific primers', 5′-CCTTACGCCGGCATCACCAT-3′ and 5′-ACCTAAACTTAAGCTTCCCCCATCAAAAAG-3′) were used for cloning a full-length cDNA based on the 1.2 kb DNA fragment by 5′/3′ RACE. A full-length cDNA, named *VrRH1*, was obtained and sub-cloned into pGEM T-easy vector (Promega). The vector harboring *VrRH1* was named pVrRH1 and subjected to DNA sequencing to verify the identity of *VrRH1*. The nucleotide sequence reported in this paper has been registered in the GenBank nucleotide sequence databases under the accession number AF156667 (Li and Chen, 2000).

Construction of expression vector pVrRH1EN

Two oligonucleotide primers were synthesized: VrRHF (5′-CTTACGCCGGGAATTCCATGGCCT-3′) and VrRHR 3′-CCCCACCTTTTTCTACCCGCCGGCG-5′. VrRHF has a sense orientation and an *Eco*RI site whereas VrRHR has an antisense orientation and a *Not*I site. PCR was performed with pVrRH1 as template. The sequence of PCR-amplified product was confirmed by nucleotide sequencing. The amplified product and pGEM-T plasmid, which has *Eco*RI and *Not*I sites on its multiple cloning sites, were digested with *Eco*RI and *Not*I. The two digestion mixtures were combined and ligated with T4 DNA ligase (Promega). Positive clones were screened with colony PCR, examined for their correct cutting sites with *Eco*RI and *Not*I and verified by DNA sequencing. The genuine construct thus obtained was named pVrRH1EN.

Expression and purification of VrRH1

The *VrRH1* insert in pVrRH1EN was subcloned into pET-28b(+) (Novagen) to generate pET-VrRH1 containing His-tagged VrRH1. *E. coli* BL21 (DE3) was transformed with pET-VrRH1 and the over-expressed His-tagged VrRH1 protein (His-VrRH1) was purified as previously described by Okanami *et al.* (1999) with modification. The bacterial pellet was lysed in 50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 14 mM 2-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride (PMSF) by sonication and insoluble materials were removed by centrifugation. The supernatant was loaded onto a Ni^{2+}-agarose column (Qiagen). After washing sequentially with 50 mM Tris-HCl pH 7.5, 0.2 M NaCl and 5 mM imidazole and with the same buffer containing 30 mM imidazole, His-VrRH1 was eluted with 50 mM Tris-HCl pH 7.5, 20 mM NaCl, 14 mM 2-mercaptoethanol, 300 mM imidazole, and 50% glycerol. The purified protein was dialyzed against 10 mM Tris-HCl buffer solution (pH 7.5) and stored at −70 °C.

Preparation of double-stranded RNA

Double-stranded RNA (dsRNA) was prepared according to the previously described method (Tai *et al.*, 1996) and used as a substrate for RNA helicase. A pGEM4 and pGEM3 vector was linearized by *Xba*I and *Pvu*II and used for *in vitro* transcription to produce transcripts of 41 nt and 104 nt, respectively. The transcripts were treated with DNase to remove template DNA, extracted with phenol/chloroform (1:1, v/v), and then purified with a Chromaspin 10 column. The 41 nt RNA was labeled with [α-^{32}P]CTP during *in vitro* transcription and is referred to as the release strand, while the unlabeled 104 nt RNA is referred to as the template strand. The two strands in 20 μl each were combined at a molar ratio of release strand to template strand of ca. 1:10 in a solution of 0.2 ml containing 20 mM HEPES [N-(2-hydroxyethyl)piperazine-N′-[2-ethanesulfonic acid]]-KOH pH 7.6, 0.5 M NaCl, 1 mM EDTA and 0.1% SDS. The mixture was boiled for 10 min, transferred to 65 °C for 30 min, and then incubated at 25 °C overnight. The hybridized products were mixed with 5× RNA loading dye (0.1 M Tris-HCl pH 7.5, 20 mM EDTA, 0.5% SDS, 0.1% bromophenol blue, 0.1% xylene cynal, 50% glycerol) and electrophoresed on an 8% native polyacrylamide (acryl-bis, 30:1)-1× Tris-borate-EDTA gel. The radioactive duplex substrate band was localized by autoradiography, excised from gel, pulverized, and extracted with 0.5 M ammonium acetate pH 7.0, 0.1% SDS, 10 mM EDTA for 2 h at 25 °C. The eluted substrate was then extracted with phenol/chloroform (1:1, v/v), precipitated with 2.5 volumes of ethanol and resuspended in storage buffer (20 mM HEPES pH 7.5, 0.1 mM EDTA). The

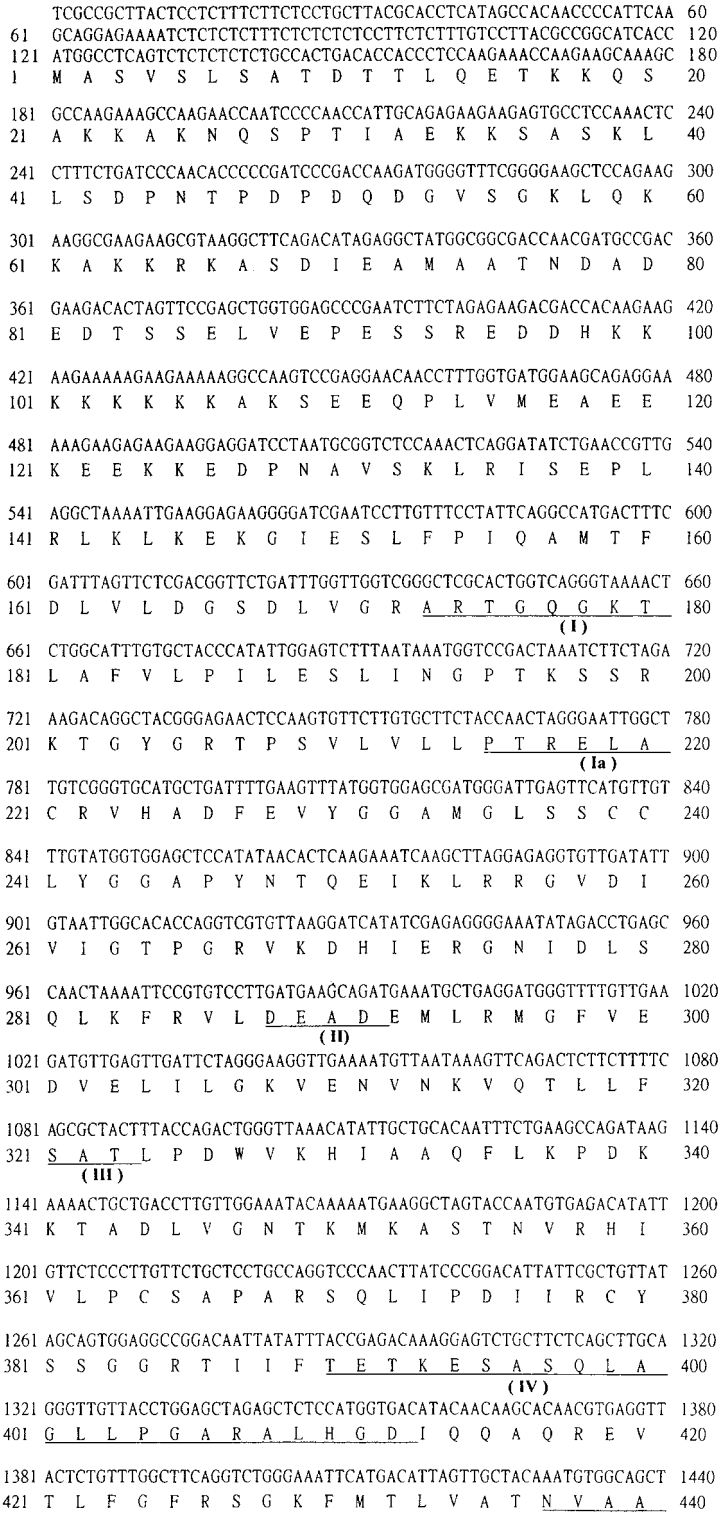

Figure 1. The nucleotide and deduced amino acid sequence of *VrRH1*. Seven conserved motifs are underlined.

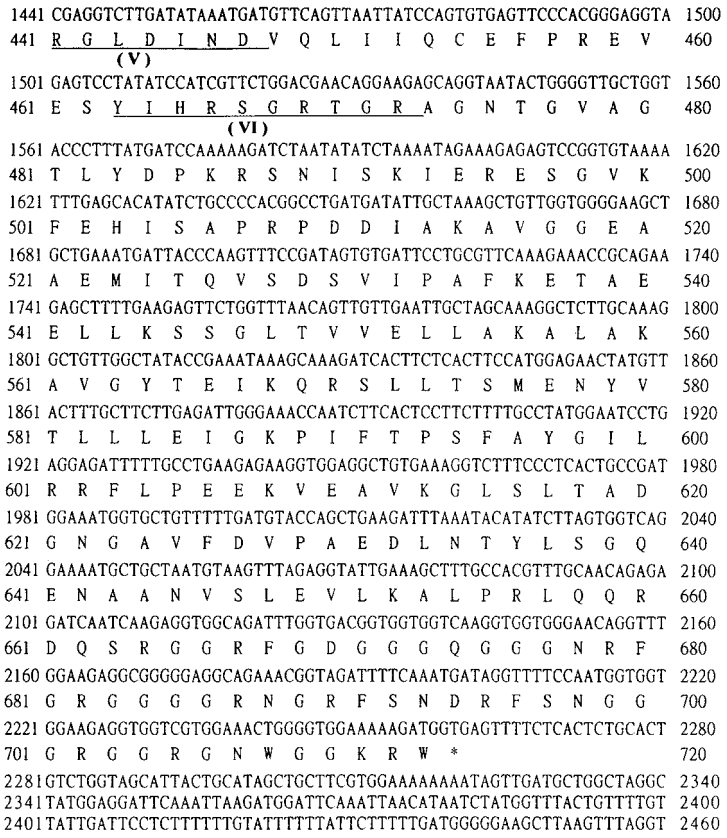

Figure 1. Continued.

specific radioactivity of the dsRNA was 1.4–1.5 × 10^3 cpm/fmol.

RNA helicase assay

The assay for dsRNA unwinding activity was carried out according to the previously described method (Tai *et al.*, 1996) with modification. The reaction mixture in a total volume of 20 μl contained 0.5 μg purified His-VrRH1, 2 mm ATP, 0.1 mg/ml BSA, 2 units RNasin, and 4.4 fmol dsRNA. The reaction was conducted at 37 °C for 1 h and then terminated by adding 5 μl of 5× RNA loading dye. A 10 μl aliquot of the reaction mixture was analyzed by 8% native polyacrylamide gel electrophoresis. The gel was dried and the radioactive double-stranded substrates and single-stranded products were detected by autoradiography.

ATPase activity assay

ATPase activity was measured by monitoring the hydrolysate of [γ-^{32}P] ATP separated by thin-layer chromatography (TLC) (Oh and Kim, 1999). Reactions were carried out in a final volume of 10 μl containing 50 mM HEPES pH 7.5, 2.5 mM $MgCl_2$, 80 μM ATP, 0.32 μM [γ-^{32}P]ATP (3000 Ci/mmol, NEN) and 160 ng of purified His-VrRH1. Reaction mixtures were incubated at 37 °C for 30 min, then terminated by adding EDTA to a final concentration of 50 mM. A 1 μl aliquot of the reaction mixture was spotted onto a plastic-tacked polyethyleneimine cellulose F sheet (Merck) and developed by ascending chromatography in 0.38 M potassium phosphate (pH 3.5). The sheets were dried and exposed to an X-ray film.

RNA blot analysis

Total RNA (25 μg) from various tissues or organs were electrophoresed on a formaldehyde-1.2% agrose gel, stained with ethidium bromide, photographed, transferred onto a Hybond-N membrane (Amersham Pharmacia Biotech), and UV cross-linked. RNA blotting was performed essentially as described by Sambrook *et al.* (1989). The full-length *VrRH1*

was randomly labeled with [α-^{32}P]dCTP by using a Rediprime labeling kit (Amersham Pharmacia Biotech). Membrane was pre-hybridized in 10 ml hybridization solution (Amersham Pharmacia Biotech) at 65 °C overnight, then the cDNA probe was added and hybridized to the membrane for at least 18 h. After hybridization, the membrane was washed twice in solution of 2× SSC, 0.1% SDS at 65 °C for 15 min. Finally, the membrane was exposed to an imaging plate for 20 h and analyzed by phosphorimager (Molecular Dynamics).

Results and discussion

cDNA cloning of VrRH1

We have established a procedure to induce accelerated aging as described above for studying the process of aging in mung bean. Group 1 seedlings were chosen for isolation of differentially expressed cDNAs because the germination rate of 9-day aged seeds markedly decreased from the original 98–99% to 37%. As a result, a differentially expressed 1.2 kb DNA fragment was found in the Group 1 seedlings. The 1.2 kb DNA fragment shares high sequence homology (71% identity) with RNA helicase cDNAs of spinach and *Arabidopsis* (Lorkovic *et al*., 1997). A full-length cDNA was isolated with PCR using this 1.2 kb fragment as a template. This cDNA, named *VrRH1*, contains an open reading frame of 2139 bp encoding a protein of 713 amino acids with an estimated molecular weight of 77 400 and an isoelectric point of 9.9 (Figure 1). The deduced amino acid sequence shows homology to the plant RNA helicases from tobacco (39–42%; Owttrim *et al*., 1992; Itadani *et al*., 1994; Owttrim *et al*., 1994), *A. thaliana* (41–71%; Metz *et al*., 1992; Lorkovic *et al*., 1997; Okanami *et al*., 1998; Gagliardi *et al*., 1999) and spinach (71%; Lorkovic *et al*., 1997). *VrRH1* shares seven highly conserved motifs with the previously described RNA helicases, including the DEAD box. These motifs are all present in the central region of *VrRH1*.

VrRH1 catalyzes unwinding of a double-stranded RNA

To determine the enzymatic activity of *VrRH1*, *VrRH1* cDNA was sub-cloned into an expression vector pET28b(+), expressed in *E. coli* BL21 and purified by a Ni^{2+}-agarose column (Figure 2). The purified His-VrRH1 was examined for its RNA unwinding and ATPase activities. A partially double-stranded RNA was used as substrate for the RNA unwinding assay (Figure 3A). The substrate RNA was stable at room temperature during the reaction and was denatured by heating at 95 °C for 10 min producing a fast-moving single-stranded RNA (Figure 3B, lanes 1 and 2). This single-stranded RNA was also formed when the double-stranded RNA substrate was incubated with the purified His-VrRH1 fractions from the Ni^{2+}-agarose column in the presence of dATP or ATP (Figure 3B, lanes 5 and 9). Generation of the single-stranded RNA from the RNA substrate was proportional to the amounts of His-VrRH1 (Figure 4A) and the passage of time during the reaction (Figure 4B). Unwinding of the substrate RNA by VrRH1 required ATP or dATP as cofactor and any (d)NTP other than ATP and dATP could not be substituted in the reaction (Figure 3B). These results indicate that VrRH1 is an ATP/dATP-dependent RNA helicase.

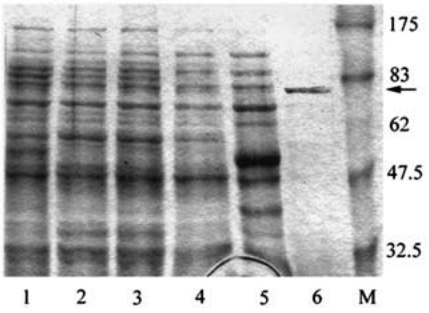

Figure 2. SDS-polyacrylamide gel electrophoresis of the purified His-VrRH1. Total proteins (103 mg) from cell-free extract of *E. coli* BL21 (DL3) carrying pET-VrRH1 were localized onto a Ni^{2+}-garose column. The column was washed and eluted as described in Materials and methods. Aliquots of the six protein peaks, 1–6, that came off the column were analyzed by SDS-PAGE and stained with Coomassie blue. An arrow indicates His-VrRH1 band. M, protein markers.

A large number of DEAD box proteins have been identified as putative RNA helicases. However, only a few members of the DEAD box family have been shown to possess unwinding activity, such as human p68 protein (Hirling *et al*., 1989), rabbit reticulocyte eIF-4A (Rozen *et al*., 1990), PPVCI (Lain *et al*., 1990), a DEAH box RNA helicase from Hela cell (Lee and Hurwitz, 1992), vaccinia virus RNA helicase (Shuman, 1992), *Drosophila* vasa protein (Liang *et al*., 1994), *E. coli* CsdA (Jones *et al*., 1996) and DbpA (Böddeker *et al*., 1997), *Xenopus* xp54 (Ladomery *et al*., 1997), An3 (Gururajan *et al*., 1997) and *Arabidopsis* AtDRH1 (Okanami *et al*., 1998). The methods and conditions used for assaying the RNA

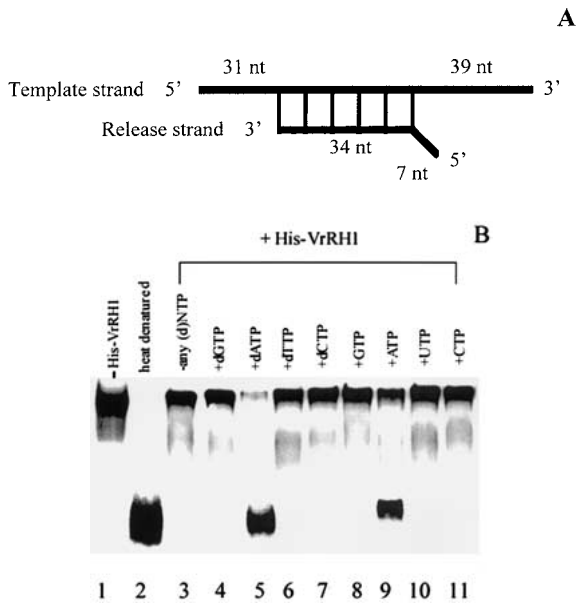

Figure 3. RNA helicase activity of VrRH1. A. Schematic representation of the partially double-stranded RNA substrate. The upper strand (template strand) of RNA substrate contains a 34 bp duplex region, 31 and 39 nt long single-strand regions at the 5′ and 3′ ends respectively. The lower strand (release strand) has 7 nt single-strand regions at the 5′ end. The lower strand was radiolabeled with [α-^{32}P]CTP. B. RNA unwinding activity of VrRH1. The RNA substrate was incubated with His-VrRH1 the presence of (d)NTPs (lanes 4–11). Experimental details are given in Materials and methods. The products of the reaction were separated by 8% native PAGE and the radiolabeled strand was visualized by autoradiography. Lanes: 1, the substrate RNA incubated under the enzyme assay conditions without His-VrRH1; 2, the substrate incubated for 10 min at 95 °C without His-VrRH1; 3, reaction in the absence of (d)NTP.

unwinding activity of these RNA helicases are quite similar with the exception of the amounts of purified RNA helicases. RNA helicases are involved in various biological processes, including transcription; mRNA splicing, maturation, export, translation and decay; ribosome biogenesis and assembly; spermatogenesis; embryogenesis; and cell growth and division. Almost all biological processes that involve RNA molecules require RNA helicase for their function and regulation. In addition to RNA unwinding activity, VrRH1 was also shown to possess ATPase activity (Figure 5A, B). We suggest that *VrRH1* may play a role in regulating the growth and development of mung bean seedlings. Identification of the natural substrate of VrRH1 may provide further insight into its physiological function.

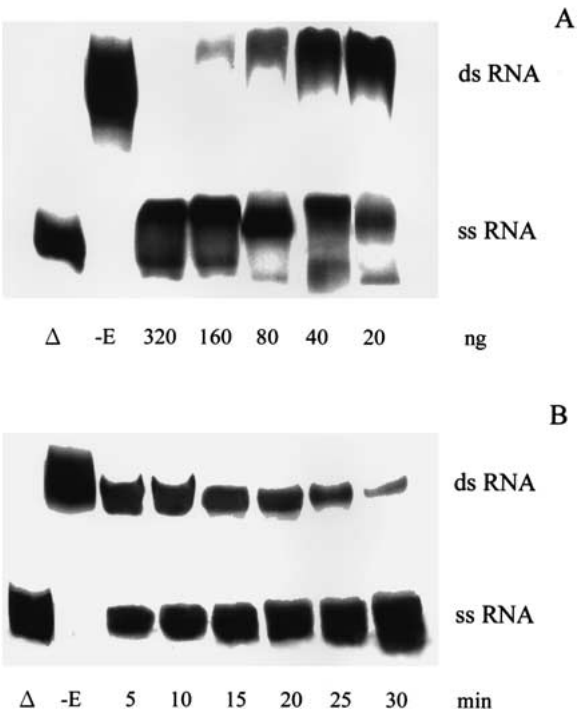

Figure 4. Effect of His-VrRH1 concentration on unwinding activity (A) and time course of RNA helicase activity (B). In A, various amounts of the recombinant His-VrRH1 (320–20 ng) were used for the assay of dsRNA unwinding activity. In B, 160 ng His-VrRH1 was used for assaying the RNA unwinding activity. The reactions were carried out at different time intervals (5, 10, 15, 20, 25, 30 min). Δ, heating at 95 °C for 10 min. -E, without His-VrRH1.

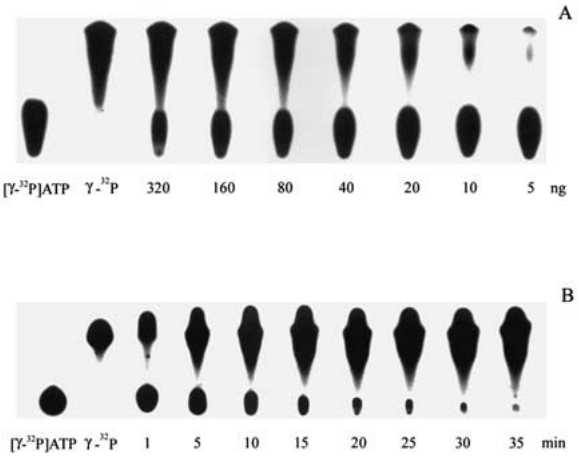

Figure 5. Chromatograms showing the dependence of ATPase activity on His-VrRH1 concentration (A) and reaction time (B). The experimental details are described in Materials and methods. In A, various amounts of the recombinant His-VrRH1 (320–5 ng) were used for the assay of ATPase activity. In B, 160 ng His-VrRH1 was used for assaying the ATPase activity.

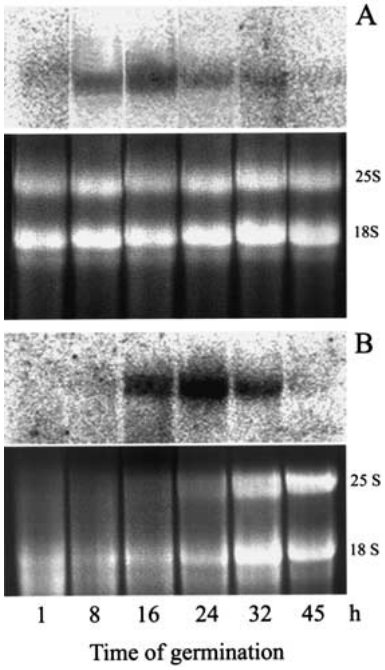

Figure 6. Comparison of *VrRH* expression patterns during germination between untreated seeds (A) and 9-day aged seeds (B). Mung bean seeds were acceleratedly aged under the standard conditions. Untreated seeds and the 9-day aged seeds were germinated for various time intervals from 1 to 45 h at 28 °C and 100% RH. Total RNAs (25 μg each) were prepared from seeds germinated for 1, 8, 16, 24, 32 and 45 h, separated on a formaldehyde/1.2% agarose gel and subjected to northern analysis with the full-length *VrRH1* as a probe.

VrRH1 has ATPase activity

VrRH1 is capable of unwinding dsRNA in the presence of ATP or dATP. To check if ATP is hydrolyzed during the reaction, [γ-^{32}P]ATP was incubated together with His-VrRH1 and then analyzed by TLC. The results indicate that [γ-^{32}P]ATP was hydrolyzed into ADP and ^{32}P-labeled inorganic phosphate. The formation of [^{32}P]phosphate was dose-dependent and proportional to reaction time (Figure 5). Those indicate that VrRH1 is also an ATPase. From the studies of binding of DNA or RNA by AtDRH1, it is supposed that the unwinding reaction would proceed in the order of RNA binding, ATP hydrolysis and release of the destabilized molecule (Okanami *et al*., 1998). Previous study indicated that RNA helicase A hydrolyzed ATP at the step of RNA release (Lee and Hurwitz, 1992). Two *E. coli* DEAD-box proteins, CsdA and DbpA, possess both RNA-dependent ATPase and RNA-unwinding activities, but the ATPase and helicase activities are not coupled (Jones *et al*., 1996; Böddeker *et al*., 1997). Thus, whether helicase activity is coupled with ATPase probably depends at least partly on the structure of RNA helicase, particularly the conserved motifs related to the binding of substrates and activities of RNA unwinding and ATPase, and the 5′- and 3′- extension regions (Böddeker *et al*., 1997). Studies on structure versus function may help to determine the relationship between ATPase and RNA helicase in VrRH1.

Expression of VrRH1

As mentioned in Materials and methods, 9-day aging and 12 h imbibition divided the aged seedlings into three groups. Group 1 (9-d-12-h seedlings with axis $\geq$0.5 cm) is viable, whereas the other two groups are non-viable. Thus this treatment can sort out the viable aged seeds from the non-viable aged seeds. Since *VrRH1* was isolated from the viable aged seedlings versus non-aged seedlings, expression of *VrRH1* may be associated with seed vigor of mung bean. We therefore compared *VrRH1* expression patterns during germination between 9-day aged and non-aged seeds. The results are shown in Figure 6. The increase of *VrRH1* transcripts in the non-aged seeds during germination was moderate (Figure 6A) when compared with the drastic increase in 9-day aged seeds (Figure 6B). The accumulation of RNA helicase (RH) mRNA(s) in 9-day aged seedlings reached a maximum at 24 h germination and declined thereafter. In the non-aged seedlings, level of *VrRH* expression gradually reached a maximum around 16 h. Although we could not exclude the possibility that there are other RNA helicases may also contribute to northern blotting, it is of interest to note that (1) a high level of *VrRH* mRNA expression was induced in 9-day aged seedlings, whereas only low-level expression of *VrRH* mRNA was observed in the non-aged seedlings, and (2) the *VrRH* mRNA expression preceded the reappearance of 25S and 18S ribosomal RNAs (Figure 6B), implying a correlation between the mRNA accumulation and biogenesis of ribosomal RNAs. VrRH1 shares high homology (71%) of amino acid sequence with PRH75 from *Arabidopsis*. PRH75 is a nuclear-localized protein, with the crucial region for the translocation into this organelles being located within the 81 N-terminal amino acids including a nucleolus localization sequence (NLS). It was also shown that higher expression of PRH75 occurs in active organs and tissues such as apical buds, hypocotyls and roots (Lorkovic *et al*., 1997). *VrRH1*

also contains a putative NLS like sequence in its N-terminal region (Figure 1) and was shown to express specifically in axis of 24 h germinated 9-day aged and non-aged seeds in northern analysis. And the 9-d-aged axis showed higher helicase expression than the non-aged seeds (data not shown). Induction of aging in mung bean seeds (9-day aging) resulted in a decrease in germination percentage from 98–99% to 37%. Upon imbibition for 12 h, only Group 1 seedlings could continue to grow and develop into mature plants as described in Materials and methods. Group 1 seedlings that accounted for about 37% of total seedlings showed much higher level of *VrRH1* transcript (or *VrRH* transcripts) than the rest of the 9-d-12-h seedlings. These transcripts appeared to be *de novo* synthesized mRNA (data not shown). At the onset of germination, enzymes that pre-existed in the seed are activated, and new enzymes are synthesized to sustain growth and development of the seedlings. Thus protein synthesis machinery is activated during seed germination. If the potentiality of protein synthesis during seedling development determines viability of mung bean seed, then any failure in ribosome biogenesis, pre-rRNA processing or pre-mRNA splicing must lead to eventual cell death. Our results seem to suggest that *VrRH1* may be implicated in protein synthesis during growth and development of mung bean seedlings and thereby related to seed viability.

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