Domain Structure of Human Nuclear DNA Helicase II (RNA Helicase A)*

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Nuclear DNA helicase II (NDH II)* was originally isolated from calf thymus by assaying its DNA unwinding activity (1). Subsequently, it was shown that NDH II also contains RNA helicase activity (2). The cDNA sequence of NDH II (3) revealed a high homology to two previously known proteins, namely human RNA helicase A (4) and the Drosophila “maleless” protein (MLE) (5). From the cDNA sequences it was also deduced that the three proteins belong to the superfamily of DEXH helicases, all members of which possess seven conserved helicase motifs in the putative catalytically active core. The presence of the DEXH helicase core domain suggests a function in RNA and/or DNA unwinding for all three members of this superfamily. The core contains an ATP binding site with the asymmetric amino acids or arginine. Similar RGG-rich sequences (RGG-boxes) have been found as part of many nucleic acid-binding proteins, such as the heterogeneous nuclear ribonucleoproteins hnRNP A1 (13) and hnRNP U (14), nucleolin (15), yeast single-strand DNA-binding protein 1 (16), as well as other proteins from the superfAMILY of DEXH helicases (17–23). Except hnRNP U, where the RGG-box is the only nucleic acid binding domain (14), RGG-boxes cooperate with other domains to achieve an increased affinity for nucleic acids.

Here, we have designed and produced various mutants of NDH II to delineate the modular structure of this enzyme with particular emphasis on the amino- and carboxyl-terminally localized nucleic acid binding domains. Our results confirm the importance of the dsRNA binding domains by showing that they influence both ATPase and helicase activities. Furthermore, the RGG-box at the COOH terminus enhances nucleic acid binding and may be required for an increased unwinding efficiency of the helicase core domain.

EXPERIMENTAL PROCEDURES

Cloning and Sequencing of the Gene Encoding Human NDH II—A Bluescript plasmid vector containing full-length cDNA of human NDH II was obtained from a ZAP cDNA library of human T-cells primed with oligo(dT) or primed randomly (Stratagene). The library was screened with a cDNA probe for bovine NDH II (3) that was labeled with [α-32P]dCTP (Amersham Corp.). Hybridization was performed under conditions of low stringency (24). Subcloning and DNA sequencing was essentially as described earlier for bovine NDH II (3). The sequence data have been deposited at the GenBank™/EMBL Data Bank under accession number Y10656.

Baculovirus-mediated Overexpression of Human Nuclear DNA Helicase II and Its Derivatives—Full-length NDH II as well as several deletion mutants were constructed with bacmid vectors (Life Technologies, Inc.). For this, NH2- and COOH-terminal parts of the cDNA were amplified by PCR and then combined (see Fig. 9). The translation initiation codon ATG and the sequence information for six histidine residues were introduced into the NH2-terminal forward primers; the COOH-terminal reverse primers were provided with stop codons. PCR primers at both ends also contained a unique BamHI restriction site. The following PCR primers were used: F-1, starting at the initiating codon ATG (5′-CGATACGACGCGATCACCCCATGCGACATCATCATCATCATATGGGTGACGTTAAAAATTTTCTG-3′); F-2, corre-

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spooling to a start at amino acid residue (aa) 131 (5′-CGATGACGG-GGCCACGATGATCCAGCTTGGTTCTCTGGG-3′); and F-3, corresponding to a start at aa 138. The reverse primers R-1 and R-2 were combined with an upstream cDNA primer yielding a 1618 bp PCR product. All PCR products were digested with HindIII or SacI, while PCR products encoding the COOH terminus could be digested with HindIII or SacI, while PCR products encoding the COOH terminus could be cleaved by HindIII and SacI at nucleotide position 1575 (see Fig. 9). At the COOH terminus, the reverse primers R-1 and R-2 were combined with an upstream cDNA primer yielding a 1618 bp PCR product. All PCR products were digested with BamHI and ligated into the donor plasmid pFastBAC1 by using the BamHI restriction site, both underlined. The expression of recombinant proteins was induced by adding 0.5 mM isopropyl-thio-
β-galactosidase. Three hours after induction, the bacteria were harvested at 15,000 rpm for 10 min, and the supernatant was mixed with a 1 ml bed volume of glutathione Sepharose 4B (Pharmacia) that had been prepared by coupling with glutathione-methylated agarose beads. GST fusion proteins were batch-purified according to the instructions of the manufacturer. Purified fusion proteins were stored at $-70°C$.

**Expression and Purification of Recombinant Proteins**—Baculovirus stocks from human NDH II vector were used to infect 1–2.5 $\times 10^8$ insect cells in the presence of 50 $\mu$g/ml ampicillin to an A600 value of 0.6–0.8. The expression of recombinant proteins was induced by adding 0.5 mM isopropyl-thio-
β-galactosidase. Three hours after induction, the bacteria were harvested at 15,000 rpm for 10 min, and the supernatant was mixed with a 1 ml bed volume of glutathione Sepharose 4B (Pharmacia) that had been prepared by coupling with glutathione-methylated agarose beads. GST fusion proteins were batch-purified according to the instructions of the manufacturer. Purified fusion proteins were stored at $-70°C$.

**Northwestern blot Assay**—Northwestern blot assays were performed as described (11). Bacterial lysates from each mutant protein were prepared by suspending bacteria from 2 ml of LB medium into 500 $\mu$l of SDS-PAGE loading buffer followed by sonication. Five $\mu$l fractions of the lysate were separated on a 25% native polyacrylamide gel and electrotransferred to a Hybond-C nitrocellulose membrane (Amersham). The membrane was incubated with 8 $\mu$l urea in Tris-buffered saline (25 mM Tris, 140 mM NaCl, and 3 mM KCl). To achieve protein renaturation, there followed 10 steps of 2:3 dilutions with Tris-buffered saline. Non-specific binding sites were blocked by treating the membrane with 5% (w/v) milk powder in probing buffer (25 mM NaCl, 10 mM MgCl2, 10 mM HEPES, pH 8.0, 0.1 mM EDTA, 1 mM dithiothreitol) for 1 h at room temperature. Then one of the $^{32}P$-labeled RNA probes, poly(rC•rC), poly(rA•rA), or poly(rC) (Pharmacia), was added in probing buffer. The membrane was incubated for another 30 min at room temperature. Washes were performed by three changes of probing buffer within 15 min. The membranes were exposed to x-ray film overnight at $-80°C$.

**Nucleic Acid Matrix Binding Assay**—About 250–500 $\mu$g of human NDH II-derived NH2- and COOH-terminal GST fusion products were purified by chromatography on glutathione-Sepharose 4B and then loaded onto 250 $\mu$l of either poly(rC•rC)-agarose or ssDNA-agarose (Pharmacia). The columns were equilibrated with binding buffer containing HEPES-KOH, pH 7.9, 50 mM NaCl, 10 mM dithiothreitol, and 1 mM dithiothreitol. The columns were washed with 0.75 $\mu$l of binding buffer and then eluted with 100 mM, 200 mM, 300 mM, 500 mM, 1 $M$, and 2 $M$ NaCl in binding buffer. 500 $\mu$l fractions were taken at each NaCl concentration, and 25 $\mu$l thereof were further processed for 10% SDS-PAGE. After electrophoresis, the proteins were visualized by staining with Coomassie Brilliant Blue. Alternatively, 0.5–10 $\mu$l of the baculovirus-expressed proteins were purified on Ni2+-NTA-agarose and mixed stock.
with 30 μl of either poly(rI*rC)-agarose or ssDNA-agarose that was 
preequilibrated with 50 mM, 100 mM, 200 mM, 300 mM, 500 mM, and 1 M NaCl in 
binding buffer. After incubation for 30 min at room temper-
ature, the agarose beads were spun down at 15,000 rpm for 5 min, 
washed extensively with 20–25 bed volumes of binding buffer with the 
corresponding NaCl concentration, and finally resuspended in 30 
μl of SDS-PAGE sample buffer for protein elution. Ten 
μl of each of the 
supernatant fractions were heated to 95 °C for 3 min, centrifuged, and 
then electrophoresed through an 8% SDS-polyacrylamide gel. After 
transfer to a Hybond-C nitrocellulose membrane, proteins were de-
tected with a 1:1000 dilution of rabbit antiserum against human RNA 
helicase A in an enhanced chemoluminescence procedure (ECL, 
Amersham).

Trypsin Digestion of Human NDH II—
Recombinant full-length hu-
man NDH II (500 ng) was digested with trypsin at a weight ratio of 
trypsin to NDH II of 1:5, 1:50, and 1:500, respectively, in 10 
μl of 20 mM HEPES-KOH, pH 7.9, 50 mM NaCl, 5 mM MgCl2, and 1 mM dithiothre-
itol. Digestions were performed at 37 °C for 30 and 60 min. Then the 
digestion mixtures were mixed with 5 μl of SDS-PAGE sample buffer 
and heated to 95 °C for 5 min. Tryptic products were analyzed by

electrophoresis on an 8% SDS-polyacrylamide gel.

Other Methods—Preparation of RNA helicase and DNA helicase 
substrates as well as enzyme assays for nucleic acid-dependent NTPase 
activity, RNA helicase activity, and DNA helicase activity were 
performed as described (2).

RESULTS

Cloning and Expression of Human NDH II—Nuclear DNA 
helicase II was previously isolated from bovine tissue according 
to its DNA unwinding property and later shown to unwind 
RNA as well (1, 2). Molecular cloning of the NDH II-encoding 
gene revealed a high homology to human RNA helicase A (3, 4), 
which was described to unwind only RNA but not DNA (25). To 
redetermine the substrate specificity of human NDH II, the 
genome was cloned. This independently cloned cDNA sequence 
was very similar to the recently modified sequence of human RNA 
helicase A (PIR accession number Q08211), with the 
exception of two missense exchanges (Q970E and K1036N) and 
a slightly altered COOH terminus (Fig. 1).

Human NDH II Unwinds both DNA and RNA—The cDNA of 
human NDH II was provided with the sequence information for 
six NH2-terminal histidine residues and expressed in insect 
cells by using a baculovirus-based expression system. Recom-
binant NDH II was purified on a Ni2+-NTA-agarose column 
and subsequent chromatography on poly(rI*rC) agarose. The 
purified enzyme had the expected molecular mass of 140 kDa,
i.e. it was clearly bigger than the previously isolated forms of bovine NDH II (Fig. 2). As its bovine homologue, the recombinant human enzyme unwound both dsRNA and double-stranded DNA in an ATP-dependent manner (Fig. 3).

**Limited Proteolysis of Human NDH II**—Bovine NDH II preparations usually consisted of two distinct protein bands with apparent molecular masses of 130 and 100 kDa that were active in both DNA and RNA unwinding (1, 2). To define a smaller form of the protein that still contains its unwinding activity, we attempted to digest recombinant NDH II in a controlled manner. A digestion pattern comparable with that of bovine NDH II could be produced by limited trypic digestion. When the weight ratio of trypsin to NDH II was adjusted to 1:500, only the 130-kDa form of the enzyme was generated (Fig. 2, lanes 6 and 7), while a ratio of 1:50 produced both the 130- and the 100-kDa forms of the protein (Fig. 2, lanes 8 and 9). Proteolized human NDH II bound to poly(rI-rC), comparable with the bovine homologue, with the 100-kDa form eluting at 0.2–0.3 M NaCl and the 130-kDa form eluting at about 0.5 M NaCl (Fig. 4). These differences in the chromatographic behavior allowed a separation of the trypic digestion products from each other. Both trypic forms of human NDH II were active in DNA and RNA unwinding (data not shown).

The course of digestion was further analyzed by probing the products with an anti-histidine antibody that specifically recognized the NH2 terminus. The 130-kDa form reacted well with the antibody (Fig. 5), indicating that it contained an intact amino terminus. Therefore, it is reasonable to conclude that the carboxyl-terminal amino acids representing the RGG-box were removed first. In contrast, the 100-kDa product did not react with this antibody, revealing the deletion of at least the NH2-terminal histidine tag. At most, 30 kDa (~270 amino acids) might have been removed from the NH2 terminus as the second trypic cutting event. This region would contain both dsRBDs that have been attributed to the amino acid residues 3–72 and 180–253, respectively (Fig. 7) (6). It was surprising that full-length NDH II reacted more weakly with the anti-histidine antibody than the COOH-terminally deleted form (Fig. 5).

A more quantitative treatment of the influence of COOH- and NH2-terminal deletions on the unwinding properties of

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**FIG. 5.** Analysis of the tryptic products of human NDH II by using a monoclonal antibody directed to the NH2-terminal histidine tag. Recombinant human NDH II was treated with trypsin at a mass ratio of 50:1 for 60 min. The digestion products were separated on an 8% SDS-polyacrylamide gel and subsequently blotted onto a Hybond-C nitrocellulose membrane. Then the nitrocellulose was probed with a monoclonal antibody recognizing the histidine tag (Qiagen). The left lane represents highly purified NDH II; the bands at 130 and about 80 kDa were not visible by Coomassie staining of a corresponding SDS gel. The right lane represents the digestion products; here a predominant band at 100 kDa was clearly visible by Coomassie staining (data not shown).

**FIG. 6.** Nucleic acid-dependent ATPase activities of human NDH II and NDH II-derived protein fragments. A, NDH II and protein fragments thereof were first purified by chromatography on Ni2+-NTA-agarose followed by purification on poly(tyr(rC))-agarose. Lanes 1 and 2, full-length NDH II after Ni2+-NTA- and poly(tyr(rC))-agarose, respectively; lanes 3 and 4, the fragment consisting of dsRBD I and II plus DEXH core after Ni2+-NTA- and poly(tyr(rC))-agarose, respectively; lanes 5 and 6, the DEXH core alone after Ni2+-NTA- and a second Ni2+-NTA-agarose purification, respectively; lane 7, the DEXH core with its amino-terminal extension including the RGG-box. This fragment was not further purified on poly(tyr(rC))-agarose because of severe losses during this step. The arrow on the right side indicates the protein band that, in a separate experiment, cross-reacted with antibodies directed against RNA helicase A. Purified recombinant proteins were separated on an 8% SDS-polyacrylamide gel and visualized by staining with silver. B, 300 ng (each) of the most purified fractions were used for measuring the ATPase activities in the presence of poly(tyr(rC)), MS-2 phage ssRNA, and M13mp18 ssDNA (50 μg/ml each).
NDH II was difficult to accomplish, mainly because minor differences in nucleic acid unwinding were hard to quantify. Therefore, the nucleic acid-dependent ATPase activity was taken as an indicator for the unwinding activity of NDH II. The ATPase activities of the 130- and 100-kDa forms were stimulated by poly(rI•rC) and by ssDNA (Fig. 6). With these effectors, the turnover rate of ATP hydrolysis was about 1.5–2-fold higher for the 100-kDa tryptic product than for the 130-kDa form (Fig. 6). Hence, once the RGG-box has been removed, the subsequent removal of parts of the NH₄ terminus stimulated ATP hydrolysis.

The Isolated dsRBD Domains of Human NDH II Bind Preferentially to dsRNA—To study the relative contribution of either of the potential dsRNA binding domains at the amino terminus to nucleic acid binding, dsRBDs I and II were expressed in E. coli as three GST fusion proteins comprising the amino acid residues 1–130 (dsRBD I), 131–318 (dsRBD II), and 1–318 (dsRBD I + II) (Fig. 7A). The RNA binding abilities of all three polypeptides were determined by chromatography on a 250 µl of poly(rI•rC)-agarose column and analyzed by electrophoresis through a 10% SDS-polyacrylamide gel and subsequent Coomassie staining.

The RGG-box of NDH II Binds Preferentially to Single-stranded Nucleic Acids—To study the nucleic acid binding properties of the carboxyl terminus, three C-terminal fragments of NDH II were expressed as GST fusion proteins (Fig. 8A). Binding of these fusion products to dsDNA and ssRNA was analyzed by Northern assays. Both RGG-box-containing fusion proteins bound to poly(rI) and poly(rI•rC), while the fragment comprising aa 953–1160 (without an RGG-box) did not display binding to either nucleic acid (Fig. 8B). The RGG-box-containing fusion proteins were eluted with 50–100 mM salt from a poly(rI•rC)-agarose column (data not shown). Hence, this type of binding was highly salt-sensitive and may have been caused by electrostatic interactions. In striking contrast, the RGG-box-containing fragments bound strongly to ssDNA-agarose, even in the presence of 1–2 M NaCl (data not shown).

Baculovirus Expression of Truncated Forms of NDH II—Full-length human NDH II comprising aa 1–1269 as well as the truncated fragments shown in Fig. 9A were all expressed in insect cells by using recombinant baculoviruses. Each of the recombinant proteins carried an N-terminal histidine tag to allow an easy purification on nickel columns. The yield of the purified proteins varied considerably between the different recombinants, most likely because some of the proteins were rapidly degraded. All recombinant proteins were analyzed by Western blotting with serum against human RNA helicase A. The observed molecular weights were in accordance with the calculated ones (Fig. 9B).

The nucleic acid binding affinities of the recombinant proteins, were probed by binding to poly(rI•rC)-agarose (Fig. 10). Full-length NDH II (data not shown) as well as the DEXH helicase core domain (aa 313–952) bound dsRNA even in the presence of 1 M NaCl (Fig. 10, left). Comparable binding was
observed when the complete NH₂ terminus was fused to the core domain (Fig. 10, middle). However, when only the dsRBD II domain was fused to the core, a reduced binding to dsRNA resulted (Fig. 10, right). Binding could not be further increased when the amino acids 953–1160 were fused to the dsRBD II-core construct (data not shown). All these constructs bound comparably well to ssDNA-cellulose. While the RGG-box hardly had an influence on RNA binding, binding to ssDNA was best with all constructs that contained an RGG-box (data not shown).

**ATPase and Helicase Activities of NDH II-derived Fragments—**Baculovirus-expressed NDH II as well as the NDH II fragments consisting of aa 1–952, 313–952, and 313–1269, were purified on nickel-agarose and poly(rI·rC)-agarose (Fig. 6A). With these proteins as well as the tryptic products, shown in Fig. 4, ATPase assays were performed in the presence of poly(rI·rC), MS-2 phage ssRNA and M13mp18 ssDNA (Fig. 6B). The ATPase activity of full-length NDH II was most stimulated by poly(rI·rC), followed by ssRNA from the bacteriophage MS2, followed by ssDNA from the bacteriophage M13. Furthermore, both tryptic products, p100 and p130, displayed nucleic acid-dependent ATPase activities, which however seemed to be less well stimulated by MS-2 RNA. In contrast, the amino-terminal part of NDH II, including the DEXH core, and the DEXH core alone were devoid of nucleic acid-stimulated ATPase activity, although all these constructs were able to bind RNA and DNA and all contained a Walker-type ATP binding site. In agreement with the failure to observe nucleic acid-stimulated ATPase activity, neither of the truncated products could unwind RNA or DNA (data not shown).

**DISCUSSION**

Nuclear DNA helicase II was originally purified and characterized as an ATP-dependent DNA helicase and subsequently shown to unwind both DNA and RNA (1, 2). Sequencing of the NDH II-encoding gene revealed a high homology to two previously known proteins, namely human RNA helicase A and the Drosophila MLE protein (3). RNA helicase A has been characterized as an RNA helicase only with no propensity to unwind DNA (25). Because of the high homology between RNA helicase A and NDH II (96% identity, 92% similarity), the apparent different substrate specificity of both enzymes was surprising. To solve this discrepancy, we independently cloned the gene of human NDH II/RNA helicase A and overexpressed it to measure its substrate specificity. The human NDH II gene obtained in this screening showed two nucleotide exchanges to the previously published (and recently corrected) primary sequence of human RNA helicase A (accession number Q08211) in addition to a different COOH terminus (see Fig. 1). Expression of the human NDH II clone in baculovirus-infected insect cells and subsequent purification yielded sufficient amounts of NDH II to redetermine its substrate specificity. It turned out that human NDH II, like its bovine homologue, unwind both DNA and RNA. In theory, the different COOH termini of both molecular clones may explain the different substrate specificities for NDH II and RNA helicase A. This, however, is unlikely, since the COOH terminus is dispensable for unwinding of either nucleic acid (see below).

Both NDH II and MLE belong to the superfamily of DNA/RNA helicases displaying a DEXD/H helicase motif in the core domain (26–29). For all helicases, it is believed that nucleic acid binding plays a critical role in coordinating NTP hydrolysis and the unwinding process (30). Nucleic acid binding has been attributed to the seven conserved ATPase/helicase motifs. Experimental evidence for this came from studies on the eukaryotic translation initiation factor 4A as well as the CI protein from plum pox virus. For these two proteins, the nucleic
acid binding domain has been assigned to the amino acid consensus sequence (H/Q)RIGRXX present in motif VI (31, 32). Alternatively, nucleic acid binding may involve all seven regions of the DEX(D/H) core (33). In any case, binding of the seven conserved DEX(D/H) motifs to nucleic acid seems to determine both affinity and specificity, conferring either RNA or DNA unwinding, to the members of this protein family.

In addition to the central core motif, many DEX(D/H) proteins contain further nucleic acid binding domains, such as the arginine-serine (RS)-rich domain of two other human RNA helicases (34, 35), a motif for binding to ribosomal RNA of yeast PRP22 (36), and finally a glycine-rich motif, called RGG-box (17–23). NDH II contains, in addition to the DEIH core, two copies of a dsRNA binding domain at its NH2 terminus (6) and an RGG-box at its COOH terminus. We have examined the contributions of these motifs to the enzymatic mechanism of NDH II.

Neither the (proteolytic) deletion of the RGG-box nor the deletion of the two dsRBDs abolished the unwinding activity. Rather, the 100-kDa form of NDH II could act as ATP-driven nucleic acid helicase for both DNA and RNA. On the other hand, the overexpressed and purified helicase core (aa 313–952, 72 kDa) as well as genetically engineered and purified NDH II fragments consisting of aa 1–952 (107 kDa) and 313–1269 (105 kDa) had no detectable ATPase or helicase activity. Further attempts to confine the amino acid sequence to a shorter but still active “minihelicase” have been unsuccessful so far. Proteolytic degradation was no longer controllable when NDH II became smaller than the 100-kDa fragment; genetic constructs with various NH2- and COOH-terminal extensions of the (stable) core were highly unstable when expressed in insect cells. Although not definitely proven, from the sum of our data we can deduce that a minimal helicase most likely consists of aa 313–1160 (95 kDa).

Although dispensable for the basic enzymatic properties of DNA and RNA unwinding, the very COOH and NH2 termini of NDH II are phylogenetically conserved and also present in the MLE protein of Drosophila. Therefore, the properties of the NH2- and COOH-terminal domains were analyzed in a more detailed way. Northwestern assays and chromatography on poly(rI·rC)-agarose demonstrated dsRNA binding for the isolated dsRBDs. For efficient binding to dsRNA, both dsRBDs were necessary. Furthermore, dsRBD-containing fragments bound poly(rI·rC) in the presence of up to 0.5 M NaCl. In this respect, the dsRBDs from human NDH II were comparable with other dsRBDs, such as those of the dsRNA-dependent protein kinase DAI (7), the dsRNA-specific adenosine deaminase DRADA (37), and the E3L protein from vaccinia viruses (12, 38). For DAI it has been shown that its two N-terminal dsRBDs are responsible for specific binding to dsRNA. In this case, binding to dsRNA induces a conformational change, which in turn activates the catalytic domain of DAI (7). In the case of DRADA, the deletion of one of its three dsRBDs did not affect RNA binding but abolished the catalytic function of the deaminase domain (8). By analogy, the two dsRBDs of NDH II may have regulatory functions in the unwinding activity (see below) rather than direct effects on nucleic acid binding.

The RGG domain mediated binding to ssRNA and ssDNA as revealed by Northwestern assays and nucleic acid affinity chromatography. A preferred affinity for single-stranded nucleic acids has also been found for the RGG domains from hnRNPA1 (13), hnRNPU (14), and nucleolin (15). The RGG-box of nucleolin displays a β-spiral structure that binds single-stranded nucleic acids with an induced base unstacking effect (39), most likely with the consequence of duplex unwinding (40). In the case of hnRNPU, the RGG domain is the only nucleic acid binding domain; nevertheless, it mediates binding to ssDNA even in the presence at 0.5–1 M NaCl (14). Such a high affinity for ssDNA may be a prerequisite for the observed binding of hnRNPU to nuclear matrix elements (SAR/MAR) (41, 42); it may also help to unwind SAR/MAR elements (43). Similar to hnRNPU, the RGG-box of human NDH II bound to ssDNA-agarose at salt concentrations of more than 0.5 M NaCl. However, we did not observe unwinding or destabilization of double-stranded DNAs or RNAs by the sole action of the individually expressed RGG domain from NDH II (data not shown).

All three additional nucleic acid binding domains might be neighbors in the three-dimensional structure of NDH II (11). This suggestion arose from combining the results of the limited proteolysis experiments and monoclonal antibody studies for detecting the histidine-tagged NH2 terminus. The removal of the RGG-box from the COOH terminus apparently enhanced binding of the anti-histidine antibody to the NH2 terminus, suggesting a close neighborhood between the RGG-box and the dsRBDs. The proteolytic removal of the RGG-box diminished the nucleic acid-stimulated ATPase activity of NDH II. This, however, could be partially regained by further deleting the dsRBDs. A cooperation between the RGG domain and the dsRBDs may be the first step to recognize a single-
stranded/double-stranded junction. An initial protein-nucleic acid contact with the RGG domain and the daRBDs then might trigger the activation of the ATPase/helicase activity, analogously to the allosteric activation effects observed with DRADA (8) and with DAI (7). Further experiments on possible cooperative effects during nucleic acid unwinding, involving both full-length NDH II and the 100-kDa form, are necessary to further substantiate this speculation.

The molecular dissection of the domain structure of NDH II resulted in a rather complex picture of its enzymatic function(s); a highly conserved but dispensable COOH terminus mediates binding to single-stranded nucleic acids, particularly DNA, while the also dispensable NH2 terminus brings about binding or recognition of double-stranded nucleic acids, particularly RNA. Analogous to DRADA and DAI, this type of recognition might regulate the ATP-driven unwinding activity localized in the center of the molecule. The supposed control mechanism for the enzymatic activity of NDH II, the phylogenetically conserved structure of the corresponding domains from Diptera to mammals, the relative abundance of NDH II in the nuclei of all tissues studied so far, and its propensity to unwind DNA and RNA may give some hints about its physiological function. A nuclear helicase that unwinds any nucleic acid might be particularly suited for melting out DNA:RNA hybrids, such as those occurring during transcription and thereby help to remove the nascent transcript from the DNA strand. During this process, single-stranded DNA of the open complex might be first recognized by the RGG domain, while one or both of the daRBDs dock onto the RNA:DNA hybrid. This might switch on the ATPase/unwinding activity of the helicase core domain, which in turn is a prerequisite for melting out the DNA:RNA duplex structure. Certainly, further experiments are required to further substantiate our current point of view.

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