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Minireview

Multiple Functions of Nuclear DNA Helicase II (RNA Helicase A) in Nucleic Acid Metabolism

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Abstract Nuclear DNA helicase II (NDH II), or RNA helicase A (RHA), was initially discovered in mammals by conventional protein purification methods. Molecular cloning identified apparent sequence homologies between NDH II and a *Drosophila* protein named maleless (MLE), the latter being essential for the *Drosophila* X-chromosome dosage compensation. Increasing amounts of evidence suggest that NDH II is involved in multiple aspects of cellular and viral DNA and RNA metabolism. Moreover the functions of NDH II may have potential clinical implications related to viral infection, autoimmune diseases, or even tumorigenesis.

Key words DNA and RNA helicase; maleless (MLE); X-chromosome dosage compensation; dsRBD; RGG-box; transcription; RNA processing; RNA transport; actin; nucleolus; SLE (systemic lupus erythematosus); virus

Secondary structures of nucleic acids play an important role in regulating their transactions as carriers of the genetic information, including DNA replication, transcription, RNA processing, RNA transport, and translation. Resolving double-stranded (ds) DNA or RNA is usually an energy-dependent process that can be accomplished by proteins termed DNA or RNA helicases, which are present in all prokaryotic and eukaryotic organisms. Earlier attempts to find mammalian helicases led to the detection of NDH II from bovine thymus [1] and RHA from cultured human cells [2]. Indeed, the subsequent biochemical characterization demonstrated many similarities between NDH II and RHA [3], while their homologous nature only was recognized after molecular cloning of the respective gene [4,5]. This also revealed an orthologue of NDH II and RHA in *Drosophila* named maleless (MLE), a protein involved in dosage compensation of the *Drosophila* X-chromosome [6]. Now, many studies suggest that NDH II is a protein with diverse functions in nucleic acid metabolism.

Characterization of the Helicase Activities of NDH II

Initially, NDH II was purified from calf thymus by monitoring the unwinding of a partially annealed dsDNA [1]. RHA, on the other hand, was purified from human cells by following its RNA unwinding activity [2]. NDH II displays a preference for a dsDNA substrate that carries at least one noncomplementary stand ahead of the DNA duplex [3]. This substrate requirement of NDH II may be due to its preferential binding to single-strand (ss) DNA. Indeed partially exposed ssDNA occurs *in vivo* at replication forks, transcription bubbles, promoters, or nuclear matrix attachment sites on chromatin loops. The DNA unwinding direction of NDH II is from 3′ to 5′. Similarly, RNA unwinding by RHA is enhanced when a single-stranded RNA protrusion flanks a RNA duplex [2]. The polarity of RHA during RNA unwinding is also 3′ to 5′ like that of DNA unwinding. NDH II (or RHA) utilizes all four NTPs or dNTPs as energy-delivering co-factors for the melting of both DNA and RNA [2,3]. The lengths observed so far for NDH II-catalyzed DNA or RNA unwinding range from 20 to 30 nucleotides [2,3]. Nevertheless, until now there is nearly no data available on the exact unwinding processivity, i.e. the number of unwound base pairs per nucleic acid binding event of NDH II.

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Domain Structure of NDH II

The gene encoding human NDH II maps to chromosome 1q25 near the major susceptibility locus for prostate cancer, while a pseudogene is located on chromosome 13q22 [7,8]. Full-length human NDH II has a molecular weight of 142 kD and comprises a helicase catalytic domain belonging to the helicase superfamily 2 (SF2) [4–6]. The conserved helicase motifs of NDH II include the classical Walker-type nucleotide binding sites A (GCGKT) and B (FILDD) in motif I, as well as the DEIH signature in motif II [4–6]. A lysine-rich nuclear localization signal (NLS) is present in mammalian NDH II between the helicase motif III and IV, which however is not found in the *Drosophila* maleless protein [6] and in NDH II from *Caenorhabditis elegans* [9].

NDH II contains two copies of dsRNA binding domains (dsRBD) at its N-terminus [3,10]. The dsRBDs from NDH II are conserved among a group of dsRNA-binding proteins that include dsRNA-dependent protein kinase (DAI), dsRNA-specific adenosine deaminase (DRADA), *Escherichia coli* RNase III, and the vaccinia virus E3L protein [11]. The biochemical characterization of the dsRBDs confirmed a specificity for dsRNA [12], although the dsRBDs of NDH II also display a limited affinity for single-stranded DNA [12] and probably also for dsDNA [13]. In addition to the dsRBDs, NDH II contains striking GY-rich sequence repeats at the C-terminus, the so-called RGG-box that is characterized by a preferential binding to ss nucleic acids [12]. Notably, an unusually elongated RGG-box is present at the C-terminus of murine NDH II, although its possible species-specific functions remain unclear [14].

The structural features of NDH II point to a model in which the nucleic acid unwinding activity can be regulated by the nucleic acid binding domains flanking both sides of the helicase's catalytic center [12]. A phylogenetic analysis suggests that NDH II may have been evolved from the DEXH helicase family by acquiring auxiliary nucleic acid binding domains, i.e. the dsRBDs and the RGG-box [15]. Most likely this has led to the divergence of NDH II from the other DEXH proteins, such as a group of yeast RNA splicing factors including prp2, prp16, prp22 and prp 43, all of which contain helicase motifs (with DEAH as signature) resembling those of NDH II [4–6]. In addition to these yeast prp proteins NDH II based on its helicase motifs is highly similar to a vaccinia virus-encoded helicase named NPH II [5]. Biochemically vaccinia NPH II catalyzes a 3′ to 5′ unwinding of dsDNA or dsRNA, using any of the nucleotides, i.e. all four rNTPs or dNTPs, as energy-delivering cofactors [16]. These catalytic properties of vaccinia NPH II and NDH II are quite similar [3] and support the view that there might have been a common ancestor of these two helicases.

Physiological Functions of NDH II

Transcription

The function of NDH II in transcription can be inferred from the physiological role of the *Drosophila* MLE protein in X-chromosome dosage compensation. In this process MLE is one of the *Drosophila* genes essential for maintaining the hypertranscriptional activity of the single male X-chromosome, so that its transcriptional output is twice as much as that of the two female X-chromosomes [6]. Cytologically MLE can be visualized by immunostaining at hundreds of discrete bands decorating the single male X-chromosome [6]. These bands coincide with the distribution of further proteins involved in dosage compensation, such as the three male-specific-lethal (Msl) gene products Msl1, Msl2, Msl3, as well as male absent on first (MOF). MOF belongs to the MYST acetyltransferase gene family and is responsible for the acetylation of histone H4 at lysine 16. This histone H4 isoform (H4Ac16) has a similar distribution on the male X-chromosome as the above mentioned proteins. Therefore H4Ac16 may be a marker for enhanced transcription [17–19].

X-chromosome dosage compensation in *Drosophila* is initiated in early embryonic development as a response to the ratio of X-chromosome (X) to the haploid set of autosomes (A), i.e. the X:A ratio. This decides a sex regulatory pathway that controls the activity of the sex lethal (Sxl) gene. Female development results from activating Sxl when the X:A ratio is 1.0. On the other hand Sxl remains inactive when the X:A ratio is 0.5. This in turn releases the Sxl-suppressed downstream pathway for establishing X-chromosome dosage compensation and induces the development of males. Msl2 is the only Msl product specific for males and it is expressed when Sxl is inactive. Msl2 co-operates with Msl1 for opening the X-chromosome at 30 to 40 entry sites, into which Msl3, MLE and MOF are subsequently recruited. Among the X-chromosomal entry sites are two genes that encode non-translated RNAs, i.e. *roX1* and *roX2*. The *roX* RNAs are found on the male X-chromosome in the same distribution pattern as the above-mentioned proteins [18]. This led to the hypothesis that Msl1 and Msl2 may initially target *roX* genes, where they

may capture the synthesized roX RNAs. The *roX* RNAs may form a scaffold for assembling the dosage compensation complex, which then is competent for spreading to other positions on the male X-chromosome. In all these processes MLE may act as a helicase to facilitate the X-chromosomal targeting of Msl proteins or the assembly of the dosage compensation complex [19]. Indeed, the maleless protein lost its function in dosage compensation when a mutation was introduced into its nucleotide binding site that abrogated its helicase/NTPase activities, although this mutation did not affect its physical binding to nucleic acids or the X-chromosome [20]. Interestingly, MLE dissociated from the male X-chromosome after RNase digestion [21]. Therefore, the physical binding of MLE to the X-chromosome most likely occurs via RNA. In mammals, dosage compensation is achieved by suppressing the transcription of genes located on one of the two X-chromosomes in females [19]. So far there is no evidence for a participation of NDH II in this process, although the inactivation of one female X-chromosome is also based on RNA. Here, X-chromosome inactivation is mediated by the non-coding *Xist* RNA that leads to a chromosome-wide suppression of X-linked gene expression. Nevertheless, NDH II is essential for early embryonic development since NDH II knockout mice die because the embryonic ectoderm fails to differentiate normally during gastrulation [22].

Evidence for a transcriptional role of NDH II in mammals came from the finding that NDH II is a bridging factor between the transcriptional co-activator CPB/p300 and RNA polymerase II [23]. Two regions at the N-terminus of NDH II are responsible for the interaction with CPB/p300 and RNA polymerase II, respectively [23]. For the latter interaction NDH II contains a minimal transactivation domain (MTAD) that is enriched in aromatic amino acids, e.g. tryptophan [24,25]. Interaction with CBP/p300 is mediated by another part of NDH's N-terminus. CPB/p300 binds to the cAMP-responsive element binding protein (CREB) by recognizing its phosphorylated serine 133. Thereby NDH II helps stimulate transcription via the transmission of the CREB/CBP-specific binding signal to RNA polymerase II. NDH II also activates transcription by interacting with the breast cancer specific tumor suppressor protein BRCA1 [26], the rat mineralocorticoid receptor (MR) in a ligand (aldosterone)-dependent manner [27], and the CpG binding protein MBD2a that regulates the transcription of cAMP-responsive genes according to their methylation state [28].

Some data suggest that the gene product of spinal muscular atrophy (SMA), named survival motor neuron (SMN) protein, interacts with NDH II and RNA polymerase II [29]. Since SMN is a component of small nuclear ribonucleoproteins (snRNPs) involved in pre-mRNA splicing, the two nuclear events, i.e. transcription and RNA processing, may be coordinated by a global complex that tethers both transcription and RNA processing factors to the transcribed gene [30]. Interestingly, after treatment with interferon α (IFN-α) NDH II is found in nuclear bodies called PML (promyelocytic leukemia nuclear bodies, NB) [31]. This is consistent with the suggested transcriptional function of NDH II, since PML is a subnuclear compartment where transcription of IFN-α-induced genes takes place. NDH II is also able to bind dsDNA, where it co-operates with DNA topoisomerase IIα for transcriptional regulation [32]. NDH II even may bind dsDNA in a sequence-specific manner, e.g. the promoter DNA of the tumor suppressor $p16^{ink4a}$ [33].

RNA processing

Genetic studies in *Drosophila* identified a recessive mutation of maleless that led to a paralytic phenotype called nap^{ts} (no action potential, temperature sensitive). Nap^{ts} is due to the insufficient expression of an X-linked sodium channel (*para* Na^+) that is required for maintaining a normal membrane excitability of neuronal cells [34]. Nap^{ts} affects both sexes and is unrelated to the sex-specific function of MLE in dosage compensation as discussed above. A transversion mutation converting C to G causing a threonine to serine exchange near the maleless consensus sequence for NTP binding (GxGKTT to GxGKTS) seems to be responsible for nap^{ts}. Later studies ascribed nap^{ts} to aberrant RNA splicing occurring at an exon-intron junction of *para* Na^+ pre-mRNA. The same region also contains dsRNA that is subjected to an adenosine-to-inosine (A-to-I) conversions by RNA editing [35]. Here MLE seems to co-operate with RNA editing to mitigate a local secondary RNA structure that conceals a splice donor site. This ensures correct splicing of *para* Na^+. Similarly, NDH II acts together with the RNA editing enzyme (ADAR) for a coordinated editing and splicing of the glutamate receptor pre-mRNA [36]. So far RNA editing has been found to be a ubiquitous phenomenon both in eukaryotes and their viruses [37]. Thus there seems to be a general co-operation of NDH II with RNA modifying enzymes to process cellular or viral dsRNAs. However, to warrant this hypothesis further studies are needed.

An earlier chemical extraction of mammalian nuclei identified the presence of NDH II in the outer nuclear membrane fraction [38]. Based on a special affinity matrix NDH II has been isolated as a component of human prespliceo-

somes [39]. Immunofluorescence studies revealed that the intranuclear localization of NDH II is similar to that of other proteins known to bind pre-mRNA or mRNA, e.g. the hnRNP proteins [40]. Moreover, NDH II directly binds to filamentous actin (F-actin) in the nucleus [41]. This supports the view of an involvement of nuclear actin in RNA processing or transport events that are mediated by NDH II. Moreover, NDH II has been found to be associated with the dsDNA-end binding protein Ku antigen within heterogeneous ribonucleoprotein complexes [42]. Surprisingly, both DNA and RNA stimulate the phosphorylation of NDH II by the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), which is brought to its protein substrates by the DNA or RNA binding subunit of Ku antigen. Hence, both the DNA- or RNA-related activities of NDH II may be regulated by DNA-PK. Phosphorylation may lead to an altered nucleic acid binding affinity and a switch of NDH II between transcription and other aspects of RNA metabolism.

In most human cells NDH II is found in the nucleoplasm, while in mouse cells it prefers to reside in the nucleolus [43]. Here NDH II is enriched at positions where ribosomal RNAs are synthesized. However, NDH II can also be found in the nucleolus of human primary cells [44]. Moreover, in human tumor cells NDH II has a propensity for a situation at or in the nucleolus, and especially at the nucleolar periphery [45]. Coincidentally, F-actin can also be detected at the nucleolar boundary and its depolymerization by gelsolin promotes an entry of NDH II into the nucleoli [45]. This raises the possibility that NDH II may be sequestered to perinucleolar structures consisting of F-actin. From that F-actin shell NDH II apparently shuttles into the nucleolus with a rate determined by the kinetics of ribosomal RNA production [45]. The nucleolar elements that retain NDH II are apparently ribosomal RNAs [45]. However other data suggest the presence of a nucleolar anchor protein that bears similarities to BRCA1 [46] to which NDH II has an apparent affinity [26] (see also below).

NDH II and Human Diseases

Viral gene expression and replication

NDH II is one of the cellular proteins that specifically bind to a region of retroviral RNA termed the constitutive transport element (CTE) [47]. The CTE represents a *cis*-acting sequence of retroviral RNAs that gains access to the host's RNA transport machinery and promotes the nuclear export of unspliced or incompletely spliced viral RNAs. Because of its association with CTEs, NDH II is considered to be involved in RNA transport. This is further supported by the identification of a C-terminal region of NDH II that enables a translocation across the nuclear membrane in both directions [48]. NDH II-mediated RNA export is insensitive to leptomycin B that, on the other hand, blocks the well-characterized RNA export pathway via the leucine-rich nuclear export signal (NES) and the export receptor CRM1. The NES-CRM1 pathway for unspliced RNA requires the presence of the so-called Rev protein that is encoded by complex retroviruses, e.g. the human immunodeficiency virus (HIV). Rev promotes the nuclear export of intron-containing viral RNAs by the recognition of a corresponding Rev response element (RRE).

There is also evidence for an effect of NDH II on retroviral gene expression that is either promoted by the CTE or by the RRE [49], although for the latter NDH II may need to co-operate with other cellular proteins that also function (perhaps synergistically) in RNA export [50]. These include HAP95 (helicase A binding protein 95), which shares homology with AKAP95 [51,52] (a nuclear matrix-binding protein from the A kinase-anchoring protein family [53]), and another CTE-binding protein named Tap (herpesvirus saimiri Tip-associated protein) [54]. A regulatory effect of NDH II on viral gene expression can also be mediated by an interaction with the *cis*-acting transactivation response element (TAR) of HIV-1's nascent RNA [55]. In this case NDH II specifically binds to the unique stem-loop structure of TAR via its dsRBDs. This leads to an activated transcription of HIV-1 DNA.

NDH II also binds to RNA from human adenoviruses. Adenoviruses encode two classes of virus-associated (VA) RNAs, i.e. VA RNA_I and VA RNA_{II}. NDH II selectively binds to VA RNA_{II}, whereas the dsRNA-dependent protein kinase (PKR) is preferably targeted by VA RNA_I [56]. Interferon-β induces PKR to phosphorylate the translation initiation factor-2 α, leading to a shut-off of protein synthesis. This host antivirus response can be abolished by VA RNA_I because it specifically binds to PKR and consequently inhibits its function. Similarly, VA RNA_{II} inhibits the helicase activity of NDH II, which increases virus survival possibly by turning down antiviral host responses [56]. VA RNA_{II} also binds to other dsRNA binding proteins belonging to the NFAR (nuclear factor associated with RNA) group. These proteins interact with NDH II and stimulate transcription from cellular and viral promoters [57]. Moreover, both NDH II and NFAR proteins bind to the 5′ and the 3′ non-translated region (NTR) of the bovine viral diarrhea virus, a close relative of the human

hepatitis C virus, with implications for viral replication and translation [58].

Genome instability

The relevance of NDH II to genome integrity may be deduced from its physical interaction with the tumor suppressor protein BRCA1 [26]. BRCA1 is a multifunctional molecule involved in various nuclear processes including transcription, chromatin remodelling, DNA repair, and the *Xist* RNA-mediated inactivation of the X-chromosome [59]. Given that an actively transcribed region is more susceptible to genomic stress and DNA damage than a closed double strand, the NDH II-promoted recruitment of BRCA1 to the RNA polymerase II holoenzyme may be critical for the surveillance of active transcription loci. This may ensure an immediate sensing of DNA lesions by RNA polymerase II and BRCA1, where the latter either replaces the transcriptional complex by the DNA repair apparatus or induces apoptosis in the case of an irreversible DNA damage [60]. Further support for a physical interaction between NDH II and BRCA1 comes from a study that used a dominant-negative construct of NDH II to block the cellular function of BRCA1 [61]. This resulted in a series of abnormalities such as reduced BRCA1 foci formation after DNA damage, a binuclear morphology, tetraploidy, and defects in centrosome replication.

NDH II is an autoantigen from patient with autoimmune disease

Co-immunoprecipitation identified NDH II as an autoantigen of the sera of patients with the autoimmune disease systemic lupus erythematous (SLE) [62]. The NDH II-positive autoantisera also co-immunoprecipitate ribonucleoprotein complexes from human cells. Moreover, Ku antigen (which was initially found in patients suffering from SLE) is also associated with NDH II within nuclear ribonucleoprotein complexes [42]. This not only indicates a similar function of NDH II and Ku antigen in cellular nucleic acid metabolism, but also provides a clue to a common etiology of autoimmune diseases. Since NDH II is a specific substrate of caspase 3 during apoptosis [33,62] it is tempting to speculate that caspase 3-generated degradation products of NDH II may trigger the autoimmune response and the development of autoimmune diseases [63]. Clinically autoantibodies against NDH II appear more frequently in patients with lupus nephritis [62]. Moreover, studies on Lupus mice using a chemical inducer suggest a different production of autoantibodies against NDH II and to the other nuclear autoantigens [64,65], in which cytokines, such as interleukin-12 (IL-12), seem to play a more important role.

Concluding Remarks

Although NDH II has been well characterized with respect to its catalytic center and its additional nucleic acid binding domains, there is accumulating evidence for the existence of various protein-protein interactions that specify diverse functions of this enzyme in RNA and possibly also in DNA metabolism. Previous studies have not only contributed to a better understanding of NDH II's various physiological roles but also to an advanced knowledge of its cellular structure-and-function-relationships. The amazingly high sequence conservation of NDH II from nematodes via dipterans to man and the many cross-interactions with viral RNAs and proteins point to a common ancestor that might even have arisen from a virus. NDH II is mainly involved in heavy-duty transcriptional tasks, such as the X-chromosome dosage compensation in *Drosophila* or rRNA synthesis in mice. This could be the afterglow of a bold and undifferentiated general transcription activator in an ancient primitive organism that subsequently has evolved into the highly specialized and versatile regulatory tool we observe today. A resolution of the still remaining puzzles about NDH II will certainly contribute to a better understanding of many biological phenomena and, at least in some cases, to a better explanation of human cancer and autoimmune diseases.

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