

SURVEY AND SUMMARY

Remodeling of ribonucleoprotein complexes with DExH/D RNA helicases

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ABSTRACT

The DExH/D protein family is the largest group of enzymes in eukaryotic RNA metabolism. DExH/D proteins are mainly known for their ability to unwind RNA duplexes in an ATP-dependent fashion. However, it has become clear in recent years that these DExH/D RNA helicases are also involved in the ATP-dependent remodeling of RNA–protein complexes. Here we review recent studies that highlight physiological roles of DExH/D proteins in the displacement of proteins from RNA. We further discuss work with simple RNA–protein complexes *in vitro*, which illuminates mechanisms by which DExH/D proteins remove proteins from RNA. Although we are only beginning to understand how DExH/D proteins remodel RNA–protein complexes, these studies have shown that an ‘RNA helicase’ does not *per se* require cofactors to displace proteins from RNA, that protein displacement does not necessarily involve RNA duplex unwinding, and that not all DExH/D proteins are able to disassemble the same range of ribonucleoproteins.

INTRODUCTION

In the last two decades it has become increasingly clear that a diverse range of RNAs play numerous critical roles in the regulation of gene expression (1–3). It has also become apparent that RNAs hardly function alone in a cellular environment. Instead, RNAs are invariably found in complexes with proteins (1,4). Prominent examples include the pre-mRNA splicing machinery, the apparatus that assembles ribosomes and mRNA storage particles (5–8). A wealth of data indicate that structure and composition of such ribonucleoprotein complexes (RNPs) are not static, and that changes in RNP structure and/or composition need to be accurately timed to ensure correct RNP function (9–11).

One group of enzymes pivotal for facilitating rearrangements of cellular RNPs are the DExH/D proteins (11,12).

These enzymes are known to manipulate RNA structure in an ATP-dependent manner (13). Members of the DExH/D protein family are present in all forms of cellular life and also in several viruses (12,14). DExH/D proteins are highly conserved, sharing at least eight characteristic sequence motifs (12). The characteristic sequence motif II often takes the form DEAD, DEAH or DExH [in single letter amino acid code (15)]. These signatures provide the names for the three subgroups of the protein family (12). The name DExH/D proteins stems from ‘averaging’ the signatures of the three subgroups.

DExH/D proteins are involved in virtually all aspects of RNA metabolism (12). Significant evidence suggests that these proteins act as ATP-driven motors or switches at very specific points in processes such as pre-mRNA splicing or during ribosome biogenesis (12). Yet, it is unknown where precisely the vast majority of DExH/D proteins bind to their targets and which exact conformational changes these proteins catalyze in their respective substrates. Nonetheless, physiological functions of many DExH/D proteins correlate with the ability of the enzymes to hydrolyze ATP in an RNA-stimulated manner, and/or to unwind RNA duplexes *in vitro* in an ATP-dependent fashion (16). For these reasons, and because of the intuitive connection between the unwinding of RNA secondary structure and conformational changes in RNPs, it has long been assumed that RNA helicase activity is central to the biological function of DExH/D proteins. In fact, DExH/D proteins are frequently referred to as RNA helicases.

However, because cellular RNAs are invariably complexed with proteins, DExH/D proteins are most likely to encounter RNA–protein complexes, rather than the pure RNA duplexes that are commonly used to measure RNA helicase activity *in vitro* (4,16,17). Yet, we are only beginning to understand how DExH/D proteins remodel RNPs. Here we review recent results indicating that DExH/D proteins can directly target RNA–protein interactions both *in vivo* and *in vitro* and that RNP remodeling does not necessarily require RNA duplex unwinding. Based on the available data, we outline a basic mechanism by which DExH/D proteins may displace proteins from RNA.

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DEXH/D PROTEINS THAT TARGET RNA-PROTEIN INTERACTIONS: PHYSIOLOGICAL EXAMPLES

The first specific examples of DEXH/D proteins targeting RNA-protein interactions emerged for Prp28p, Sub2p, Prp5p and Dbp5p (18–21). Dbp5p is involved in mRNA export (22), the other three proteins are essential components of the pre-mRNA splicing machinery (17). The DEAD-box protein Prp28p is involved in exchanging U1 snRNA with U6 snRNA on the 5' splice site and has been implicated specifically in the removal of the U1 snRNP from the 5' splice site (23) (Figure 1A). The U1 snRNP binds to the 5' splice site by forming a short RNA-RNA helix that is stabilized through several RNA-protein interactions (24,25). One of these stabilizing proteins is U1Cp, and Prp28 is thought to counteract the stabilizing effect of U1Cp (18,26). If U1Cp contains a mutation that reduces its affinity for the RNA, the otherwise essential Prp28p becomes dispensable, suggesting that Prp28p is responsible for the removal of U1Cp (18) (Figure 1A).

A similar bypass suppressor strategy illuminated the involvement of the DEAD-box protein Sub2p in the displacement of the protein Mud2p (19). Among other functions, which include a role on RNA export (27), Sub2p participates in early spliceosome assembly by promoting the exchange of the branch point-binding protein (BBP) with the U2 snRNP at the pre-mRNA branch site (28) (Figure 1B). The binding of BBPp to the branch site is presumably stabilized by the non-essential protein Mud2p (29). Deletion of Mud2 obviates the need for the essential Sub2p, consistent with a role of Sub2p in Mud2p displacement (19).

Prp5p is required for the stable addition of the U2 snRNP to the branch site, which normally depends on the ATPase activity of Prp5p (30). However, the interaction of the U2 snRNP with the branch site can also occur with ATPase-deficient Prp5p, but only if the non-essential protein Cus2p is deleted (20). Thus, the essential Prp5p cannot be completely bypassed by deletion of Cus2p, yet the normally essential ATPase activity of Prp5p can be made obsolete (20), suggesting that Prp5p dislodges Cus2p in an ATP-dependent manner (Figure 1C).

Recently, it has been shown that the DEAD-box protein Dbp5p, which functions in late steps of mRNA export on the cytoplasmic side of the nuclear pore complex, is also likely to be specifically required for displacement of the protein Mex67 from RNA (21). It was shown that in *dbp5* mutant cells, the mRNA export receptor Mex67 accumulates on mRNA, and that these Mex67 bound RNAs were enriched at the nuclear rim (21). The accumulation of Mex67 bound RNAs in *dbp5* mutant cells were suppressed by a *mex67* mutation, consistent with a scenario where Dbp5p removes Mex67 from the RNA (Figure 1D).

Although the observations for Prp28p, Sub2p, Prp5p and Dbp5p strongly suggested the involvement of these enzymes in the removal of other proteins from RNA, the mechanisms by which DEXH/D enzymes caused protein displacements remained unclear (17). For example, the data obtained with Prp28p, Sub2p, Prp5p and Dbp5p did not illuminate whether DEXH/D proteins rely on other cofactors or on a specific context to displace proteins, or whether DEXH/D helicases alone

are sufficient to dislodge other proteins. It also remained unclear whether DEXH/D proteins are able to act directly on the respective RNA-protein interaction, or whether the enzymes displace proteins only indirectly, possibly through the remodeling of RNA secondary structure.

RNP REARRANGEMENT BY DEXH/D PROTEINS *IN VITRO*

To elucidate the basic mechanism(s) of protein displacement by DEXH/D proteins, it is critical to quantitatively analyze RNP remodeling reactions. Since it is unknown where precisely the vast majority of DEXH/D proteins bind to their targets and which exact conformational changes the enzymes catalyze in their respective substrates (12,15), it has not yet been possible to devise model systems that recapitulate a physiological RNP remodeling reaction that can be analyzed quantitatively as well. For example, complex *in vitro* systems such as pre-mRNA splicing extracts provide invaluable qualitative information about DEXH/D protein function, but the limited control over parameters such as concentrations of individual factors precludes the use of these systems for quantitative mechanistic studies. Thus, it has been only possible to obtain quantitative mechanistic information about RNP remodeling by DEXH/D proteins with simple, yet non-physiological RNP models (31–33).

The first RNP used for such model studies consisted of two RNA strands which formed a binding site for the prototypical RNA-binding protein U1A (31) (Figure 2A). U1A is part of the pre-mRNA splicing machinery, and it also acts as a feedback inhibitor for its own gene expression (34,35). In the model RNP, U1A forms a homo-dimer on the RNA, contacting predominantly the single-stranded loops embedded in the helical regions of the RNA (36) (Figure 2A). It was tested whether the DEXH RNA helicase NPH-II could displace U1A from the RNA (31). NPH-II, which is involved in the replication of vaccinia virus (37), was selected for these protein displacement studies because a basic mechanism existed for the RNA unwinding activity of this enzyme (38).

NPH-II increased the dissociation rate constant of U1A from the RNA by more than three orders of magnitude in an ATP-dependent fashion (31). That is, NPH-II did not 'wait' until U1A dissociated spontaneously to then unwind the U1A-binding site; rather, NPH-II actively displaced U1A (31). Further kinetic analysis of the RNP remodeling showed that U1A displacement was in fact faster than RNA unwinding by NPH-II. The processivity of NPH-II was decreased, but not completely eliminated by the U1A displacement event, i.e. the enzyme could continue to unwind RNA duplexes after dislodging U1A without first leaving the RNA (31).

These results established that DEXH/D proteins could directly and actively displace stably bound proteins from RNA in an ATP-dependent reaction. Thus, in principle, no other cofactors are necessary to adapt 'RNA helicases' to displace proteins from RNA (16). However, observations made with this U1A-based model RNP do not preclude the requirement of other factors or a specific context for protein displacement by other DEXH/D proteins.

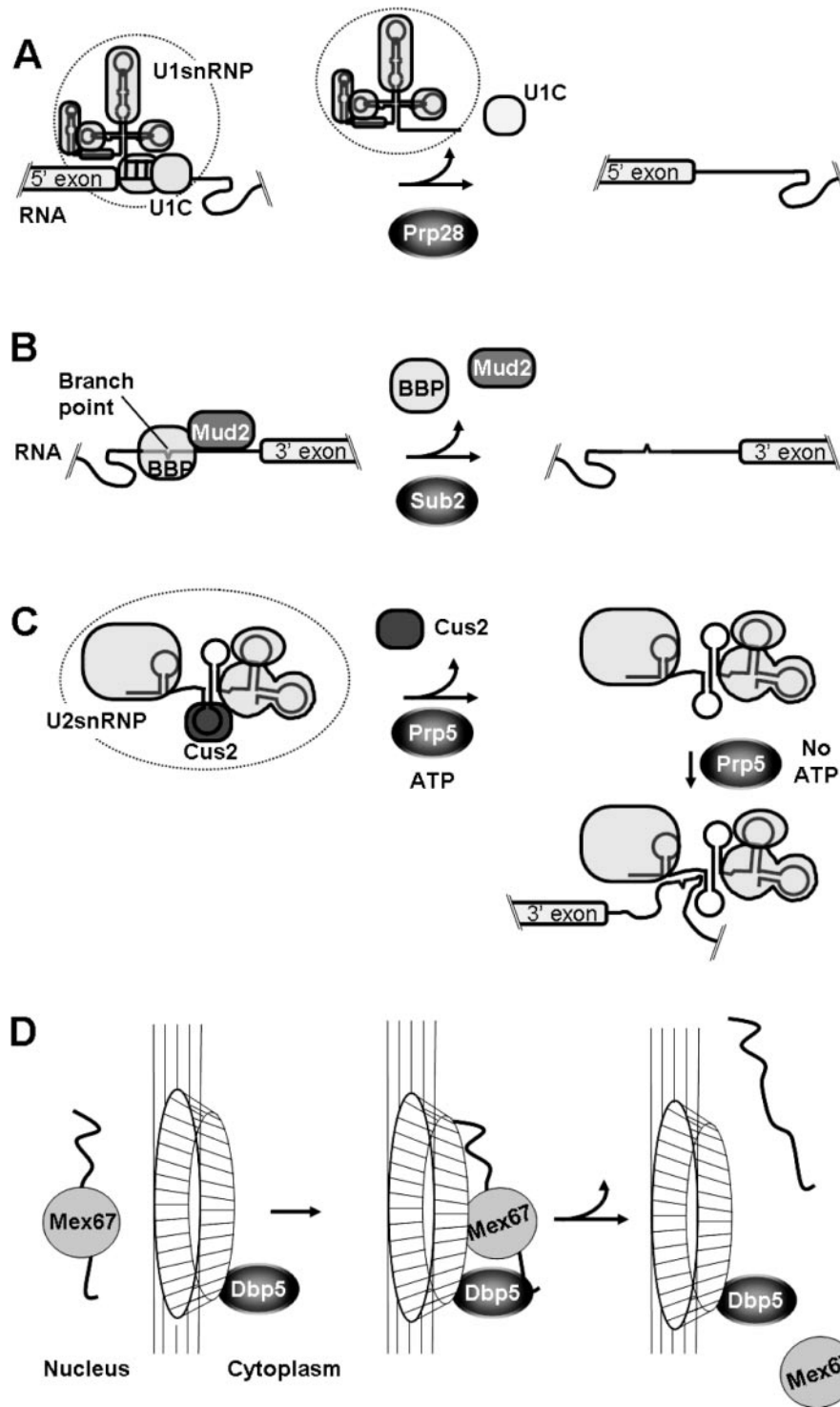


Figure 1. DEXH/D proteins targeting RNA–protein interactions: physiological examples. (A) Displacement of the U1snRNP from an RNA containing a 5' splice site by Prp28p. (B) Displacement of Mud2p by Sub2p. (C) ATP-dependent displacement of Cus2p by Prp5p. The second step shows the ATP-independent binding of U2 snRNP to the branch site of the pre-mRNA, which is facilitated by Prp5 in an ATP-independent fashion. (D) Displacement of Mex67p by Dbp5p. Dbp5p is bound to the nuclear pore complex on the cytoplasmic side of the nuclear rim. As mRNAs are transported through the nuclear pore, Dbp5p specifically facilitates the release of Mex67.

While the above results clearly showed active protein displacement by NPH-II, it remained unclear whether protein displacement required the unwinding of RNA secondary structure, which in the U1A-based RNP surrounded the

protein-binding site (Figure 2A). To test whether DEXH/D RNA helicases could also displace proteins from RNA without unwinding any duplexes, two model RNPs were devised where RNA secondary structure played no role in

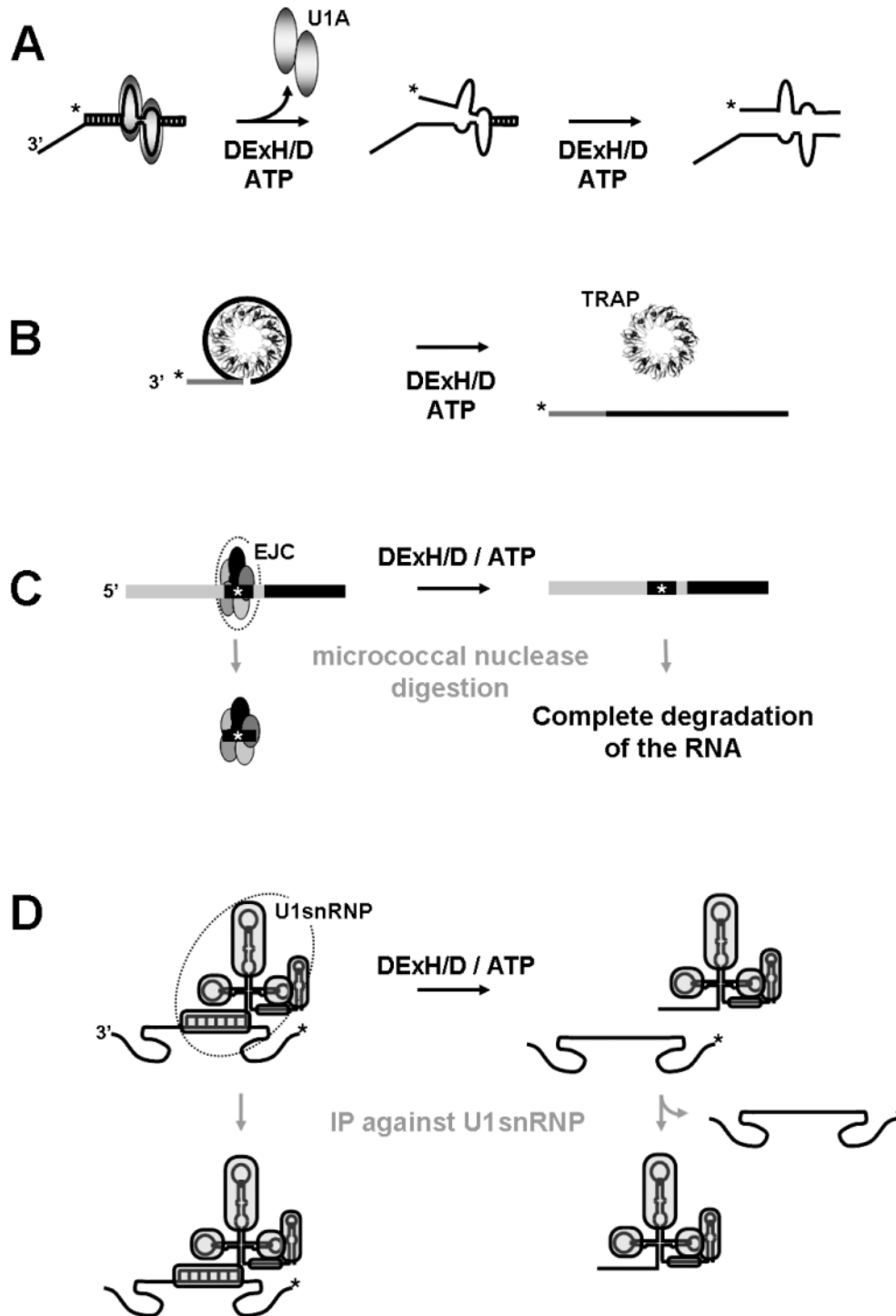


Figure 2. Model systems for measuring RNP remodeling by DExH/D proteins *in vitro*. (A) U1A-based RNP. Ovals indicate U1A, lines the RNA, and arrows represent the reactions catalyzed by the DExH/D protein. RNA–protein complex and free RNA is visualized on non-denaturing PAGE. (B) Trap-based RNP. RNA–protein complex and free RNA is visualized on non-denaturing PAGE. (C) EJC-based RNP. The RNA contains specific radiolabel (asterisk) in the EJC-binding region. Removal of the EJC renders the region protected previously from nuclease digestion by the EJC susceptible to degradation. Degradation was visualized on denaturing PAGE. (D) Removal of the U1 snRNP from a radiolabeled (asterisk) RNA with a 5' splice site. Removal of the U1snRNP was monitored by immunoprecipitation of U1snRNP and subsequent quantification of radioactivity in supernatant and immunoprecipitate.

protein binding (32). One complex was formed between the tryptophan RNA-binding attenuation protein (TRAP) and its specific 53-nt-long cognate RNA (Figure 2B). TRAP binds to this RNA in a sequence-specific manner as a 11-unit

oligomer, and its affinity can be modulated by tryptophan (39). The second complex was the multi-component exon junction complex (EJC) that is deposited on mRNAs as a consequence of splicing (40) (Figure 2C). While the exact

Table 1. Remodeling of four different RNPs by DED1 and NPH-II

RNP	Binding site	K_d^a	k_{off}^b	NPH-II $k_{displace}^c$	Rel. accel. ^d	DED1 $k_{displace}^c$	Rel. accel.	References
U1A	15 nt ^e	5.1 nM	$1.7 \times 10^{-3} \text{ min}^{-1}$	$>50 \text{ min}^{-1}$	$> 2.9 \times 10^4$	$7.9 \times 10^{-4} \text{ min}^{-1}$	<1	(31,33)
TRAP	53 nt	0.5 nM	$2.0 \times 10^{-3} \text{ min}^{-1}$	$>8 \text{ min}^{-1}$	$> 4 \times 10^4$	$<2 \times 10^{-3} \text{ min}^{-1}$	<1	(32)
U1snRNP	7 bp/~30 nt ^f	4 nM	$1.2 \times 10^{-3} \text{ min}^{-1}$	$>6 \text{ min}^{-1}$	$> 5 \times 10^3$	$>6 \text{ min}^{-1}$	$>5 \times 10^3$	(33)
EJC	8/10 nt ^g	— ^h	$<10^{-5} \text{ min}^{-1}$	$3.2 \times 10^{-2} \text{ min}^{-1}$	$> 3.2 \times 10^3$	$2.8 \times 10^{-2} \text{ min}^{-1}$	$>2.8 \times 10^3$	(32)

^a K_d , equilibrium binding constant of the protein(complex) to the RNA substrate.

^b k_{off} , dissociation rate constant of the protein (complex) from the RNA substrate.

^c $k_{displace}$, rate constant of protein displacement by the DEXH/D protein.

^dRel. accel., relative enhancement of the dissociation rate constant by the DEXH/D protein.

^eBinding site size judged from the NMR structure of the U1A–RNA complex (31).

^fThe U1snRNP forms a 7 bp helix and with the substrate RNA. In addition, ~30 nt in the mRNA substrate are protected from digestion with micrococcal nuclease (33), suggesting an U1snRNP-binding site on the mRNA substrate of ~30 nt.

^gAn equilibrium dissociation constant (K_d) cannot be determined because the EJC is deposited on the RNA during pre-mRNA splicing (32).

^hDigestion of the RNA with bound EJC using micrococcal nuclease yields two bands of 8 and 10 nt (32).

composition of the EJC is presently unclear, the complex is known to contain at least five distinct proteins that bind tightly in a non-sequence-specific manner ~20 nt upstream of exon junctions (40). The EJC plays a variety of roles in postprocessing mRNA metabolism, including nonsense-mediated decay and translational efficiency (41).

In an ATP-dependent fashion, NPH-II accelerated the dissociation rate constants for both the TRAP and the EJC-based complexes by several orders of magnitude (32). Thus, NPH-II actively displaced protein complexes from unstructured RNA, indicating that unwinding of RNA duplexes was not required for the removal of proteins from RNA (32). However, while NPH-II actively disrupted both RNPs, the EJC was displaced at a significantly slower rate than TRAP, suggesting that the properties of a given RNP might affect the rate by which it can be remodeled by DEXH/D proteins (32) (Table 1).

The ability of a DEXH/D protein to perform ATP-driven conformational work on single-stranded RNA is thought to resemble the movement (tracking) of the enzyme on single-stranded nucleic acids that has been observed for some SF1 DNA helicases (42). A notable difference may be the inability of many DEXH/D proteins to move with high processivity on the RNA. Many DEXH/D proteins may only be able to track one or a few steps before dissociating from the RNA.

The active displacement of U1A, TRAP and the EJC by NPH-II indicated that DEXH/D proteins could efficiently disrupt RNA–protein interactions. However, many physiological RNPs, such as the spliceosomal complexes that contain the small nuclear RNAs, bind to their targets through a combination of RNA–RNA and RNA–protein interactions (11). To illuminate the range of RNPs which DEXH/D proteins can disassemble, it was tested whether NPH-II could displace the U1snRNP, a complex that bound its target through such a combination of RNA–RNA and multiple RNA–protein interactions (33) (Figure 2D). The U1snRNP is part of the eukaryotic splicing apparatus where it is involved in the recognition of the 5' splice site (43). NPH-II accelerated the dissociation of purified U1snRNP from a substrate RNA (with an authentic 5' splice site) in an ATP-dependent fashion, indicating that the enzyme could actively disrupt a more complex RNA–protein interface (33) (Figure 2D).

Experiments with four simple model RNPs had demonstrated the ability of the DEXH/D protein NPH-II to actively

displace a diverse range protein complexes from RNA (Table 1). It was then critical to test whether the efficient RNP remodeling seen with NPH-II was also observed for other DEXH/D proteins, especially for enzymes that displayed lower ATPase and RNA helicase activities than NPH-II (32). Therefore, protein displacement was measured for the DEAD-box protein DED1 from *Saccharomyces cerevisiae*, which is phylogenetically distant from NPH-II (32,33). [For a detailed review on DED1 see Ref. (44).] DED1 efficiently unwinds short RNA duplexes (45), but the enzyme appears significantly less processive than NPH-II (E. Jankowsky, *et al.*, unpublished data). The RNA-stimulated ATPase activity of DED1 is also considerably lower than that of NPH-II (45).

Nevertheless, DED1 actively displaced the EJC and the U1snRNP from their respective RNAs, even with an efficiency comparable to that seen with NPH-II (Table 1). However, DED1 was unable to accelerate the dissociation of U1A or TRAP from their target RNAs (Table 1). In fact, the kinetics for separation of the RNA strands of the U1A-based RNP with U1A bound closely matched the kinetics of spontaneous U1A dissociation, indicating that DED1 did not actively displace U1A from the RNA (33). Collectively, these data demonstrated that the ability of DEXH/D proteins to actively remodel RNPs is not restricted to NPH-II. Yet, different enzymes do not necessarily disrupt the same range of RNP substrates in an active fashion.

To understand the functional differences between the two helicases, it will be critical to elucidate which features make an RNP susceptible to an active disruption by DED1. Although no results have yet been reported that specifically address this question, the available data suggest that basic macroscopic physicochemical properties of the RNP such as the affinity for the RNA and the dissociation rate constant are unlikely to dictate whether a given RNP complex will be actively disassembled by DED1 (Table 1). However, it is worth noting that DED1 actively disrupted the two RNPs that bind their target RNA through multiple different proteins, whereas the enzyme failed to actively displace the two homo-oligomers U1A and TRAP. Perhaps the arrangement of individual protein units within an RNP may determine whether DED1 can actively disrupt a given RNA–protein complex.

A POSSIBLE MECHANISM FOR ACTIVE PROTEIN DISPLACEMENT BY DEXH/D PROTEINS

To reconcile the observations made during the remodeling of the four tested RNPs by both NPH-II and DED1, it is instructive to propose a basic mechanism by which DEXH/D proteins actively displace proteins from RNA. We emphasize that although the currently available data set is very limited with respect to the diversity of RNPs and DEXH/D enzymes, a mechanism needs to be consistent with a rather complex set of observations, including (i) the ability of both NPH-II and DED1 to displace proteins without unwinding RNA secondary structure, (ii) the ability of NPH-II to actively displace a greater range of proteins than DED1, and (iii) the capacity of DED1 to actively disassemble the stable multi-component RNPs but not the stable homo-oligomeric RNPs tested (Table 1).

We propose that the ability of a DEXH/D protein to actively disrupt a given RNP is based on the capacity of the enzyme to track on single-stranded RNA in an ATP-dependent fashion, which explains the ability of NPH-II and DED1 to displace proteins without unwinding RNA secondary structure. During the tracking, the DEXH/D protein is able to capture nucleotides that are normally part of the RNA–protein interface of the RNP. This reduction in the number of RNA–protein contacts increases the propensity of the protein(s) to dissociate from the RNA (Figure 3A, step 1). Additional advance of the enzyme on the RNA and the sequestering of more nucleotides further reduces the number

of RNA–protein contacts in the RNP, eventually leading to the dissociation of the RNP at a rate greater than that of spontaneous RNP dissociation, i.e. the protein is actively displaced from the RNA (Figure 3A, step 2). If the DEXH/D protein dissociates from the RNA before capturing the critical number of nucleotides necessary to accelerate dissociation of the RNP, no active protein displacement is observed (Figure 3A). Therefore, a DEXH/D protein tracking fast and processively (e.g. NPH-II) should be able to actively disassemble a wider range of RNPs than an enzyme that tracks slowly and/or with low (or no) processivity (e.g. DED1). This prediction is consistent with our observations: the processive NPH-II actively displaces a greater range of proteins than the less processive DED1.

However, active protein displacement is observed even with a less processive enzyme, provided the capture of one (or few) nucleotides from the RNA–protein interface suffices to accelerate the dissociation of the RNP (Figure 3B). For example, a small decrease in the RNA–protein interface in a multi-component complex such as the EJC might lead to dissociation of one critical component that in turn unravels the entire RNP (Figure 3B). This scenario would provide a basis to explain why DED1 actively disassembles the stable multi-component RNPs but not the stable homo-oligomeric RNPs tested (Table 1).

It is unclear how exactly the nucleotides are captured by the DEXH/D proteins. Conceivably, the DEXH/D helicase

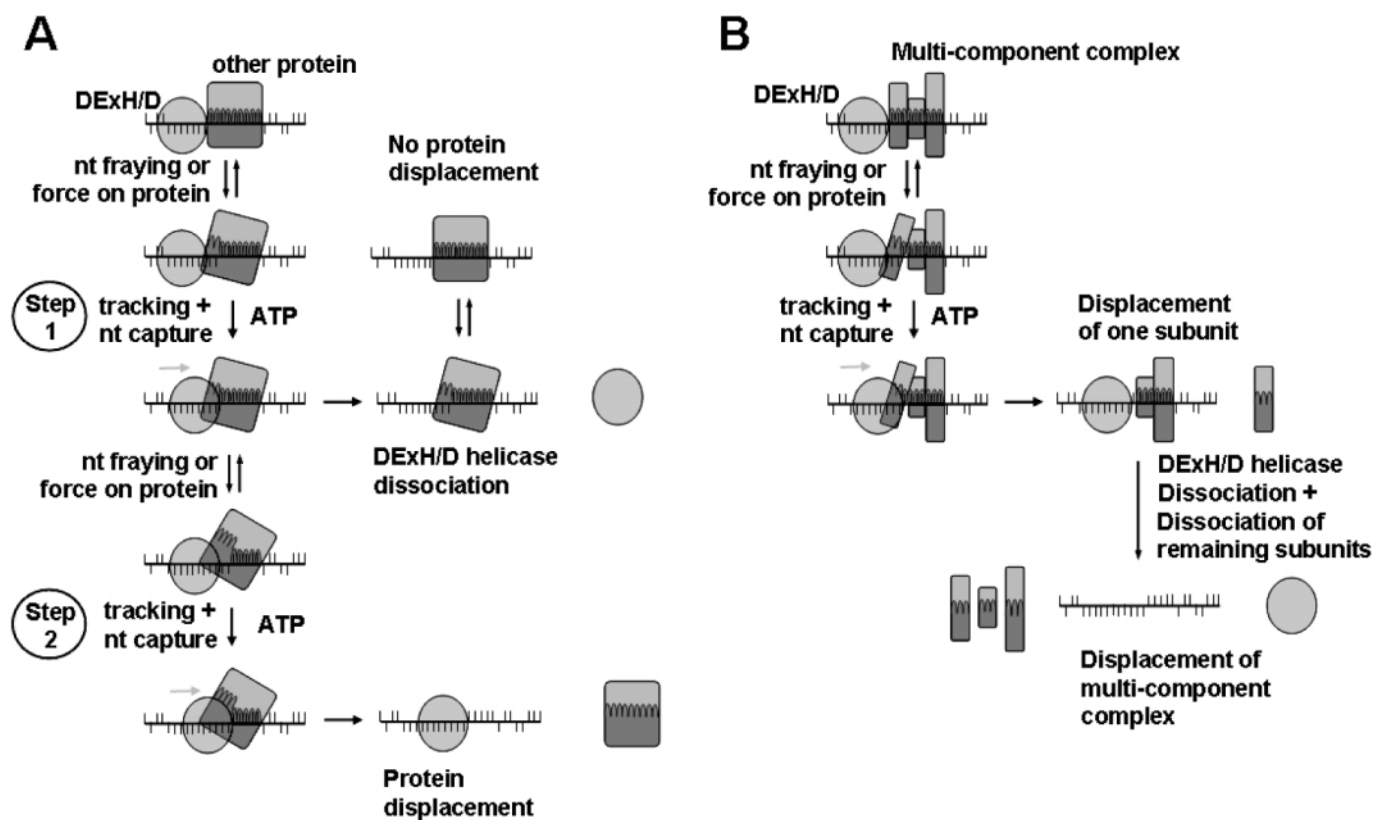


Figure 3. Possible mechanism for active protein displacement by DEXH/D proteins. (A) Schematic representations for the displacement of a single (homopolymeric) protein from RNA. The line represents the RNA. (B) Schematic representations for the displacement of a multi-component protein complex from RNA.

could exert force on the other protein to ‘free’ one or several nucleotides, although it is not known whether DExH/D proteins can produce sufficient force when tracking on RNA. Alternatively, the DExH/D helicase could simply sequester transiently fraying nucleotides during the tracking on RNA. We note that tracking of a ‘helicase’ on single-stranded nucleic acid and capture of fraying nucleotides is also considered to be important for the unwinding of DNA or RNA duplexes by these enzymes (46). There, a tracking helicase is proposed to capture fraying nucleotides from the termini of the helix interface (46). It is perhaps not surprising that similar mechanisms may underlie both duplex unwinding and protein displacement by ‘helicase’ enzymes.

Because of the limited amount of data for RNP remodeling by DExH/D proteins, other mechanisms by which these enzymes cause active protein displacement should not be discounted. For example, it may be possible that, instead of capturing one or few nucleotides at a time, DExH/D proteins may simply force the entire protein off the nucleic acid by physical clashes between protein domains. While it is unknown whether DExH/D proteins can exert sufficient force when tracking on RNA, as mentioned above, it is well established that DExH/D proteins change their domain orientations upon ATP binding/hydrolysis (47). Such large-scale conformational changes could be used to induce a physical clash between the DExH/D enzyme and other proteins. However, this scenario is more difficult to reconcile with the similar rate constants by which DED1 and NPH-II displace the EJC, given the different rates by which both enzymes turn over ATP.

SUMMARY AND PERSPECTIVE

Using simple RNP models to study protein displacement by DExH/D proteins has provided three major insights. First, DExH/D proteins can directly remodel RNPs, i.e. the enzymes do not *per se* require specific cofactors that turn ‘RNA helicases’ into RNP remodelers. Second, RNP remodeling does not necessarily require RNA duplex unwinding. Third, not all DExH/D proteins are able to actively disassemble the same range of RNPs. The proposed mechanism by which DExH/D proteins actively displace proteins from RNA may present a starting point for more targeted questions about the mechanism(s) of RNP remodeling by DExH/D proteins such as defining conformational changes that DExH/D proteins induce in RNA in order to displace proteins. Moreover, it will be critical to investigate the rearrangement of more diverse RNPs by a larger range of DExH/D proteins. It also remains of central importance to elucidate how DExH/D proteins remodel RNPs *in vivo*. Finally, it will be illuminating to compare mechanisms of protein displacement by DExH/D proteins with mechanisms that DNA helicases use to remove proteins from DNA (48–50).

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