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Distinct RNA unwinding mechanisms of DEAD-box and DEAH-box RNA helicase proteins in remodeling structured RNAs and RNPs

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Abstract

Structured RNAs and RNA-protein complexes (RNPs) fold through complex pathways that are replete with misfolded traps, and many RNAs and RNPs undergo extensive conformational changes during their functional cycles. These folding steps and conformational transitions are frequently promoted by RNA chaperone proteins, notably by superfamily 2 (SF2) RNA helicase proteins. The two largest families of SF2 helicases, DEAD-box and DEAH-box proteins, share evolutionarily conserved helicase cores but unwind RNA helices through distinct mechanisms. Recent work has advanced our understanding of how their distinct mechanisms enable DEAD-box proteins to disrupt RNA base pairs on the surfaces of structured RNAs and RNPs, while some DEAH-box proteins are adept at disrupting base pairs in the interior of RNPs. Proteins from these families use these mechanisms to chaperone folding and promote rearrangements of structured RNAs and RNPs, including the spliceosome, and may use related mechanisms to maintain cellular mRNAs in unfolded or partially unfolded conformations.

Introduction

Structured RNAs carry out a wide range of regulatory and catalytic functions in all domains of life. RNA folding is a hierarchical process in which rapid formation of local secondary structure, *i.e.* short helices, allows for formation of tertiary contacts between elements that are widely separated in sequence. With only four standard bases and the potential for non-canonical pairs beyond the standard Watson-Crick pairings, structured RNAs are plagued by a tendency to form non-native secondary structure elements (1, 2). Local non-native structures can be further stabilized by tertiary structure, resulting in misfolded structures that require large-scale remodeling by RNA chaperone proteins to form native conformations at biologically relevant rates (3). Even messenger RNAs (mRNAs), which must adopt single-stranded conformations to be actively translated by processing ribosomes, contain many sequences capable of forming local structures that must be at least transiently unfolded to permit movement of the ribosome in translation (4, 5).

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Additionally, many processes in RNA metabolism rely on base pairing between multiple RNAs or formation of RNPs to mark the correct positions for processing steps. Nowhere is this more evident than in eukaryotic pre-mRNA splicing. In each cycle of splicing, small nuclear RNAs (snRNAs) and protein splicing factors must assemble to form a functional spliceosome RNP and then must be extensively remodeled through the splicing process. These assembly steps and rearrangements are accelerated by a set of DEAD-box and DEAH-box proteins (6, 7). There is a clear division of labor between the two helicase families, with DEAD-box proteins accelerating early assembly steps and DEAH-box proteins accelerating RNP rearrangements in the downstream catalytic steps. This striking division raises the possibility that different unwinding mechanisms are required for forming the active spliceosome versus carrying out the splicing reaction (7).

DEAD-box Proteins: Remodeling, One Duplex at a Time

DEAD-box proteins, named for the amino acid sequence of a highly conserved motif, form the largest family of SF2 helicases, with 37 family members in humans and 26 in *S. cerevisiae* (8, 9). These enzymes function primarily as ATP-driven, non-processive helicases, binding and unwinding short, exposed RNA duplexes before releasing the RNA and repeating the process on another duplex segment (10). All DEAD-box proteins share a highly conserved helicase core that consists of two RecA-like domains (abbreviated D1 and D2) tethered by a short, flexible linker (Fig. 1). The core contains 13 conserved sequence motifs, many of which are implicated in specific steps of substrate binding and RNA duplex unwinding (11). In most DEAD-box proteins, the core is flanked by additional N- and/or C-terminal extensions, which contribute to the functional diversity of this protein family. Many of these extensions direct individual DEAD-box proteins to their functional targets by interacting with protein or RNA components of the targets, and some extensions modulate the activity of the helicase core (12, 13). The core itself binds short RNA duplexes without significant sequence specificity. It also binds ATP, with high specificity relative to other nucleoside triphosphates, to power cycles of RNA duplex unwinding (Table 1) (10, 14).

Work with the *S. cerevisiae* DEAD-box protein Mss116, which is required for efficient splicing of mitochondrial, self-splicing group I and group II introns, has suggested a mechanism by which the two core domains interact to unwind dsRNA. Attached by a flexible linker, the two domains remain ordered but spatially separated prior to substrate binding (Fig. 2A) (15, 16). ATP initially binds to D1, a property that seems to be universal among DEAD-box proteins, and dsRNA binds to D2 of Mss116 (15). In some DEAD-box proteins, initial RNA binding involves interactions with both domains, but upon formation of the ternary complex (enzyme, ATP, and dsRNA), unwinding proceeds similarly (17). A core closure step, which brings the two domains together and creates a functional ATPase active site, is driven by formation of inter-domain contacts between conserved amino acid motifs (18). Core closure significantly distorts the bound RNA, resulting in one strand becoming kinked and retained in a tight complex with the enzyme, while the other strand is released from its base pairs and from the complex (19–25). Mss116 and its homologs are unique among DEAD-box helicases in that they possess a C-terminal extension of D2 that forces a second kink in the retained strand, giving a ‘crimped’ conformation of this strand that may increase unwinding activity (Fig. 2B) (20). Recycling of the enzyme requires hydrolysis of

the bound ATP and release of inorganic phosphate (P_i) to allow efficient release of the tightly bound RNA strand (26–29).

This cycle produces a nonconventional helicase mechanism for DEAD-box proteins that does not involve translocation. Instead, unwinding is limited to short RNA duplexes and achieved in a single cycle of ATP-dependent conformational changes (28, 30). Instead of advancing along the RNA duplex, DEAD-box proteins rely on stochastic separation of the base pairs adjacent to the unwound section. Consequently, the unwinding process becomes less efficient as duplex length increases and re-annealing becomes more favorable upon incomplete unwinding by the DEAD-box protein. Though the basic mechanism is similar to unwinding, some DEAD-box proteins function primarily as nucleotide dependent RNA clamps and RNP assembly platforms. In these cases, associated protein factors trap the helicase core in the RNA-bound state by regulating steps in the ATPase cycle. A notable example is eIF4A-III, which forms the core of the exon junction complex (EJC) by remaining stably bound to mRNA following splicing. The MAGOH-Y14 dimer, also made up of EJC components, interacts with eIF4A-III and stabilizes its ADP-P_i-bound state, which binds tightly to RNA and enables the ‘clamping’ activity within the EJC (9, 31, 32).

In vitro experiments using the *Tetrahymena* ribozyme, a well-studied group I intron derivative, have shown how the local unwinding activity of a DEAD-box helicase can lead to remodeling of a complex, structured RNA (Fig. 2C). *In vitro*, the *Tetrahymena* ribozyme predominantly folds into a stable, misfolded state that is structurally similar to the native state but requires significant remodeling of secondary and tertiary structure to refold into an active conformation (33, 34). CYT-19, an Mss116 homolog from *Neurospora crassa*, was shown to accelerate this transition significantly in the presence of ATP, suggesting that it acts as a general RNA chaperone and raising the questions of how a helicase can accelerate folding transitions that include loss of tertiary contacts, and how a general chaperone is able to favor the formation of native structure over misfolded structure (35, 36). Single molecule FRET experiments showed that instead of prying open tertiary contacts, CYT-19 relies on a mechanism of “helix capture,” in which the enzyme binds to transiently exposed helical structures, preventing them from re-forming tertiary contacts with the larger RNA structure and weakening any other secondary or tertiary contacts that form cooperatively (37). CYT-19 can then unwind the captured helix, further disrupting local structure and exposing more helices for binding. This result was bolstered by the finding that ribozyme mutations that decrease tertiary stability and lead to less compact structures also increase stimulation of CYT-19’s ATPase activity (38). The dependence of CYT-19 activity on exposed duplex structures suggests that DEAD-box proteins act preferentially on nonnative structures that lack the extensive compaction and tertiary contacts found in natively folded RNAs.

For larger RNAs, some remodeling steps likely require multiple cycles of helix capture, unwinding, and ATP hydrolysis, which can be accelerated by tethering the DEAD-box helicase to the RNA substrate. Both CYT-19 and Mss116 contain arginine-rich C-tails that bind introns non-specifically and are thought to tether the helicase cores to perform multiple rounds of unwinding on helices within an RNA structure (39, 40). Recently it was shown that decreasing the number of arginines in the CYT-19 C-tail led to decreased unwinding activity on an exposed *Tetrahymena* ribozyme duplex, and increasing the number of

arginines increased the unwinding activity (41). These changes tracked directly with changes in functional RNA affinity. Although the basic C-tail is not ubiquitous among DEAD-box proteins, a similar mechanism of tethering the helicase core to structured RNAs or RNPs could be mediated by other unstructured domains, by assembly of the DEAD-box protein into a larger protein complex, or by specific interactions between an ancillary domain and a substrate RNA or RNP. A well-studied example of this last mechanism is the bacterial protein DbpA/YxiN, in which a folded ancillary domain localizes the protein by interacting specifically with an RNA helix within the large ribosomal subunit during ribosome biogenesis (42, 43). Recent results have suggested that the human DEAD-box protein DDX43 may follow a similar pattern, with an N-terminal K-homology nucleic acid-binding domain interacting with flanking RNA and imparting a preference for 5′-tailed dsRNA substrates (44).

DEAH-Box Proteins as Molecular Winches

DEAH-box proteins share many sequence and structural similarities with DEAD-box proteins, but they have adopted a very different mechanism of duplex unwinding (7). The DEAH-box core consists of two RecA-like domains, which contain the same set of conserved motifs as DEAD-box proteins but have notable differences in their sequences (Fig. 1). These core domains are flanked by N- and C-terminal extensions (8, 45). The N-terminal extensions show little conservation, and, as with enzymes from other families in SF2, often seem to be involved in recruiting the helicase to the correct complex or subcellular location (46). Unlike DEAD-box proteins, which contain a wide variety of non-conserved C-terminal extensions, DEAH-box proteins share a highly-conserved C-terminus made up of three domains: a winged helix (WH), a ratchet-like domain, and an oligosaccharide binding (OB) fold (45). Crystal structure show that these C-terminal domains interact strongly with the helicase core, yielding an enzyme with less flexibility in the core than DEAD-box proteins (47–49).

Whereas DEAD-box proteins use simple cycles of RNA duplex binding, unwinding, and release, DEAH-box proteins function as translocating helicases, advancing in the 3′–>5′ direction to disrupt nucleic acid structures (25, 50, 51). Instead of binding directly to structured RNA elements, DEAH-box helicases require 3′ single-stranded regions for unwinding activity (50, 52, 53). Unlike DEAD-box proteins, which are highly specific for dsRNA, some members of the DEAH-box family can act on both DNA and RNA, leading to unwinding of helices and, for some DEAH-box proteins, four-stranded G-quadruplex structures (54, 55). DEAH-box proteins also lack specificity for ATP, binding and hydrolyzing all four NTPs to power cycles of directional movement (Table 1) (8, 52, 56). Although many questions remain, recent structural and biochemical data have provided new insights into how these enzymes load onto a nucleic acid strand, couple ATP hydrolysis with translocation, and remodel RNP structures.

Recent structures of Prp43 from *C. thermophilum* have provided insight into how DEAH-box proteins load onto ssRNA adjacent to their target structures to initiate unwinding (57). Among other roles, Prp43 is required in the final step of pre-mRNA splicing, where it disrupts base pairing between the U2 snRNP and the intron branch point, releasing the U2,

U5, and U6 snRNPs from the lariat intron (58–62). Like other DEAH-box proteins, Prp43 lacks sequence specificity for RNA substrates, a property that was rationalized from the crystal structure of the enzyme bound to ADP-BeF_3 and U_7 RNA, as the majority of contacts are formed with the sugar-phosphate backbone of the RNA, not the uracil bases (57, 63). A similar structure without RNA showed that large conformational changes in the C-terminal domains, particularly the ratchet-like and OB-fold domains, lead to an opening of the RNA-binding tunnel between D2 and the C-terminal domains (Fig. 3A,B). When this opening step was blocked by a disulfide bridge, the mutant Prp43 was decreased substantially in RNA unwinding ability, suggesting that opening is required for efficient substrate loading (57).

Structural and biochemical experiments with Prp43 have also suggested a model for ATP-dependent translocation. Two extended amino acid hairpin structures serve as 3′ and 5′ bookends to the bases of a bound RNA stack, and their movement relative to the RNA strand during cycles of NTP hydrolysis is suggested to drive 3′->5′ translocation (51). The 3′ hairpin includes an extended motif Ib on domain 1, which is highly conserved among DEAH-box helicases and related DExH helicases, but varies significantly from those of other SF2 families (51, 64). The 5′ hairpin is a D2 structure that does not correspond to previously identified SF2 core motifs but is present in other DEAH/RHA proteins, the viral NS3 helicase, and as a shorter version in the related ski2-like helicases (64–67). These bookends trap the bound nucleic acid bases between them, and they move in cycles to incorporate a new base on the 5′ side and exclude one on the 3′ side, resulting in directional translocation of the helicase. This cycle is driven by changing interactions between conserved motifs as NTPs are bound, hydrolyzed, and released (64). The directional translocation is suggested to confer RNA unwinding activity by displacing the complementary strand(55).

In pre-mRNA splicing, several DEAH-box proteins are required to promote a series of base-pairing changes between spliceosomal snRNAs and the mRNA substrate within a larger RNP during the splicing process. While the basic biochemical properties of DEAH-box proteins outlined above would suggest that they would be capable of unwinding these helices, the burial of the helices inside the spliceosome has led to the question of how the helicase proteins gain access to their substrates. Recent biochemical experiments with the yeast DEAH-box proteins Prp16 and Prp22 have provided an exciting new insight, suggesting a model in which DEAH-box proteins can unwind helices without traversing through them (Fig 3C) (68). Following the branching step of splicing, Prp16 is required for repositioning the RNA substrate to enable recognition of the 3′ splice site, and it also plays a role in branch site selection (69). Prp22 serves similar functions downstream in the process, regulating selection of the 3′ splice site and releasing mRNA from the spliceosome following exon ligation (70). The key observation was that as long as Prp16 and Prp22 could load onto a downstream segment of ssRNA, their activity was not abrogated by DNA nucleotide substitutions between the loading site and the remodeled regions, even though the proteins are unable to translocate through these DNA stretches. These seemingly paradoxical results were reconciled with a model in which the DEAH-box proteins load onto a 3′ extension and begin translocating. However, when they are prevented from moving relative to the spliceosome by contact with the protein surface, the result of continued translocation

is to pull on the RNA strand, disrupting base pairs in a process termed winching (68). This novel model explains how DEAH-box proteins can promote unwinding of RNA helices that are buried inside the spliceosome without the helicases burrowing into the RNP. It remains to be determined whether this mechanism is shared by other DEAH-box proteins involved in splicing, such as Prp2 and Prp43, and whether it extends beyond the spliceosomal DEAH-box proteins.

Conclusions

We have discussed two distinct mechanisms by which RNA helicases can act as chaperones for RNAs and RNPs by disrupting RNA base pairs to permit remodeling steps. DEAD-box proteins rely on binding to exposed RNA duplexes and local, non-processive strand displacement to promote stepwise loss of compaction and ultimately allow RNAs a chance to refold. Though this activity is ubiquitous in all cells, the division of labor between DEAD-box and DEAH-box proteins in eukaryotic splicing suggests that this mechanism is not sufficient to remodel RNA structures occluded within the center of a larger RNP complex. The discovery that a subset of DEAH-box proteins use translocation to winch an RNA strand out of a larger structure, disrupting upstream base-pairing without translocating through the complex, suggests a simple solution to this problem.

It remains unclear whether these two mechanisms are universal for enzymes in their respective families. Although several well-characterized DEAD-box proteins have shown non-processive unwinding activities, partner proteins can dramatically alter their properties. Such appears to be the case for human eIF4A, which has been reported to undergo stepwise, processive duplex unwinding in the presence of eIF4G and eIF4B accessory proteins (71). It is also possible that some DEAD-box and/or DEAH-box proteins have evolved specifically for their individual functions in ways that endow them with properties quite distinct from the conventional properties of their families. Analysis of RNA and RNP remodeling reactions of a broader set of helicases from each family will ultimately shed light on the universality of these mechanisms.

Beyond the pathways discussed here, recent high-throughput experiments have shown that for mRNAs, duplex and G-quadruplex structures are actively unwound by robust, ATP-dependent RNA chaperone activities (72, 73). We still know relatively little about which RNA helicases are responsible, but DEAD-box and DEAH-box helicases are strong candidates for these activities. Indeed, the local duplex unwinding and winching mechanisms we have described for these helicases are likely to play important roles in these and other steps of RNA metabolism.

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Abbreviations

dsRNA	double-stranded RNA
ssRNA	single-stranded RNA
FRET	Förster resonance energy transfer
lncRNA	long non-coding RNA
P_i	inorganic phosphate
snRNP	small nuclear ribonucleoprotein complex

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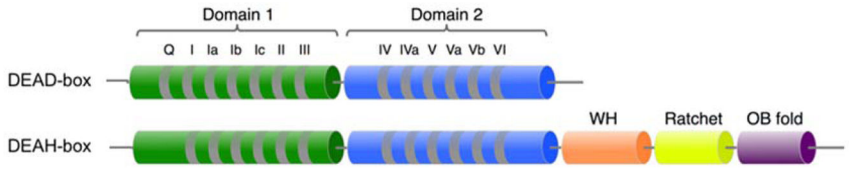

Figure 1.

Structural arrangement of conserved domains in DEAD-box and DEAH-box helicases. The protein families share two conserved, RecA-like domains with numbered sequence motifs that are highly conserved within each family. Many DEAD-box proteins have additional N- and C-terminal extensions that are required for their specific functions but are not conserved between different DEAD-box proteins and are not shown here. In contrast, DEAH-box proteins share conserved C-terminal domains consisting of winged helix (WH), ratchet-like, and oligosaccharide binding fold (OB fold) domains.

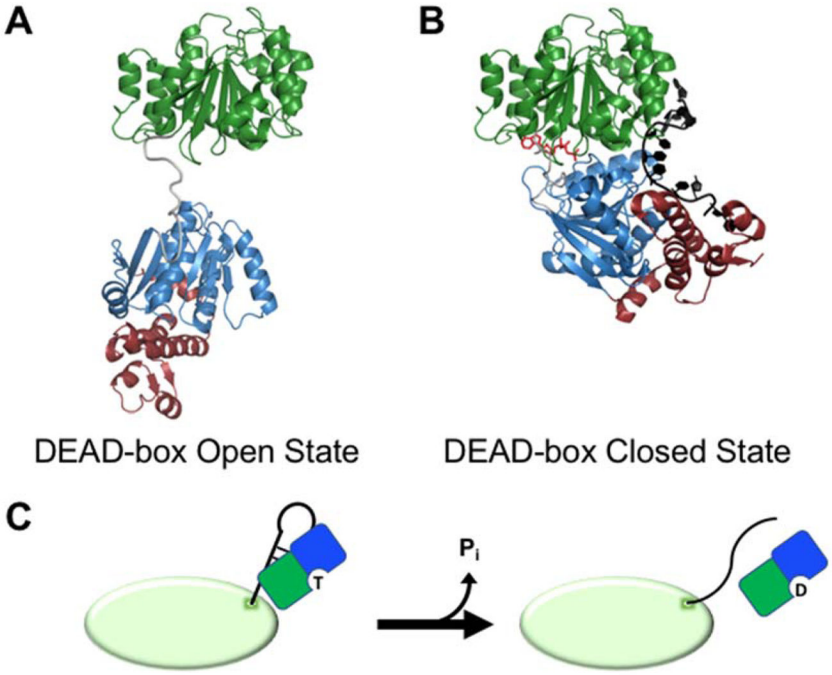

Figure 2.

Remodeling of exposed RNA duplexes by DEAD-box proteins. (**A**) Structural model for the open conformation of a DEAD-box protein (*S. cerevisiae* Mss116) prior to substrate binding (16). Conserved domains 1 and 2 are colored as in Figure 1. Mss116 and its homologs have an additional C-terminal extension (magenta) that is not present in all DEAD-box proteins. (**B**) Crystal structure representing the closed state of a DEAD-box protein (Mss116) following strand displacement (PDB: 3I5X) (20). The bound ssRNA strand is shown in black and the bound AMP-PNP molecule is red. (**C**) Model for unwinding of an exposed RNA duplex from a larger RNA or RNP by a DEAD-box helicase (shown as green and blue D1 and D2 respectively). The ATP-bound DEAD-box protein interacts with an exposed RNA helix on the surface of a structured RNA or RNP (green oval). Protein binding results in unwinding of the helix, allowing the partner strands to form new inter- or intramolecular interactions. ATP hydrolysis and release of P_i then allow the DEAD-box protein to release from the liberated ssRNA.

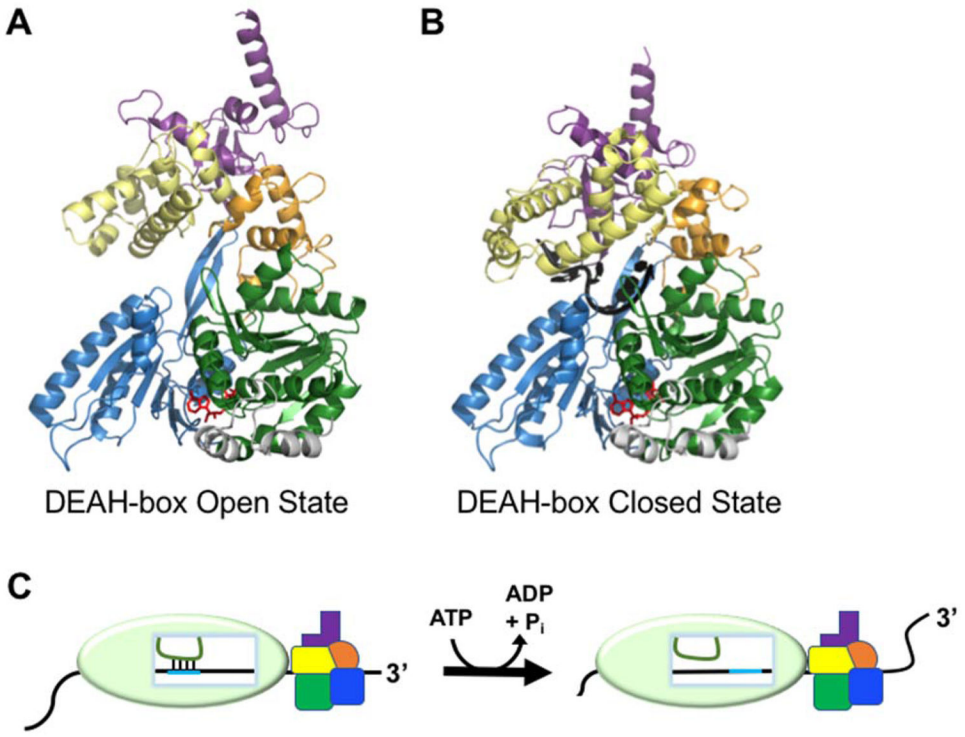

Figure 3.

Remodeling of internal RNA duplexes by DEAH-box proteins. (**A**) Crystal structure of Prp43 with bound ADP-BeF_x showing the open conformation, which facilitates RNA loading (PDB: 5LTK)(57). Domains are colored as in Fig. 1. (**B**) Crystal structure of the closed conformation of Prp43. The structure includes bound ADP-BeFx and U7 RNA and reflects the conformation that follows RNA loading (PDB: 5LTA)(57). (**C**) Model for DEAH-box helicase-catalyzed disruption of an RNA duplex within a larger RNP complex by molecular winching (68). Translocation of the protein against the RNP surface results in movement of the RNA (black) relative to the interior of the RNAP. Because its base-pairing partner (green) is held in place relative to the RNP, the movement of the black RNA results in disruption of the base pairs between the two RNAs.

Table 1

General Properties of DEAD-box and DEAH-box Helicases

Family	Unwinding Substrate	NTP Preference	Tail Preference	Translocation	Polarity
DEAD-box	RNA	ATP	none	none	none
DEAH-box	RNA or DNA	Any NTP	3′	Yes	3′ – 5′