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REVIEW

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Roles of DEAD-box proteins in RNA and RNP folding

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Key words: DEAD-box protein, RNA chaperone, RNA folding, RNP remodeling, RNA-protein interaction, misfolded RNA, RNA unwinding, RNA helicase, group I intron, pre-mRNA splicing

Abbreviations: RNP, ribonucleoprotein complex; SF2, superfamily 2

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RNAs and RNA-protein complexes (RNPs) traverse rugged energy landscapes as they fold to their native structures, and many continue to undergo conformational rearrangements as they function. Due to the inherent stability of local RNA structure, proteins are required to assist with RNA conformational transitions during initial folding and in exchange between functional structures. DEAD-box proteins are superfamily 2 RNA helicases that are ubiquitously involved in RNA-mediated processes. Some of these proteins use an ATP-dependent cycle of conformational changes to disrupt RNA structure nonprocessively, accelerating structural transitions of RNAs and RNPs in a manner that bears a strong resemblance to the activities of certain groups of protein chaperones. This review summarizes recent work using model substrates and tractable self-splicing intron RNAs, which has given new insights into how DEAD-box proteins promote RNA folding steps and conformational transitions, and it summarizes recent progress in identifying sites and mechanisms of DEAD-box protein activity within more complex cellular targets.

Introduction

Functional RNAs are ubiquitous in processes that make up the central dogma of molecular biology, including pre-mRNA and tRNA processing, protein synthesis, and the regulation of gene expression. They also play critical roles in processes that extend well beyond the central dogma, such as intracellular protein trafficking and the maintenance of chromosome ends.[1-6] Indeed, recent findings of pervasive transcription of prokaryotic and eukaryotic genomes have established that non-coding RNAs greatly exceed mRNAs,[7,8] and even large, complex RNAs continue to be discovered.[9,10] Many cellular RNAs have functions that require folding into highly specific three-dimensional structures, and many of these RNAs must also cycle or exchange between functional structures during their reactions. This requirement creates a fundamental challenge because local RNA structure can be independently stable, and thus the energy barriers between alternative structures are often large enough to block interconversion on the biological timescale.[11,12] Consideration of these basic properties led to the prediction that RNA chaperone proteins would be necessary to interact transiently with RNAs and RNA-protein complexes (RNPs) and accelerate transitions between alternative conformations,[12] and since then RNA chaperone activities have indeed been demonstrated for several groups of proteins (reviewed in ref. 13–16).

One critical group is the DEAD-box proteins. It is the largest family within the superfamily 2 (SF2) RNA helicases, and present in all three branches of life.[17] At least one DEAD-box or related protein is required for essentially all RNA-mediated processes.[18,19] These proteins have diverse roles and biochemical activities, but many of them use a cycle of ATP-dependent conformational changes to facilitate folding and remodeling transitions of RNAs and RNPs. Here we review the properties of DEAD-box proteins as RNA helicases and chaperones, and we outline current views on how DEAD-box proteins resolve misfolded RNAs and use a range of mechanisms to promote RNA and RNP folding transitions and remodeling. We focus on work using group I intron RNAs, with other RNAs and related RNA helicase proteins noted briefly. The roles of DEAD-box proteins in group II intron folding have been recently reviewed,[20-22] as have the biochemical activities and cellular roles of other classes of RNA chaperones.[13,15]

DEAD-box Proteins: Superfamily 2 RNA "Helicases"

DEAD-box proteins share with other superfamily 1 and 2 helicases a conserved core of two RecA-like domains connected by a flexible linker.[17-19,23] At least eleven conserved motifs lie near the interface of these two domains (**Fig. 1**).[23-29] ATP binds on one side of the interface, forming interactions with several motifs. The triphosphate moiety interacts with motif I (specifically the P-loop), motif II including the eponymous D-E-A-D sequence, and motif VI. In addition, motif V is thought to contact the ribose of ATP,[23] and the adenine base forms specific interactions with the Q-motif, which is diverged in most other SF2 helicases and gives rise to a strong preference in DEAD-box proteins for ATP over other nucleoside triphosphates.[30,31] Motif III lies near the γ-phosphoryl group of ATP and is suggested to assist in positioning it for hydrolysis.[32] On the opposite side of the domain

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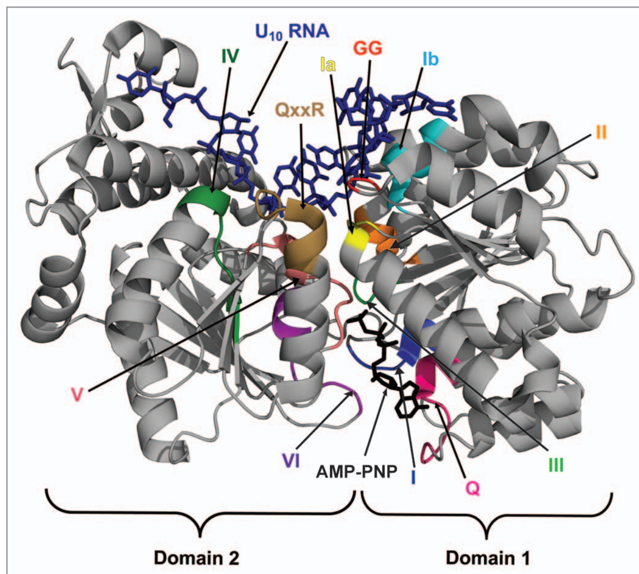

Figure 1. DEAD-box protein structure and RNA unwinding mechanism. (A) Crystal structure of DEAD-box protein Mss116p with bound AMP-PNP (black, at the bottom of the domain interface) and U_{10} ssRNA (dark blue, at the top of the domain interface).[24] The conserved motifs are highlighted and labeled. Domain 1 is on the right.

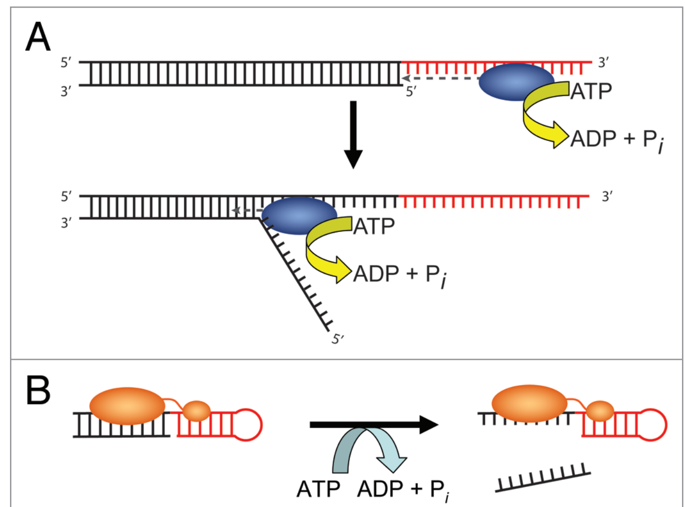

Figure 2. Models for duplex unwinding by conventional helicases and DEAD-box proteins. (A) Conventional helicases load onto single-stranded overhangs of a defined polarity (red) and then translocate into the duplex region (black), displacing the complementary strand. (B) DEAD-box proteins unwind short duplexes in an ATP-dependent but nonprocessive manner by initiating unwinding internally, within a duplex region (black). Some DEAD-box proteins form additional interactions with adjacent single-stranded or double-stranded segments (red), which are suggested to tether the core in position to disrupt nearby helices.

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interface, motifs Ia, GG and Ib from domain 1, along with motifs IV, QxxR and V from domain 2, mediate binding of a segment of single-stranded RNA (ssRNA).[23]

A conserved feature of DEAD-box proteins is that the binding sites for ssRNA and adenosine nucleotide interact energetically to give coupled binding of the two ligands. Studies using ATP and non-hydrolyzable analogs revealed a conformational change, coordinated by a network of interactions between several motifs, that "closes" the two domains of the helicase core, such that they interact extensively with each other and give tight binding of nucleotide and ssRNA.[32-36] Upon hydrolysis of ATP, release of inorganic phosphate (P_i) is a key step.[37] Prior to release of P_i, nucleotide and RNA binding are positively coupled and RNA is bound at least as tightly with ADP and P_i as with ATP.[38] In contrast, upon release of P_i, a conformational change occurs that results in much weaker binding of RNA in the ADP-bound state due to anti-cooperativity.[33]

This cycle of regulated ssRNA binding affinity is thought to be critical for most or all of the activities of DEAD-box proteins, including RNA unwinding activity. Although this unwinding activity can crudely be referred to as helicase activity, the mechanisms of DEAD-box proteins are substantially different from conventional helicases in ways that make them poorly suited to unwind long, continuous helices but very well-suited to physiological roles in manipulating structured RNAs and RNPs (**Fig. 2**). First, whereas conventional helicases unwind helices by translocating along one of the strands and typically have processivity measured in the range of hundreds to thousands of base pairs,[39,40] DEAD-box proteins have very low processivity, displaying little or no unwinding of helices longer than about 25–40 base pairs.[41-43] Increased duplex stability, without increased length, also severely inhibits DEAD-box proteins, and they are proposed to use an unwinding mechanism that does not involve translocation.[43-46] Instead of moving along the duplex, DEAD-box proteins can initiate unwinding internally and unwind short duplexes using a single cycle of ATP-dependent changes in RNA affinity.[45-47]

Analysis of structures has been tremendously useful toward understanding the differences in unwinding between DEAD-box proteins and processive helicases. DEAD-box proteins lack additional domains present in many helicases that form interactions with the bases of the bound ssRNA and are involved in directional translocation.[48-52] In contrast, they contact ssRNA only along its sugar-phosphate backbone.[24-29] DEAD-box proteins also lack a β-hairpin that is present in conventional helicases and is thought to facilitate duplex disruption by acting as a "pin" or "wedge" to separate the two strands during translocation.[17,49] Thus, DEAD-box proteins are apparently not set up structurally for efficient and directional translocation along ssRNA. Recent crystal structures of DEAD-box proteins with both ligands have shown that bound ssRNA includes one or two sharp bends (termed a "crimp"), maintaining it in a conformation that would prevent base-pairing with a complementary strand.[24-29] This bending is likely to be critical for strand separation and provides a reasonable mechanism for the internal initiation of strand separation by DEAD-box proteins. This structural feature also provides a good explanation for observations that local strand separation can precede hydrolysis of ATP, with subsequent hydrolysis and product release being coupled to release of the DEAD-box protein from the tightly bound strand of short duplexes.[45,46]

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A second key difference between DEAD-box proteins and conventional helicases concerns the role of structures that flank the helix. Most conventional helicases strongly prefer a single-stranded extension, and this extension must have a defined polarity because it serves as a starting point for directional translocation and unwinding (**Fig. 2A**).[39,40] In contrast, some DEAD-box proteins can unwind blunt-ended helices just as efficiently as those with extensions,[44,53,54] again suggesting the unwinding is initiated by a direct interaction with a duplex, and although other DEAD-box proteins are activated by extensions, there is no requirement for a defined polarity (reviewed in refs. 17 and 48). Further, the extension is not even required to be single-stranded, as double-stranded extensions and even a structured RNA "extension" enhance unwinding,[55] and the extension can be separated from the duplex through a streptavidin linker.[56] Together, these results indicate that the extensions do not serve as a starting point for translocation but suggest instead that they provide an additional point of contact to tether the helicase core where it can unwind nearby helices (**Fig. 2B**).[55,56]

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Features of DEAD-box protein structures and sequences also give insight into this behavior. Many DEAD-box proteins have ancillary sequences in addition to the core helicase domains, and these ancillary regions are suggested, and in some cases shown, to form the tethering interactions with RNA substrates (**Fig. 2B**).[57-61] Some ancillary regions are highly basic and predicted to be unstructured in solution, most likely interacting with RNA non-specifically.[57,58] On the other hand, DEAD-box proteins can also be appended with domains that recognize specific RNA structures and direct the proteins to these structures. The archetypal protein of this group is the bacterial DbpA/YxiN protein, which has an ancillary C-terminal domain that adopts an RNA recognition motif fold (RRM) and binds specifically to a structure within the large ribosomal RNA, allowing the protein to function in large subunit assembly.[59,60,62,63] In keeping with their versatile nature, there is strong evidence that other DEAD-box proteins rely on a tethering interaction formed by the helicase core of a second monomer in a transiently-formed dimer or multimer.[46,61]

These characteristics of DEAD-box protein structure and activity suggest an unwinding mechanism in which they are localized by specific or non-specific tethering interactions, disrupt nearby helices in an inherently non-specific manner, and then release the liberated RNA strands upon ATP hydrolysis and release of products. Although this mechanism is very different from conventional helicases and would be clearly ineffective for tasks involving unwinding of long, continuous duplexes, it allows DEAD-box proteins to efficiently promote conformational transitions of RNAs and RNP complexes, as described in the sections below.

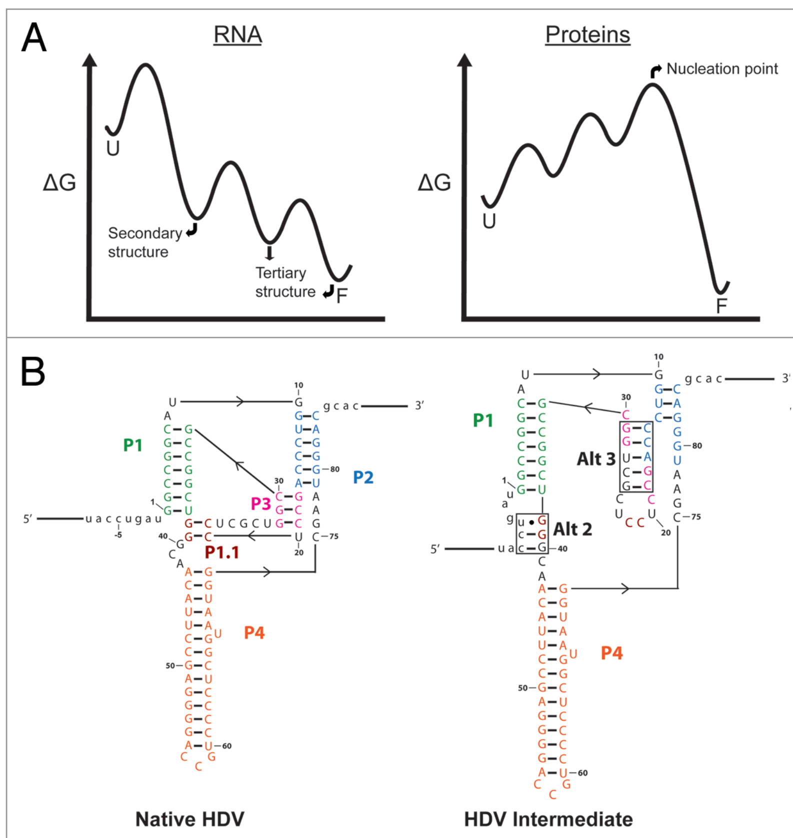

Figure 3. (A) Free energy diagrams comparing RNA and protein folding. Folding of RNA typically proceeds through intermediates of increasing stability, with secondary structure forming first from unfolded structures (U), followed by steps of tertiary contact formation to give a fully folded conformation (F). Many small proteins, on the other hand, do not accumulate intermediates because partially-ordered conformations are less stable than unfolded forms even under conditions that favor global folding. (B) Secondary structures of the native hepatitis delta virus (HDV) and an intermediate with non-native base pairings.[68] Nucleotides are color-coded according to their native fold. Alternate base pairings in the HDV intermediate are boxed and labeled.

RNA Secondary Structure and Folding Transitions

Short helices are pervasive in structured RNAs, so much so that they typically encompass most of the nucleotides in the RNA and constitute the basic unit of RNA secondary structure. Even helices of modest length (five or six base pairs) can form stably in the absence of higher-order, enforcing structure. The stability depends principally on local interactions, hydrogen bonding and base stacking, such that helical stability can be predicted by considering only the composition of each base pair and its immediate neighbors.[64] Secondary structure can form extremely fast during folding, such that it is very useful to consider RNA folding as a hierarchical process in which secondary structure forms first, and then tertiary connections are established between pre-formed secondary structure elements.[65] This mechanism generates a folding landscape in which sequential intermediates have progressively greater stability (**Fig. 3A**, left). These models have been generated largely from in vitro experiments monitoring folding of full-length RNA, but the basic features are also likely to be relevant in vivo, where folding of local helices can occur during transcription before longer-range tertiary

partners are synthesized.[66-68] This behavior is in striking contrast with a common feature of protein folding. Although it is by no means a universal property, many small proteins and domains fold without accumulating intermediates. Because its local secondary structure and tertiary contacts tend to be unstable, protein folding does not become "downhill" until these locally unstable structural elements coalesce into a tertiary fold that is stabilized by global cooperativity (**Fig. 3A**, right).[69]

With only four standard RNA bases, there is potential for at least a short alternative helix in even the smallest RNA sequences. Thus, non-native secondary structures are likely to form at least transiently for most RNAs. While an interaction of one or two base pairs would be expected to dissociate spontaneously without causing problems for folding, helices of as few as five or six base pairs can create substantial blocks for subsequent rearrangement to the native state. Early work demonstrated that several different tRNAs can adopt two different conformations, only one of which is active for aminoacylation,[70,71] and further work on diverse tRNAs indicated that the inactive conformations can have substantial differences in secondary structure from the native "cloverleaf" arrangement.[72,73] An alternative secondary structure was also suggested for 5S rRNA,[74] and more recently misfolded conformations with secondary structure changes have been identified for several RNAs, including group I self-splicing introns[75-78] and the hepatitis delta virus (HDV) ribozyme (**Fig. 3B**).[68,79]

Beyond folding to the native state, conformational transitions involving secondary structure changes are also required for the reaction cycles of many cellular RNAs and RNP complexes. RNA secondary structure changes underlie the regulatory roles of some groups of riboswitch RNAs.[80] Analogously, miRNAs, siRNAs and small regulatory RNAs in bacteria rely on a functional cycle of base pair formation and dissociation from target mRNAs.[2,81] The functional cycle of the spliceosome includes a series of extensive secondary structure rearrangements involving changes in base pairing of the snRNA components of the spliceosome with each other and the pre-mRNA.[82] Indeed, conformational changes in the spliceosome have proven to be fertile ground for exploring specific roles of DEAD-box proteins and related SF2 helicases. Proper splicing dictates that the conformational transitions proceed in a defined order, and it has been shown that certain mutations in the helicases, typically leading to cold sensitivity, allow accumulation and isolation of relatively homogenous intermediates stalled just upstream of the conformational transition mediated by the mutated protein.[83-85] With this strategy as a starting point, specific secondary structure rearrangements have been identified for the DEAD-box protein Prp28,[86,87] the DEAH-box proteins Prp16,[88,89] and Prp22,[90] and the Ski2-like helicase Brr2.[91] In particular, Prp22 has been shown directly to disrupt base pairs between the newly-spliced mRNA and U5 snRNP, allowing release of the spliced product from the spliceosome.[90]

Misfolding of Large, Multi-domain RNAs: Group I Introns

In addition to forming the full set of native base pairs, structured RNAs must form tertiary interactions that bring together the secondary elements into a precise native arrangement. As with secondary structures, RNA tertiary contacts are frequently modular and can have substantial independent stability. Some tertiary contacts are formed by base-pairing interactions involving loops, termed pseudoknots and these have the same thermodynamic properties as secondary structure. Other tertiary contacts are formed by motifs in terminal or internal loops that form specific contacts with helical segments.[92,93] In part because the structural elements that form these contacts are largely preformed at the level of secondary structure, the tertiary contacts are also relatively stable in isolation. Indeed, a tetraloop-receptor interaction has been removed from its natural context and used in extensive quantitative studies on the kinetics and energetics of tertiary-contact formation.[94,95]

With stable, modular tertiary contacts, it is perhaps not surprising that RNA can misfold at the level of tertiary structure as well as secondary structure. Extensive work has been done on folding and misfolding of group I introns, particularly one from *Tetrahymena thermophila* and its shortened "ribozyme" form (**Fig. 4**). Upon addition of Mg^{2+} in vitro, the ribozyme folds through a complex landscape along pathways that lead to the native state and a long-lived misfolded conformation that is similar in overall size and shape.[96-103] Although a non-native secondary structure, termed alt P3, biases folding toward the misfolded conformation,[104] chemical footprinting and fluorescence experiments showed that the misfolded RNA has all of the native secondary structures and all five long-range tertiary contacts (arrows in **Fig. 4**). This result suggested that the non-native alt P3 is resolved to the correct pairing during folding to the misfolded conformation.[105,106] In spite of its predominently native structure, extensive unfolding of the misfolded ribozyme is required to refold it to the native state, and this unfolding includes disruption of all five tertiary contacts. This leads to the important conclusion that even native tertiary contacts can stabilize misfolded intermediates by "locking in" non-native structure elsewhere,[107] which for this misfolded conformation has been suggested to be a non-native topology within the core.[105] Although the detailed structure and physical origin of the misfolded intermediate remain unproven, its formation and resolution have been useful in dissecting the roles of DEAD-box proteins in RNA folding transitions (see *Acceleration of group I intron folding by RNA chaperones* below).

Other group I introns have also been shown to misfold, broadening the conclusion that misfolding is common while suggesting that the details can vary substantially between related RNAs. A *Candida albicans* intron from the same subgroup as the Tetrahymena intron adopts a misfolded conformation that is linked to the P3 pseudoknot and can be avoided by pre-incubation in monovalent ions, analogous to results for the Tetrahymena ribozyme.[67,108] On the other hand, an intron from the mitochondria of *Saccharomyces cerevisiae* misfolds to a conformation that includes an extensive non-native secondary structure and drastic changes in tertiary structure relative to the functional structure.[78,109] A smaller group I intron from the bacterium Azoarcus folds under standard conditions without accumulating misfolded intermediates detectable by global or local structural probes,[110,111] although small changes in the RNA sequence or

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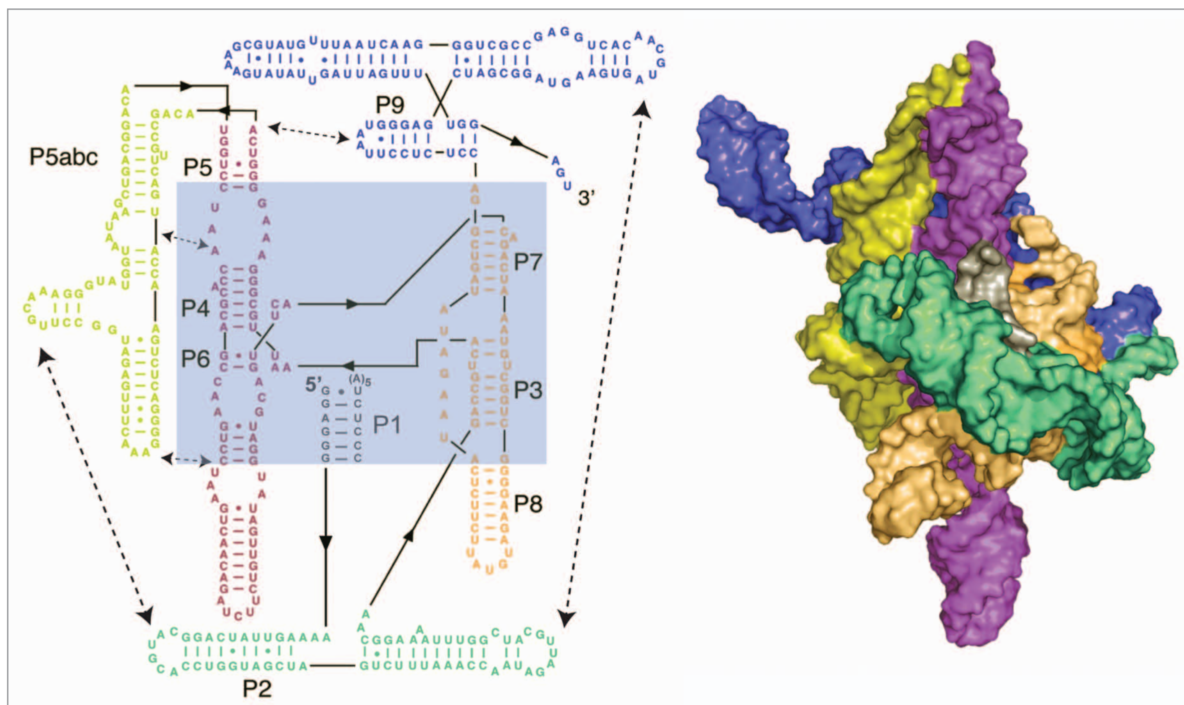

Figure 4. Secondary and tertiary structures of the Tetrahymena group I intron. The shortened "ribozyme" version is shown, in which the exons are removed and the ribozyme forms a core helix, termed P1, with an oligonucleotide that mimics the 5'-splice site. The ribozyme core is highlighted in blue in the secondary structure. Regions of the secondary structure are color-coded according to structural domains, and tertiary contacts are indicated with dashed lines. At the right is a tertiary structure model of the intron,[149] with domains in the same colors as in the secondary structure diagram.

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solution conditions result in accumulation of intermediates that are thought to include non-native structure,[112,113] underscoring the prevalence of misfolding for large RNAs.

Acceleration of Group I Intron Folding by RNA Chaperones

Group I introns have served as a valuable system to explore protein-facilitated RNA folding, dating back to the mid 1990s when it was shown that several *E. coli* ribosomal proteins have the ability to accelerate splicing of phage T4 introns by participating in folding of the introns.[114] Since then, several groups of proteins have been demonstrated to possess RNA chaperone activity using a systematic assay in which overexpression of a chaperone protein rescues self-splicing of a T4 intron that is otherwise trapped by non-native secondary structure.[115,116] Proteins that have been demonstrated to possess RNA chaperone activity for group I introns, using this and other approaches, include the HIV protein NC, the *E. coli* histone-like protein StpA, and several of the ribosomal proteins.[117-119] These proteins are small and quite positively-charged, and they are thought to function in RNA folding by binding strongly to single-stranded segments, thereby stabilizing intermediates required for exchange of alternative structures or contacts.[14-16]

More recently it was discovered that DEAD-box proteins also participate in folding of complex, structured RNAs including group I and group II introns.[120,121] The *Neurospora crassa* protein CYT-19 was shown to be essential in vivo for efficient splicing of several mitochondrial group I introns of different structural sub-groups and to participate in other RNA processing reactions. Extending earlier work,[122] the *S. cerevisiae* ortholog Mss116p was also shown to be required for efficient splicing in vivo of all nine mitochondrial group I introns and all four group II introns.[121] Further, splicing defects in a strain lacking Mss116p were rescued by co-expression of CYT-19, underscoring the breadth of RNA structures on which CYT-19 can act productively.

Further biochemical work in vitro probed the mechanisms by which DEAD-box proteins promote group I and group II intron folding. Using the misfolded conformer of the Tetrahymena group I ribozyme, described above, it was shown that CYT-19 gives ATP-dependent acceleration of refolding to the native state.[55] Upon reaching the native state, the ribozyme is fully active, and no further assistance from CYT-19 is needed. Thus, CYT-19 acts as a true chaperone in this process, interacting transiently with the RNA to promote folding to the native state.[55,120] Chaperone activity of DEAD-box proteins was also demonstrated for group II introns in reverse branching and reverse splicing reactions, with CYT-19 being proteolyzed prior to initiation of the reaction by substrate addition.[123] DEAD-box proteins can also function as chaperones to facilitate conformational changes between or following the splicing steps, as shown for Mss116p in splicing of one of its cognate group I introns.[124]

Further studies using the Tetrahymena ribozyme have provided additional insights into the mechanism of CYT-19 in promoting folding. Because refolding of the misfolded ribozyme requires extensive transient unfolding,[105] it was inferred that

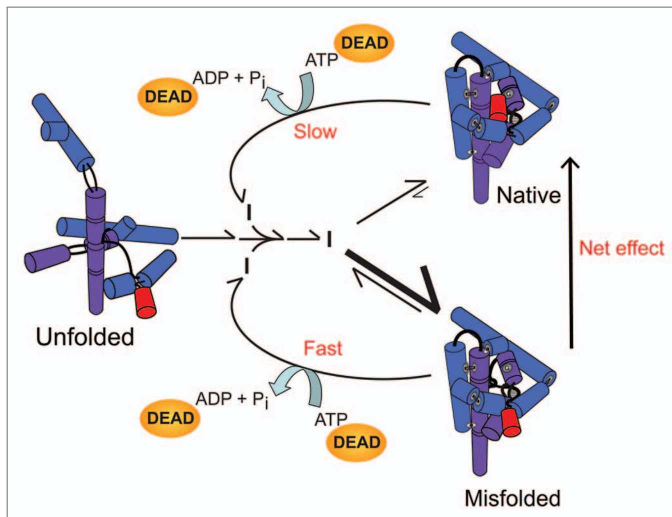

Figure 5. RNA chaperone activity of DEAD-box proteins by non-specific disruption of local structure. The RNA, shown in cartoon form as helical cylinders, represents the Tetrahymena group I intron ribozyme. The ribozyme folds primarily to the long-lived misfolded conformation (bottom; larger rate constants are shown with longer, thicker arrows), which therefore accumulates to high levels despite being less stable than the native state (top). DEAD-box proteins facilitate local structure disruptions of the native and misfolded RNA species in an ATP-dependent manner, without recognizing any structural features of the misfolded RNA. The RNA then folds again without any further influence of the DEAD-box protein. Despite the lack of specific recognition by the DEAD-box protein, the misfolded RNA is disrupted with greater efficiency than the native ribozyme because of its lower stability, and this difference allows the non-specific activity of DEAD-box proteins to accelerate net refolding to a population of predominantly native ribozyme (indicated by arrow at right). Modified from reference 125 with permission.

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CYT-19 promotes refolding by accelerating partial unfolding of the RNA. Further work supported and extended this model by demonstrating that CYT-19 and Mss116p are inherently non-specific, efficiently unfolding the native state as well as the misfolded conformer of mutant RNAs in which the large natural energy gap between these two structures is decreased.[125,126] After promoting unfolding, CYT-19 appears to allow subsequent folding along the same pathways and rates as in its absence. This activity leads to a distribution of native and misfolded conformers in which the relative populations depend on both kinetics and thermodynamics (**Fig. 5**).[125] For the wild-type ribozyme, this activity leads to rapid accumulation of the native state, whereas for destabilized mutants the same activity favors the misfolded state because the reduced energy gap is not sufficient to overcome a substantial kinetic bias for misfolding.[125] An analogous redistribution was observed for model duplexes by the *S. cerevisiae* DEAD-box protein Ded1p.[127]

Together, the results above lead to a model in which DEAD-box proteins facilitate exchange between alternative conformations by using ATP to disrupt base pairs locally[37,45,46] and then releasing the liberated strands one at a time to give them a chance to form new contacts. This mechanism bears a strong resemblance to one used by protein chaperones, particularly the Hsp70 group. Akin to DEAD-box proteins, Hsp70 proteins bind short polypeptides in an extended conformation, preventing the bound segment from forming other interactions.[128,129] Also in analogy to DEAD-box proteins, the peptide binding affinity of Hsp70 proteins is coupled to ATP binding and hydrolysis and the ATPase cycle is regulated in turn by proteins that accelerate or inhibit specific steps.[130] The resulting cycles of regulated peptide binding and release are used by Hsp70 proteins to promote protein folding, to translocate proteins across membranes and to disrupt protein aggregates alone or in combination with additional chaperone proteins.[131-135]

Distinguishing Misfolded and Native RNAs

Any RNA chaperone protein that functions with multiple RNAs faces a fundamental challenge. How can the native, functional RNAs be distinguished from misfolded intermediates? The same conceptual challenge is faced by protein chaperones and an important part of their general mechanism is that they bind preferentially to exposed hydrophobic sequences, which are typically buried in functional, folded proteins. RNA chaperones probably cannot use an analogous strategy because misfolded RNAs can strongly resemble their native counterparts, being compact and monomeric and differing principally in local secondary structure or the local arrangement of helices.[105-107] Thus, RNA chaperones are capable of disrupting native RNA species in addition to misfolded ones, as described above.[125]

Nevertheless, one possible strategy has been suggested from work on CYT-19 and the Tetrahymena ribozyme. CYT-19 accelerates strand separation of the P1 duplex, which is formed between the ribozyme and its oligonucleotide substrate (see **Fig. 4**), but CYT-19 is strongly inhibited by docking of P1 into tertiary contacts with the ribozyme core.[55] The extent of inhibition tracks with the docking stability, suggesting that CYT-19 does not disrupt the tertiary contacts, but only separates the strands after spontaneous undocking. Although it remains to be determined whether sensitivity to tertiary contacts is general for other DEAD-box chaperones and other RNAs, it could be an important strategy for biasing DEAD-box proteins to act on misfolded RNAs, which are less likely than their native counterparts to pack their structural elements into a globular structure.[109] Further, by disrupting loosely-associated structural elements, DEAD-box proteins may be biased to disrupt the non-native regions, allowing them to form stable, native tertiary contacts and then be protected from further chaperone activity.

Other Activities of DEAD-Box Proteins that Promote RNA Folding and Conformational Transitions

Detailed biochemical work has shown that DEAD-box proteins have activities extending beyond the local disruption of RNA duplexes, and there is growing evidence that these additional activities are used in vivo to promote folding and rearrangements of RNAs and RNPs. Ded1p was shown to promote release of the exon junction complex of proteins (EJC) from an RNA oligonucleotide in an ATP-dependent manner and to release the U1

snRNP from RNA in a reaction that requires disruption of both RNA-protein and RNA-RNA contacts.[136,137] DDX42, a human DEAD-box protein, can displace the single-stranded binding protein T4gp32 from ssRNA.[138] Although these activities do not necessarily reflect the in vivo functions of these proteins, they establish that remodeling RNA-protein complexes is within the catalytic repertoire of DEAD-box proteins. The ability to remodel complexes by displacing proteins most likely arises from the basic RNA binding properties of the helicase core. In protein displacement, tight binding to a segment of ssRNA sequesters it away from its former protein partner.

There is strong genetic evidence and emerging biochemical evidence that some DEAD-box proteins function in vivo by remodeling or disassembling RNPs. In the spliceosome, Prp5p is thought to displace the protein Cus2p from the branchpoint to allow association of the U2 snRNP,[139] and Prp28p is thought to remove U1C from the RNA helix formed between U1 snRNA and the 5'-splice site in addition to disrupting the helix.[86,87] The DEAD-box protein Dbp5p (DDX21 in humans), which shuttles between the nucleus and cytosol and functions in nuclear export of mRNAs, has been the subject of intense recent scrutiny.[140-143] Genetic and biochemical evidence suggests that Dbp5p promotes loss from newly-exported mRNA of at least two proteins, Mex67p and Nab2p, which bind mRNA in the nucleus and promote export (**Fig. 6A**).[140,141] The displacement activity appears to be directed to the right time and place by Gle1p, which is located on the cytosolic face of the nuclear pore complex (NPC) and stimulates the ATPase activity of Dbp5p.[144,145] Thus, a model has emerged in which Dbp5p travels with mRNA and its associated proteins through the NPC and then interacts with Gle1p, which promotes dissociation of the proteins, blocking re-entry of the mRNA into the nucleus and conferring directionality on the export process.

DEAD-box proteins have also been shown to accelerate duplex formation or annealing of complementary oligonucleotides present at low concentrations in vitro (**Fig. 6B**).[41,138,146,147] The acceleration by Ded1p and Mss116p is several orders of magnitude, such that the rate constant for duplex formation approaches the diffusion limit for a bimolecular reaction.[146,147] The annealing activities of both proteins are enhanced by positively charged C-terminal regions,[58,146,147] a feature of many DEAD-box proteins that functions in manipulation of RNA and RNP structures.[23,41,42,57,58,146]

Strand annealing activity may also be important for the functions of DEAD-box proteins as RNA chaperones. It is intuitive that the formation of RNA secondary structure is an intramolecular version of duplex formation between two ssRNAs, and it is possible that annealing activity accelerates formation of intramolecular RNA secondary or tertiary structure (**Fig. 6B**). This role is difficult to establish experimentally, however, because a folding step that gives accumulation of structure may nevertheless be rate-limited by a local structure disruption, which then allows the larger-scale movements that lead to the new structure. More generally, because intramolecular RNA segments are held in proximity by their covalent connections, it is not clear that the process of bringing these regions together to form a contact is

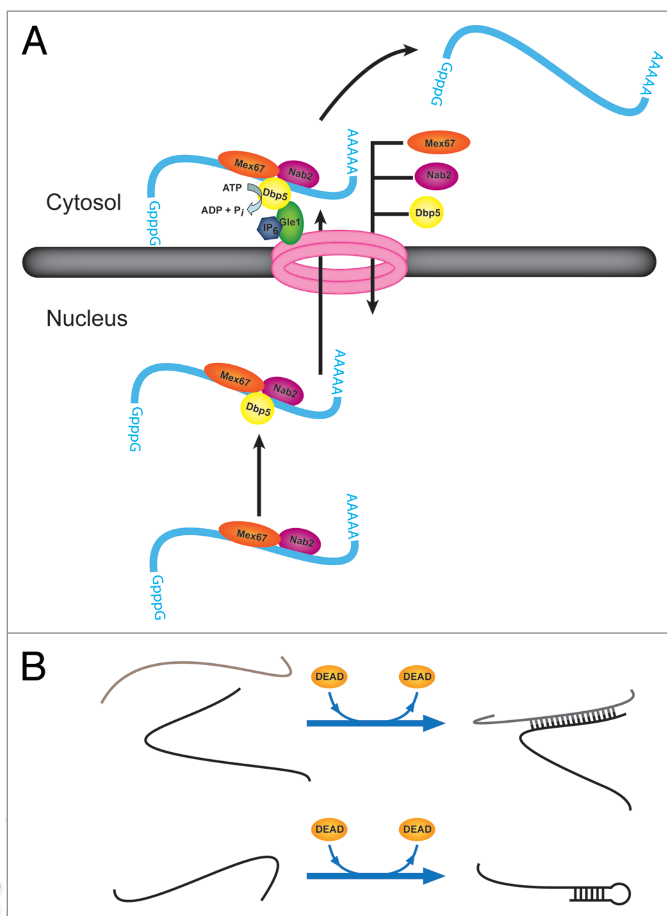

Figure 6. Additional activities of DEAD-box proteins in promoting RNA rearrangements and RNP remodeling. (A) Dbp5p facilitates transport of mRNA from the nucleus to the cytosol. The role of Dbp5p is thought to include removal of the nuclear export factor Mex67p and the nuclear RNA-binding protein Nab2p. Upon protein removal, the mRNA is blocked from re-entering the nucleus. (B) Strand annealing capabilities of DEAD-box proteins. DEAD-box proteins have been shown to accelerate intermolecular duplex formation by two strands of RNA in solution without a requirement for ATP (top). It is possible that this activity is important for intramolecular RNA folding by facilitating formation of local or long-range secondary structure (bottom).

rate-limiting for folding, even for individual steps, so acceleration of annealing may not typically lead to accelerated folding. This remains an interesting question for the future.

Conclusions and Perspective

In the last decade, remarkable progress has been made toward understanding how DEAD-box proteins and related SF2 helicases function in RNA folding, conformational transitions and RNP complex remodeling. Work on the structures and mechanistic capabilities of DEAD-box proteins has generated a detailed physical understanding of the basic properties of these proteins. Further, the discovery that some DEAD-box proteins function as general RNA chaperones in folding of the relatively simple group I and group II introns produced model systems that have allowed biochemical dissection of the roles of DEAD-box proteins

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in RNA folding. This work has led to a model in which some DEAD-box proteins function as general RNA chaperones by using their ATP-dependent RNA binding and unwinding activities to disrupt local structure non-specifically, providing further opportunities for formation of stable, functional RNA structures that are resistant to further action of DEAD-box chaperones. Major challenges remain, however, in determining more precisely what local contacts are disrupted, what folding intermediates are subsequently formed, and how these intermediates are resolved, with or without additional help from DEAD-box proteins, to generate the functional structures.

Over the same period, great progress has been made in identifying molecular targets for a diverse set of DEAD-box proteins and their relatives, and it is now possible to link with good confidence most of these proteins to specific processes and molecular targets.[148] While the sheer size and complexity of many of these target complexes create substantial challenges in dissecting the roles of helicases during folding, assembly, and the catalytic cycles, recent work is making dramatic inroads toward identifying specific steps that are promoted by DEAD-box proteins and the biochemical activities used to promote them. Given the current pace in these areas, the next decade is likely to be at least as rewarding.

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