Published in final edited form as:

Wiley Interdiscip Rev RNA. 2011 ; 2(1): 135–152. doi:10.1002/wrna.50.

DEAD-box proteins as RNA helicases and chaperones

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Abstract

DEAD-box proteins are ubiquitous in RNA-mediated processes and function by coupling cycles of ATP binding and hydrolysis to changes in affinity for single-stranded RNA. Many DEAD-box proteins use this basic mechanism as the foundation for a version of RNA helicase activity, efficiently separating the strands of short RNA duplexes in a process that involves little or no translocation. This activity, coupled with mechanisms to direct different DEAD-box proteins to their physiological substrates, allows them to promote RNA folding steps and rearrangements and to accelerate remodeling of RNA-protein complexes. This review will describe the properties of DEAD-box proteins as RNA helicases and the current understanding of how the energy from ATPase activity is used to drive the separation of RNA duplex strands. It will then describe how the basic biochemical properties allow some DEAD-box proteins to function as chaperones by promoting RNA folding reactions, with a focus on the self-splicing group I and group II intron RNAs.

Keywords

ATPase; CYT-19; DExD/H-box protein; Group I intron; Group II intron; Mss116; RNA folding

DEAD-box proteins are the largest family of superfamily 2 (SF2) helicases. They share with other SF2 proteins a helicase core composed of two RecA-like domains and a common set of sequence motifs. In the DEAD-box proteins, motif II includes the sequence D-E-A-D, which is the origin of their name (1). DEAD-box proteins are found in all three domains of life and perform a diverse set of functions in an even more diverse set of cellular processes, including assembly and function of macromolecular machines like the ribosome and spliceosome, quality control mechanisms in gene expression, nuclear export of mRNA, folding of self-splicing RNA introns, and others (2,3) (Table 1). The broader term “DExD/H-box” has been used to include other proteins with related motif II sequences (2,3), but a recent comparative sequence analysis has led to questions as to the relevance of this designation, because DEAD-box proteins are not more closely related to DExH helicases than to other families of SF2 helicases (4).

In keeping with the broad range of functions for DEAD-box proteins, there is also considerable diversity in their mechanisms. Nevertheless, common themes are emerging. Since their discovery more than 20 years ago (1), the central defining biochemical activity of DEAD-box proteins has been their ability to bind and hydrolyze ATP in a cycle that is stimulated by binding to single-stranded or double-stranded RNA (ssRNA or dsRNA).

Further Reading

<http://www.helicase.net/>

Information on RNA and DNA helicases, maintained by Eckhard Jankowsky

http://medweb2.unige.ch/~linder/RNA_helicases.html

Information on RNA helicases, maintained by Patrick Linder

Cross-References

RNA chaperones, RNA folding in cells, group I/II introns

Subsequent work has shown that the energetic communication implied by the stimulation of ATPase activity by RNA binding forms a common set of properties that underlies the diverse mechanisms of DEAD-box proteins. Progression through the ATPase cycle leads to changes in ssRNA affinity, allowing DEAD-box proteins to bind and release RNA in a regulated manner. This regulated binding is then used by different DEAD-box proteins in different ways. It can be used to separate the strands of short duplex regions of RNA, to promote rearrangements of complex, structured RNAs, to remodel RNA-protein (RNP) complexes, or even to form a highly stable complex on ssRNA. Some DEAD-box proteins are tuned to operate in the context of specific RNA or RNP substrates and even to promote specific rearrangements, whereas others function more broadly, interacting productively with a range of substrates.

This review outlines the basic properties of DEAD-box proteins as RNA strand separators or helicases and describes how the fundamental mechanistic feature, ATP-dependent cycling of ssRNA affinity, allows the proteins to perform this function. More global aspects of DEAD-box protein structures have been reviewed recently (4,5) and are covered here only in the context of the helicase mechanism. The review then covers work that probes how DEAD-box proteins use their RNA helicase activity to promote RNA structural rearrangements and folding events, focusing on self-splicing introns as a model.

The distinctive RNA helicase activities of DEAD-box proteins

Given their substantial sequence similarity with other families of RNA and DNA helicases, it is not surprising that DEAD-box proteins can use ATP to separate the strands of helices. They act efficiently on short RNA duplexes or RNA-DNA heteroduplexes, and this basic activity is certain to underlie many of their functions, especially those involving structural changes in RNA. Interestingly, although there is considerable mechanistic overlap between DEAD-box proteins and conventional helicases, the properties of unwinding differ in fundamental ways, both in terms of the features required of the substrates and the mechanisms of unwinding.

Declining efficiency with increasing helical length or stability

The general mechanism that conventional helicases use to separate the strands of a duplex is to bind at one end of the duplex and then use energy from cycles of ATP binding and hydrolysis to move, or translocate, directionally along one of the strands (6,7). Most SF1 and SF2 helicases translocate in the 3' to 5' direction, while a smaller number move toward the 3' end. By gripping tightly to one of the strands as they translocate, helicases cause the other strand to be displaced. Repeated cycles of ATP hydrolysis, translocation, and strand displacement ultimately result in complete unwinding of the helix. With each cycle, there is a finite probability that the helicase will dissociate from the strand prematurely, abruptly ending the unwinding reaction before it is completed. The probability of continuing onward rather than dissociating defines the processivity, with a more highly processive helicase being more likely to continue translocating. Therefore, a common means of evaluating the processivity of a helicase is to examine how the fraction of successful unwinding events decreases as the helix length increases.

When evaluated in this way, the DEAD-box proteins that have been tested are poor helicases. Helicase processivity is commonly measured in the hundreds or thousands of base pairs, but the DEAD-box proteins become ineffective when the helix length rises above 10–15 base pairs, and above 20–25 base pairs unwinding is often not detected at all (8–10). Further, unlike most helicases, the efficiency of strand separation for DEAD-box proteins is strongly dependent on the stability of the helix, not just its length. This behavior was first demonstrated for mammalian eIF4A, a small DEAD-box protein involved in translation

initiation. It was observed that eIF4A can readily unwind certain short duplexes but is much less efficient for duplexes that are rich in G-C pairs and calculated to be more stable (11,12). An analogous correlation has been suggested for at least one other DEAD-box protein (13).

The picture that emerges from these studies is that DEAD-box proteins are rather inefficient helicases, which is mysterious only until one considers that the helical elements within structured RNAs are rarely longer than 10 base pairs. Thus, there would be no need for great processivity in proteins that function by rearranging structured RNAs. In terms of the mechanism of unwinding, the observed differences in properties would not require a substantial departure from a conventional helicase mechanism. It could be imagined that DEAD-box proteins unwind helices in much the same way as conventional helicases, but they move more slowly when they encounter more stable structure, and they tend to dissociate before they have taken very many steps. Indeed, such mechanisms have been proposed (8,9). On the other hand, the duplexes that are unwound efficiently are so short that a single protein monomer could bind along most of the length of the duplex (see Fig. 3B–D below), raising the possibility that strand separation by DEAD-box proteins does not involve any translocation at all. This would indeed represent a substantially different mechanism from conventional helicases (11,13–17).

Enhancement of unwinding by RNA extensions or appendages

Besides processivity, a second general feature of conventional helicases is that most have a strong preference for helices that include a single-stranded extension (7). The mechanistic role of the extension is to provide an initial site of interaction, from which the helicase can begin to translocate into the helix. Thus, the extension is required to have a certain polarity, such that directional translocation will move the helicase core into the helix rather than toward the free end of the extension. Because most helicases translocate from 3' to 5', they require that the extension include a free 3' end (Fig. 1A).

In this regard as well, DEAD-box proteins behave quite differently from most other SF1 and SF2 helicases. First, some DEAD-box proteins are as efficient at separating the strands of a blunt-ended helix as the same helix with an extension, as first demonstrated for eIF4A (12). The equivalent activity on a blunt-ended duplex suggests that eIF4A can initiate unwinding by contacting a double-stranded region, most simply suggesting that unwinding could begin internally, within a duplex. Such a mechanism would be very unlikely to succeed for long duplexes, even for a processive helicase, because initiating unwinding from an internal region of the duplex would leave intact the region of the duplex that was not in the preferred direction of translocation. However, this could be a viable strategy for short duplexes. The protein could generate local strand separation within the helix, and any base pairs that remained intact at one or both ends of the helix could dissociate spontaneously (12). Indeed, it was demonstrated directly that the *Saccharomyces cerevisiae* proteins Ded1p and Mss116p can initiate an unwinding reaction by interacting with an internal region of an RNA duplex (18).

Second, many DEAD-box proteins are enhanced for unwinding by extensions, but unlike the behavior of conventional helicases, the extension can be beneficial for unwinding regardless of its polarity (Fig. 1B) (9,10,14,18–20). This non-polar activation argues strongly against a mechanism that requires directional translocation. Although a mechanism involving random, bidirectional translocation would not be ruled out, further data suggest that the extension is not used as a starting point for translocation at all, but rather as an additional handle to maintain an interaction with the duplex. Thus, the *Neurospora crassa* protein CYT-19, which functions in folding of mitochondrial group I and group II introns (see RNA Chaperone Functions of DEAD-box Proteins below), is activated by double-stranded extensions at least as well as single-stranded ones, and extensions composed of DNA are as

activating as RNA extensions (Fig. 1C) (21). DNA extensions also activate Ded1p, and this activation is preserved even if the extension is not covalently linked to the duplex but is instead held in proximity through linkages to streptavidin (Fig. 1D) (22). Finally, activation of CYT-19 is even greater upon 'extension' with a highly structured group I intron RNA (21). Together, these observations lead to a model in which the role of the extension, whether it be single-stranded, double-stranded, or higher-order RNA structure, is to provide a site of interaction that can localize the DEAD-box protein in position to disrupt nearby RNA structure.

Targeting DEAD-box proteins to certain RNAs or RNPs

Many DEAD-box proteins have specialized functions that require them to act on specific RNAs or RNPs. In analogy with the DEAD-box proteins described above that bind to helix extensions with low or no specificity, DEAD-box proteins that function specifically are thought to be directed by forming additional interactions with their targets. These interactions can be formed in two general ways, either through specific loops or surfaces within the helicase core or by the ancillary domains adjacent to the helicase core in many DEAD-box proteins (Fig. 2).

One of the best-understood DEAD-box proteins in terms of targeting is the *E. coli* protein DbpA (YxiN in *Bacillus subtilis*), which functions in biogenesis of the large ribosomal subunit and uses a C-terminal ancillary domain to recognize a specific hairpin within the 23S rRNA (Fig. 2A) (23–26). The small C-terminal domain adopts the fold of an RNA recognition motif (RRM) (27). The connections between the domains are flexible in solution, suggesting that a specific interaction of the C-terminal domain with its target helix would allow the helicase core to be localized and yet still sufficiently flexible as to interact with multiple target sites nearby on the assembling ribosomal subunit (28). Other DEAD-box proteins have ancillary domains that provide targeting but in a less specific way, such as the *Thermus thermophilus* protein Hera, which binds structured RNAs through an RRM-like domain (29,30). Analogously, CYT-19, Mss116p and other DEAD-box proteins such as Ded1p and SrmB possess a basic 'tail' region at the C-terminus that interacts with structured RNAs with no apparent specificity (16,31) (Fig. 2B). Another means of non-specific targeting may be multimerization, which could allow additional interactions to be formed by the helicase core of an interacting subunit (Fig. 2C) (18). A dimerization domain is present in Hera and may contribute to binding of several different structured RNAs or interactions with large RNA substrates (5,29,32).

On the other hand, several DEAD-box proteins form specific protein-protein interactions with the helicase core. Dbp5p (DDX19 in humans) functions in mRNA export from the nucleus, most likely by removing proteins from mRNA-containing complexes (33–35). It shuttles between the nucleus and the cytoplasm but is largely localized to the cytoplasmic face of the nuclear pore by interactions with Nup159p and Gle1p, which are present in cytoplasmic filaments of the nuclear pore complex (36–39). Recent crystal structures and functional analysis show that Dbp5p interacts with both of these proteins via specific loops and contact surfaces in its helicase core (39–41). Interactions with these proteins serve additional functions beyond simple tethering of Dbp5p. Gle1p activates the RNA-dependent ATPase activity of Dbp5p (39), and the interaction with Nup159p is competitive with binding of RNA and ATP, indicating that the ATP-dependent functions of Dbp5p require dissociation from Nup159p (36,41). The eIF4A protein, which lacks ancillary domains, also uses the helicase core to contact partner proteins, including eIF4G, which is a central component of the mRNA cap-binding complex, and the HuD protein, which stimulates cap-dependent translation (42,43). Analogously, the nonsense-mediated decay protein UPF3b forms a complex with the helicase core of the exon-junction complex (EJC) component eIF4A-III (44). Interestingly, eIF4G and UPF3b interact with the same region of the C-

terminal RecA-like domain as Gle1p uses to contact Dpb5p (42,44,45). The DDX6 protein (Dhh1p in *S. cerevisiae*) uses this same surface to interact with a peptide fragment of the P body component EDC3 (46). The emerging view from these results is that DEAD-box proteins are frequently localized by specific interactions between the helicase core and protein components of RNPs (Fig. 2D).

Tethered strand separation without translocation

In combination, the biochemical properties described above suggest a model for RNA unwinding by DEAD-box proteins that is quite different from the mechanism of conventional helicases. A tethering interaction holds the DEAD-box helicase core in proximity to targeted RNA duplexes, allowing the core to generate local separation of the strands. Different DEAD-box proteins could use this basic mechanism to promote RNA remodeling, protein displacement, or to facilitate disruption of RNA tertiary contacts, as the critical event is simply the tight, regulated binding of RNA. A critical distinction of this mechanism relative to conventional helicases, which may also be localized via interactions with other proteins, is that for the non-processive actions of DEAD-box proteins, the tethering interaction could remain intact through the entire process. Thus, a DEAD-box protein could remain positioned to disrupt the same structure repeatedly or to facilitate additional structure disruptions of intermediates that form after the initial disruption of structure. Nevertheless, it remains possible that the initial entry of DEAD-box proteins to a target duplex is followed by a small number of translocation steps. This mechanism would require either that the tethering interaction be disrupted during duplex unwinding or that the RNA could be rearranged in such a way as to allow the helicase core to track along the RNA duplex without a corresponding movement of the domain or surface making the tethering interaction.

Because of the fundamental differences between the mechanisms of DEAD-box proteins and conventional helicases, the activity of DEAD-box proteins has sometimes been referred to as 'strand separation' or 'unwinding' activity rather than helicase activity (3,47–50). This is because directional translocation is often considered to be a central, defining feature of helicase activity, whereas the strand separation activities of DEAD-box proteins involve little or no translocation.

Coupling ATP binding and hydrolysis to RNA strand separation

The helicase core of DEAD-box proteins consists of two RecA-like domains that include a series of conserved motifs (Fig. 3A–C). In three dimensions, the motifs line the protein surfaces that bind single-stranded RNA and an adenosine nucleotide. Both ligands lie in clefts between the two core domains, on opposite sides of the protein from each other. Despite their physical separation, there is intimate energetic communication between the bound RNA and nucleotide, which changes from cooperativity to anti-cooperativity during the ATPase cycle. Recent advances, described below, have greatly increased understanding of how energy from ATP binding and hydrolysis is transduced to the RNA binding site to promote strand separation.

Molecular basis for RNA and ATP recognition by DEAD-box proteins

Important insights into the mechanism of RNA strand separation by DEAD-box proteins have come in recent years from a series of crystal structures of the helicase core or the core in complex with ssRNA and a nucleotide analog (41,51–62). In structures that include bound ligands, all eleven motifs of the helicase core line the interface between the two domains and shape binding sites for the RNA and adenosine nucleotide (Fig. 3C). The bound ssRNA lies along a basic crevice that extends across both domains and contains contributions from

motifs Ia, GG, and Ib from domain 1 and motifs IV, QxxR, and V from domain 2 (41,55–57,59,61). Biochemical studies have confirmed the roles of these motifs in RNA binding and strand separation (55,63,64).

Analysis of the bound RNA reveals several features that are distinct from other RNA and DNA helicases and provide insight into mechanistic features of DEAD-box proteins (Fig. 3D). In the DEAD-box protein structures, the ssRNA is bound in a conformation that includes a sharp bend imposed by a helix that includes motif Ib (55–57), and in one structure a second bend is imposed by a C-terminal extension of domain 2 in Mss116p (59). Thus, for all of these proteins the ssRNA is bound in a conformation that is incompatible with the presence of this strand in a duplex. In all of the DEAD-box protein structures, conserved amino acids make contacts exclusively to the sugar-phosphate backbone of the RNA, including several interactions with the 2'-OH groups, explaining why binding is not sequence specific but can distinguish RNA from DNA (55). Notably absent from DEAD-box proteins are the ancillary domains or inserts found in other SF2 and SF1 helicases that form additional contacts with the nucleotide bases and are intimately involved in processive translocation (6,65), providing structural insight into the apparent lack of translocation in DEAD-box proteins. Along these lines, DEAD-box proteins also lack a β -hairpin that is suggested to function in other helicases as a wedge or 'pin' to promote strand separation during directional 3'-5' unwinding (4,66).

On the opposite side of the core lies the ATP binding site. It contains a P-loop (motif I), characteristic of many nucleotide-binding proteins from a variety of superfamilies, which interacts with the triphosphate moiety. Additional contacts with the phosphates are formed by amino acids from the D-E-A-D sequence within motif II, as well as motif VI, and motif V contacts the ribose moiety. Mutations in motifs I, II, and VI give weakened or abolished nucleotide binding and hydrolysis (63,67–74). Additionally, DEAD-box proteins include a Q-motif within domain I, which forms specific contacts with adenine nucleotides (75,76). Motif III, located between the RNA and ATP binding sites, participates in positioning of the γ -phosphate and has recently been implicated in coordinating the other conserved motifs for formation of a high-affinity RNA-binding site in the ATP-bound state and for hydrolysis of ATP (50).

Crystal structures have shown that the two domains of the core are flexible relative to each other in the absence of bound ligands, with several DEAD-box proteins adopting conformations that are extended, or 'open', to varying extents (51–53,56,57). In striking contrast, the domains become packed together, or 'closed', in structures with bound RNA and ATP analog, as shown in Fig. 3B & C (41,55–57,59,61,62). This movement is also supported by solution studies in which conformational changes were detected by sensitivity to protease (77) or relative motions of the two domains were monitored by fluorescence (78). This domain closure is thought to underlie the cooperativity binding of ssRNA and ATP (see below).

RNA affinity changes upon ATP binding and hydrolysis

Early studies on eIF4A used steady-state ATPase kinetics to show that ATP and ssRNA bind in a random order with energetic coupling (48). The K_M value for ATP is decreased by up to an order of magnitude in the presence of saturating ssRNA, and likewise the half-maximal concentration for stimulation by RNA decreases with saturating ATP, indicating that each ligand tightens binding of the other at the point in the ATPase cycle that accumulates in the steady state. This cooperative effect is reversed with ADP, which inhibits ATPase activity with a K_i value that is increased by nearly an order of magnitude upon binding of ssRNA. This result indicates that bound ssRNA weakens ADP binding, *i.e.* that they bind with anti-cooperativity (48). Thus, the affinity for ssRNA changes dramatically

through the ATPase cycle, with tight binding linked to the γ -phosphoryl group. Coupled binding has also been demonstrated for other DEAD-box proteins using non-hydrolyzable nucleotide analogs such as AMP-PNP (64,71,78,79). However, questions have arisen as to the extent to which AMP-PNP faithfully mimics the binding properties of ATP (13,17), and recent work has indicated that the energetic coupling is strongest after hydrolysis of ATP, in a state in which both the ADP and P_i products remain bound (80). Together these results lead to a model in which the affinity for ssRNA is weakened upon release of P_i , leading to a cycle of strong and weak ssRNA binding with each cycle of ATP binding, hydrolysis, and release of products. Because the binding sites for RNA and nucleotide are physically separated, the most likely mechanism for energetic connectivity involves the closing of the domains upon ligand binding, as described in the previous section (55). Because each ligand favors the closed conformation, binding of one ligand tightens binding of the other, giving rise to cooperativity. Presumably this ordering is diminished or lost later in the cycle after release of the P_i product, leading to decreased affinity for ssRNA. It should be noted that the structural information does not provide a simple explanation for the observed anti-coupling between ssRNA and ADP.

When in the ATPase cycle does strand separation occur?

The coupled ATPase and strand separation activities imply that the favorable free energy change associated with ATPase activity provides the driving force for separation of the two strands of an RNA helix, which is unfavorable when one or both strands are present at concentrations above the equilibrium constant for duplex formation. In the simplest terms, the coupled processes can be described by eq. 1, in which 'E' represents a DEAD-box protein.

While eq. 1 is useful as a starting point, it does not indicate how the processes are coupled or what intermediate species are formed. The ATPase cycle consists of ATP binding, hydrolysis, and release of each product, and the strand separation process consists of an interaction with a double-stranded duplex, generation or capture of a segment of single-stranded RNA (and repetitive captures of ssRNA, if necessary, until the duplex is completely separated), and finally release of one tightly-bound ssRNA product from the DEAD-box core. Although it is tempting to imagine the ATP hydrolysis event itself as releasing the energy that drives the unfavorable process of strand separation, there is no requirement from the coupled nature of the cycles that the strand separation follow the hydrolysis event. Indeed, evidence described below suggests that local strand separation can precede ATP hydrolysis, and that for some short RNA duplexes, this local strand separation is sufficient to completely separate the strands. Thus, the entire strand separation process is accomplished in a single cycle of ATP-dependent conformational changes and includes the intermediate shown in eq. 2.

A study using a series of ATP analogs showed that Ded1p promotes complete separation of model duplexes in the presence of ADP-BeF_x with an efficiency only modestly lower than that with ATP (17), demonstrating that RNA unwinding by a DEAD-box protein is possible without ATP hydrolysis. The relative activity of ADP-BeF_x in promoting multiple rounds of unwinding was much lower, suggesting that the principal role of ATP hydrolysis is to

decrease the affinity of Ded1p for the ssRNA that remains tightly bound after the duplex is separated, allowing recycling of the protein (eq. 2). This model is consistent with eq. 1 and in no way violates the notion that the energy from ATP binding and hydrolysis is coupled to RNA unwinding. Here, the ATP hydrolysis event (and release of P_i product) is directly coupled to the production of one of the ssRNA products by facilitating its release from the DEAD-box protein rather than from the complementary strand.

The work above would leave open the possibility that, when ATP is present, it is hydrolyzed to the products, ADP and P_i , prior to strand separation. This model would propose that ADP-BeF_x can accelerate strand separation along a slower pathway without hydrolysis, but when ATP is present it is converted to the products, perhaps with transient reversals back to ATP via re-synthesis (62,80), faster than the strands are separated. Although not strictly required by the model, net conversion of ATP to the products could then promote strand separation in addition to playing a role in dissociation of the tightly bound strand. Indeed, support for such a model has come from work in which fluorescence was used to measure strand separation and ATP hydrolysis by DbpA for an 8-bp RNA duplex (81). The rate constant for ATP hydrolysis is larger than that for strand separation for this duplex, indicating that ATP hydrolysis precedes strand separation in most events. Here as well, a single cycle of ATP-dependent conformational changes apparently gives complete strand separation, because the rate constant for strand separation was the same within error as that for release of P_i . Analogous results were reported for unwinding of a short duplex by the NS3 helicase of hepatitis C virus (82).

The results above can be reconciled by a model in which strand separation may be completed before ATP hydrolysis or after ATP hydrolysis, depending on the length and stability of the duplex and on the solution conditions. This model is supported by a study in which steady-state rates of duplex separation and ATP hydrolysis were compared for a series of duplexes of different lengths under a range of conditions (13). For a 6-bp duplex at low Mg^{2+} concentration, less than one ATP is hydrolyzed per duplex unwound by the DEAD-box protein CYT-19, even as ATP gives substantial acceleration of unwinding. Thus, ATP can stimulate unwinding of this duplex even when it is not hydrolyzed, at least for a fraction of the events. The viability of the hydrolysis-independent pathway suggests that the recorded ATP hydrolysis events may have occurred after strand separation and functioned in recycling. On the other hand, for longer or more stable duplexes, or at higher Mg^{2+} concentration, the ATP consumption rises to unity and beyond unity. The latter result indicates that, for many strand separation events, ATP hydrolysis precedes strand separation. The multiple ATPs hydrolyzed would be consistent with multiple cycles arising from limited translocation, but it appears more likely to arise from cycles of ATP binding and hydrolysis that do not result in strand separation (*i.e.* futile cycles). This latter explanation is favored because of evidence described above for a lack of translocation, and because the increased ATP requirement was also observed for duplexes that are particularly stable due to their sequences yet are small enough to be entirely bound by one monomer without any translocation.

Together, these results lead to the model shown in Fig. 4. Short or unstable duplexes can be separated in the absence of ATP hydrolysis. In events that include hydrolysis, it occurs after strand separation and promotes recycling (pathways 1 and 2, respectively). Longer or more stable duplexes require ATP hydrolysis or tend to become fully separated after ATP hydrolysis (pathway 3) (80). The precise role of hydrolysis remains to be determined, but it is possible that it promotes additional strand separation in a conformational change that resembles a single step of translocation (6,83). For more stable duplexes, a fraction of the ATP hydrolysis events still do not give complete strand separation, and dissociation of the helicase core with re-zipping of the duplex gives futile ATPase cycles (pathway 4).

RNA chaperone functions of DEAD-box proteins

The behavior of DEAD-box proteins in RNA duplex unwinding assays *in vitro* leads to the conclusion that they are severely deficient as helicases and have unusual properties. They are poorly processive, inhibited by stable duplexes, even relatively short ones, and they do not translocate on ssRNA to begin duplex unwinding. On the other hand, the same properties that would seem to be liabilities for a conventional helicase can readily be imagined to be assets for DEAD-box proteins that promote RNA folding and conformational rearrangements. Most helical elements in structured RNAs are short, rarely more than a single helical turn, so high processivity is not needed. In contrast, what is needed is to be able to disrupt short helices or other contacts efficiently without first loading onto a single-stranded segment, which are rare in structured RNAs and not likely to be present adjacent to every structural element that must be rearranged. Last, local and non-processive structure disruption would offer the advantage that it could allow selective rearrangements without loss of the global RNA structure. The sections below describe evidence that some DEAD-box proteins function as RNA chaperones, facilitating native folding of structured RNAs by accelerating conformational rearrangements.

Genetic and biochemical evidence for chaperone activity with group I and group II introns

RNA chaperone activity has been recognized in diverse groups of proteins dating back as far as 35 years ago (84–86). However, the first demonstration of such activity by a DEAD-box protein was in 2002, when the CYT-19 protein was shown by genetic and biochemical approaches to function in folding of at least three mitochondrial group I introns, as well as playing roles in other RNA processing events and translation (87). Its homolog in *S. cerevisiae*, Mss116p, also functions in folding of all nine mitochondrial group I introns and all four group II introns (88). Group I and II introns form highly compact, globular structures (Fig. 5), and both intron groups have been shown to misfold substantially *in vitro*, suggesting the necessity for chaperone proteins (89,90). The diversity of RNAs that depend on CYT-19 and Mss116p suggest a general mechanism of action, a conclusion that is supported by the finding that CYT-19, when expressed in yeast, can at least partially suppress all of the defects from loss of functional Mss116p (88).

Further insight into the mechanistic role of CYT-19 came from studies of a shortened ribozyme version of the group I intron from *Tetrahymena thermophila*, which accumulates *in vitro* predominantly in a single, long-lived misfolded conformation that is globally similar to the native state (91). CYT-19 gives ATP-dependent acceleration of unfolding of this misfolded conformer, allowing its refolding to the native state. After facilitating this refolding process, CYT-19 does not contribute to the catalytic reaction of the ribozyme (21). Thus its function is that of a molecular chaperone, in direct analogy with proteins that use ATP binding and hydrolysis to promote native folding of other proteins. The comparison may be the most direct with Hsp70 proteins, which bind short peptide sequences in an extended conformation in a cycle of high and low affinity that is regulated by the ATPase cycle (92,93). Similar to the effects of CYT-19 in assisting refolding of group I introns, Hsp70 proteins use these affinity cycles for activities including the active unfolding of protein substrates (92,93).

Mechanisms of RNA chaperone activity on group I and group II introns

Further work using the *Tetrahymena* group I RNA investigated whether CYT-19 recognizes specific structural features of misfolded intron. For variants with destabilized native structures, even the native forms are readily disrupted by CYT-19, and the RNAs then accumulate in the misfolded structure because it is preferred kinetically during subsequent refolding (94). Thus, CYT-19 disrupts RNA structure without recognizing specific structural

features of this misfolded RNA, and the reason that the reaction with the wild-type RNA proceeds toward the native state is because the native state is sufficiently more stable than the misfolded form such that it is seldom disrupted by CYT-19. The inability of CYT-19 to recognize the misfolded structure specifically is not surprising, given that CYT-19 functions *in vivo* with multiple introns, at least one of which appears to adopt multiple misfolded forms (87). This may be a general property of RNA chaperones, as misfolded RNAs can contain extensive native structure and bear a strong resemblance to their native counterparts, making specific recognition unlikely.

Nevertheless, one possible source of recognition of misfolded RNA has been suggested (Fig. 6). CYT-19 efficiently unwinds a helix within the *Tetrahymena* intron known as P1, but this unwinding activity is strongly inhibited by formation of tertiary contacts between P1 and the intron body (21). Although it remains to be determined whether this sensitivity to tertiary structure is a general property for DEAD-box proteins that function as RNA chaperones, it suggests a possible mechanism to generate biased action toward misfolded structures. Misfolded RNAs may be less able to pack their structural elements together into a globular structure, so selective activity toward exposed helices may be an effective means to direct DEAD-box proteins toward misfolded RNAs. Indeed, this mechanism may even target the protein specifically to the misfolded structural elements, because these elements are less likely to form native contacts.

The mechanisms of CYT-19 and Mss116p in group II intron folding and splicing have also been explored using two group II introns from *S. cerevisiae*. Both proteins facilitate splicing of the bII intron, with Mss116p having higher efficiency than CYT-19 (20,95). CYT-19 also promotes a folding reaction of the intron that allows a reverse splicing reaction to take place after destruction of CYT-19, indicating that CYT-19 functions as a chaperone in folding of the intron (96). Both proteins also facilitate splicing of the ai5 γ intron (20,73,97), but the mechanism may be more complex. An RNA cleavage reaction by a shortened version of this intron (D135, which includes domains 1, 3, and 5) is accelerated by Mss116p in an ATP-dependent manner, but the acceleration requires Mss116p and ATP after addition of an oligonucleotide substrate, most simply suggesting that Mss116p promotes a conformational rearrangement that follows binding of the substrate (20). In this and other folding processes, Mss116p is thought to function as a chaperone by destabilizing native or non-native contacts that must be disrupted for further productive folding, as there is a strong correlation of different DEAD-box proteins and Mss116p variants in their efficiencies of RNA duplex unwinding, disruption of group I intron tertiary structure, and promotion of ai5 γ intron splicing (95,97). Nevertheless, it is possible that DEAD-box proteins play multiple roles in folding of this intron [see below; refs. (73,98)].

A broader range of mechanisms for promoting RNA rearrangements

It is becoming clear that many DEAD-box proteins function by using energy from ATP to promote local strand separation within a structured RNA, presumably permitting the liberated strand and its partner to adopt new interactions and potentially destabilizing structural elements that form cooperatively. It seems highly likely that this activity underlies much of the ability of DEAD-box proteins to function as RNA chaperones and, more generally, to promote rearrangements of structured RNAs. For example, the DEAD-box protein Prp28p appears to function in part by disrupting a helix within the spliceosome in preparation for pre-mRNA splicing (99), and eIF4A and Ded1p may disrupt structure within mRNAs to promote translation (63,100,101).

Beyond these roles, the ability to use ATP binding and hydrolysis to generate tight, yet reversible binding to ssRNA may allow DEAD-box proteins to perform a broader range of activities. It has been suggested that Mss116p functions in folding of the ai5 γ group II intron

using multiple mechanisms, including resolution of misfolded exon structures and stabilization of an early folding intermediate that gives global collapse of the major domain of the intron (98,102). This latter activity could be reconciled with the known properties of DEAD-box proteins if formation of this intermediate requires capture or sequestration of a ssRNA segment that would otherwise form an inhibitory interaction. While such an activity might simply reflect duplex unwinding, as above, if the critical event is maintenance of the interaction during subsequent folding steps, this might also be viewed as a moderate version of an activity that another DEAD-box protein, eIF4A-III, has carried to an extreme. This protein forms a very stable complex with spliced mRNAs as a component of the exon-junction complex (EJC), serving as a marker for the status and location of splicing events (56,57,103,104).

Other departures from local strand separation mechanisms are also possible. As noted above (see *Targeting DEAD-box proteins to certain RNAs or RNPs*), Dbp5p functions in export of mRNA from the nucleus and is suggested to remodel the mRNA by removing proteins (34). The ability of DEAD-box proteins to remodel RNP complexes by removing proteins has been established using model systems (105,106), and most likely arises from essentially the same mechanism as strand separation, with the key event being sequestration of a segment of ssRNA away from its former partner. A more traditional helicase reaction with 3' to 5' directionality, albeit with limited processivity, has been suggested for DbpA from experiments with an extensive set of model duplexes (83). This reaction would be analogous to that of the DEAH-box protein Prp22, which functions in pre-mRNA splicing by translocating 3' to 5' along the newly spliced mRNA, releasing it from the spliceosome (107). Last, some DEAD-box proteins have been shown to accelerate duplex formation in addition to dissociation, which may function in promoting RNA folding and rearrangements (20,108).

Conclusion

The last few years have seen enormous progress toward understanding the mechanism of RNA unwinding by DEAD-box proteins, and a basic framework has emerged. DEAD-box proteins can be tethered to substrate RNA duplexes through interactions that can be highly specific or much less specific, where they can interact directly with double-stranded RNA to induce or capture local strand separation. Hydrolysis of ATP and release of the products complete the process and allow recycling of the DEAD-box protein by releasing ssRNA. There is still much to be learned, however. It is not clear whether the paradigm of RNA unwinding by a single cycle of ATP-dependent conformational changes applies to all DEAD-box proteins and all duplexes that can be separated, or whether mechanisms involving multiple cycles and limited translocation exist for some proteins and unwinding reactions. If there are different mechanisms for different proteins, it is not clear what determines which mechanism is followed. It is possible that DEAD-box proteins follow a rather limited set of mechanisms, and proteins from other SF2 families behave more like conventional helicases. Alternatively, it remains possible that there is some overlap in the mechanisms of proteins from different families, with individual proteins employing mechanisms that are dictated by their physiological substrates and reactions.

Recent progress indicates that DEAD-box proteins have adapted their basic property – coupled cycles of ATPase activity and ssRNA binding – to perform a very diverse set of functions. One common function is the facilitation of RNA rearrangements or folding steps, and it has recently become clear that some DEAD-box proteins function as RNA chaperones, disrupting structure in RNA folding intermediates to allow their continued folding to functional structures. Nevertheless, at a molecular level, the manipulations of structured RNAs by DEAD-box proteins are not well understood. Even for a general

chaperone, which functions on a range of RNAs, there may be preferred molecular pathways for rearrangements, dictated at least in part by the individual folding transitions. Different folding transitions may also have different molecular requirements, even within the same RNA or RNP. For example, a folding step that requires extensive structure disruption may be particularly dependent on ATP hydrolysis. Thus, it is a continuing challenge to better understand RNA folding steps and intermediates and to explore how folding is facilitated by DEAD-box proteins. Catalytic RNAs, including group I and II introns, will continue to be attractive as model systems due to their relative simplicity and experimental tractability as well as their physiological importance.

Acknowledgments

We thank members of the Russell lab for helpful comments on the manuscript. Research in the Russell lab is supported by grants from the NIH (GM070456), the Welch Foundation (F-1563), and the Norman Hackerman Advanced Research Program (003658-0242-2007).

References

1. Linder P, et al. Birth of the D-E-A-D box. *Nature*. 1989; 337:121–122. [PubMed: 2563148]
2. Linder P. Dead-box proteins: a family affair--active and passive players in RNP-remodeling. *Nucleic Acids Res*. 2006; 34:4168–4180. [PubMed: 16936318]
3. Jankowsky E, Fairman ME. RNA helicases--one fold for many functions. *Curr Opin Struct Biol*. 2007; 17:316–324. [PubMed: 17574830]
4. Fairman-Williams ME, Guenther UP, Jankowsky E. SF1 and SF2 helicases: family matters. *Curr Opin Struct Biol*. 2010
5. Hilbert M, Karow AR, Klostermeier D. The mechanism of ATP-dependent RNA unwinding by DEAD box proteins. *Biol Chem*. 2009; 390:1237–1250. [PubMed: 19747077]
6. Singleton MR, Dillingham MS, Wigley DB. Structure and mechanism of helicases and nucleic acid translocases. *Annu Rev Biochem*. 2007; 76:23–50. [PubMed: 17506634]
7. Lohman TM, Tomko EJ, Wu CG. Non-hexameric DNA helicases and translocases: mechanisms and regulation. *Nat Rev Mol Cell Biol*. 2008; 9:391–401. [PubMed: 18414490]
8. Rossler OG, Straka A, Stahl H. Rearrangement of structured RNA via branch migration structures catalysed by the highly related DEAD-box proteins p68 and p72. *Nucleic Acids Res*. 2001; 29:2088–2096. [PubMed: 11353078]
9. Huang Y, Liu ZR. The ATPase, RNA unwinding, and RNA binding activities of recombinant p68 RNA helicase. *J Biol Chem*. 2002; 277:12810–12815. [PubMed: 11823473]
10. Chamot D, Colvin KR, Kujat-Choy SL, Owttrim GW. RNA structural rearrangement via unwinding and annealing by the cyanobacterial RNA helicase, CrhR. *J Biol Chem*. 2005; 280:2036–2044. [PubMed: 15542859]
11. Rogers GW Jr, Richter NJ, Merrick WC. Biochemical and kinetic characterization of the RNA helicase activity of eukaryotic initiation factor 4A. *J Biol Chem*. 1999; 274:12236–12244. [PubMed: 10212190]
12. Rogers GW Jr, Lima WF, Merrick WC. Further characterization of the helicase activity of eIF4A. Substrate specificity. *J Biol Chem*. 2001; 276:12598–12608. [PubMed: 11278350]
13. Chen Y, et al. DEAD-box proteins can completely separate an RNA duplex using a single ATP. *Proc Natl Acad Sci U S A*. 2008; 105:20203–20208. [PubMed: 19088196]
14. Bizebard T, Ferlenghi I, Iost I, Dreyfus M. Studies on three *E. coli* DEAD-box helicases point to an unwinding mechanism different from that of model DNA helicases. *Biochemistry*. 2004; 43:7857–7866. [PubMed: 15196029]
15. Iost I, Dreyfus M. DEAD-box RNA helicases in *Escherichia coli*. *Nucleic Acids Res*. 2006; 34:4189–4197. [PubMed: 16935881]
16. Grohman JK, et al. Probing the mechanisms of DEAD-box proteins as general RNA chaperones: the C-terminal domain of CYT-19 mediates general recognition of RNA. *Biochemistry*. 2007; 46:3013–3022. [PubMed: 17311413]

17. Liu F, Putnam A, Jankowsky E. ATP hydrolysis is required for DEAD-box protein recycling but not for duplex unwinding. *Proc Natl Acad Sci U S A*. 2008; 105:20209–20214. [PubMed: 19088201]
18. Yang Q, Del Campo M, Lambowitz AM, Jankowsky E. DEAD-box proteins unwind duplexes by local strand separation. *Mol Cell*. 2007; 28:253–263. [PubMed: 17964264]
19. Rozen F, et al. Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Mol Cell Biol*. 1990; 10:1134–1144. [PubMed: 2304461]
20. Halls C, et al. Involvement of DEAD-box proteins in group I and group II intron splicing. Biochemical characterization of Mss116p, ATP hydrolysis-dependent and -independent mechanisms, and general RNA chaperone activity. *J Mol Biol*. 2007; 365:835–855. [PubMed: 17081564]
21. Tijerina P, Bhaskaran H, Russell R. Nonspecific binding to structured RNA and preferential unwinding of an exposed helix by the CYT-19 protein, a DEAD-box RNA chaperone. *Proc Natl Acad Sci U S A*. 2006; 103:16698–16703. [PubMed: 17075070]
22. Yang Q, Jankowsky E. The DEAD-box protein Ded1 unwinds RNA duplexes by a mode distinct from translocating helicases. *Nat Struct Mol Biol*. 2006; 13:981–986. [PubMed: 17072313]
23. Fuller-Pace FV, Nicol SM, Reid AD, Lane DP. DbpA: a DEAD box protein specifically activated by 23S rRNA. *EMBO J*. 1993; 12:3619–3626. [PubMed: 8253085]
24. Tsu CA, Kossen K, Uhlenbeck OC. The *Escherichia coli* DEAD protein DbpA recognizes a small RNA hairpin in 23S rRNA. *RNA*. 2001; 7:702–709. [PubMed: 11350034]
25. Diges CM, Uhlenbeck OC. *Escherichia coli* DbpA is an RNA helicase that requires hairpin 92 of 23S rRNA. *EMBO J*. 2001; 20:5503–5512. [PubMed: 11574482]
26. Karginov FV, Caruthers JM, Hu Y, McKay DB, Uhlenbeck OC. YxiN is a modular protein combining a DEx(D/H) core and a specific RNA-binding domain. *J Biol Chem*. 2005; 280:35499–35505. [PubMed: 16118224]
27. Wang S, et al. The domain of the *Bacillus subtilis* DEAD-box helicase YxiN that is responsible for specific binding of 23S rRNA has an RNA recognition motif fold. *RNA*. 2006; 12:959–967. [PubMed: 16611943]
28. Wang S, Overgaard MT, Hu Y, McKay DB. The *Bacillus subtilis* RNA helicase YxiN is distended in solution. *Biophys J*. 2008; 94:L01–03. [PubMed: 17951299]
29. Linden MH, Hartmann RK, Klostermeier D. The putative RNase P motif in the DEAD box helicase Hera is dispensable for efficient interaction with RNA and helicase activity. *Nucleic Acids Res*. 2008; 36:5800–5811. [PubMed: 18782831]
30. Rudolph MG, Klostermeier D. The *Thermus thermophilus* DEAD box helicase Hera contains a modified RNA recognition motif domain loosely connected to the helicase core. *RNA*. 2009; 15:1993–2001. [PubMed: 19710183]
31. Mohr G, et al. Function of the C-terminal domain of the DEAD-box protein Mss116p analyzed *in vivo* and *in vitro*. *J Mol Biol*. 2008; 375:1344–1364. [PubMed: 18096186]
32. Klostermeier D, Rudolph MG. A novel dimerization motif in the C-terminal domain of the *Thermus thermophilus* DEAD box helicase Hera confers substantial flexibility. *Nucleic Acids Res*. 2009; 37:421–430. [PubMed: 19050012]
33. Tran EJ, Zhou Y, Corbett AH, Wentz SR. The DEAD-box protein Dbp5 controls mRNA export by triggering specific RNA:protein remodeling events. *Mol Cell*. 2007; 28:850–859. [PubMed: 18082609]
34. Stewart M. Ratcheting mRNA out of the nucleus. *Mol Cell*. 2007; 25:327–330. [PubMed: 17289581]
35. Linder P. mRNA export: RNP remodeling by DEAD-box proteins. *Curr Biol*. 2008; 18:R297–299. [PubMed: 18397738]
36. Schmitt C, et al. Dbp5, a DEAD-box protein required for mRNA export, is recruited to the cytoplasmic fibrils of nuclear pore complex via a conserved interaction with CAN/Nup159p. *EMBO J*. 1999; 18:4332–4347. [PubMed: 10428971]
37. Hodge CA, Colot HV, Stafford P, Cole CN. Rat8p/Dbp5p is a shuttling transport factor that interacts with Rat7p/Nup159p and Gle1p and suppresses the mRNA export defect of xpo1–1 cells. *EMBO J*. 1999; 18:5778–5788. [PubMed: 10523319]

38. Weirich CS, et al. Activation of the DEXD/H-box protein Dbp5 by the nuclear-pore protein Gle1 and its coactivator InsP6 is required for mRNA export. *Nat Cell Biol.* 2006; 8:668–676. [PubMed: 16783364]
39. Dossani ZY, Weirich CS, Erzberger JP, Berger JM, Weis K. Structure of the C-terminus of the mRNA export factor Dbp5 reveals the interaction surface for the ATPase activator Gle1. *Proc Natl Acad Sci U S A.* 2009; 106:16251–16256. [PubMed: 19805289]
40. Napetschnig J, et al. Structural and functional analysis of the interaction between the nucleoporin Nup214 and the DEAD-box helicase Ddx19. *Proc Natl Acad Sci U S A.* 2009; 106:3089–3094. [PubMed: 19208808]
41. von Moeller H, Basquin C, Conti E. The mRNA export protein DBP5 binds RNA and the cytoplasmic nucleoporin NUP214 in a mutually exclusive manner. *Nat Struct Mol Biol.* 2009; 16:247–254. [PubMed: 19219046]
42. Oberer M, Marintchev A, Wagner G. Structural basis for the enhancement of eIF4A helicase activity by eIF4G. *Genes Dev.* 2005; 19:2212–2223. [PubMed: 16166382]
43. Fukao A, et al. The ELAV protein HuD stimulates cap-dependent translation in a Poly(A)-and eIF4A-dependent manner. *Mol Cell.* 2009; 36:1007–1017. [PubMed: 20064466]
44. Buchwald G, et al. Insights into the recruitment of the NMD machinery from the crystal structure of a core EJC-UPF3b complex. *Proc Natl Acad Sci U S A.* 2010; 107:10050–10055. [PubMed: 20479275]
45. Schutz P, et al. Crystal structure of the yeast eIF4A-eIF4G complex: an RNA-helicase controlled by protein-protein interactions. *Proc Natl Acad Sci U S A.* 2008; 105:9564–9569. [PubMed: 18606994]
46. Tritschler F, et al. Structural basis for the mutually exclusive anchoring of P body components EDC3 and Tral to the DEAD box protein DDX6/Me31B. *Mol Cell.* 2009; 33:661–668. [PubMed: 19285948]
47. Staley JP, Guthrie C. Mechanical devices of the spliceosome: motors, clocks, springs, and things. *Cell.* 1998; 92:315–326. [PubMed: 9476892]
48. Lorsch JR, Herschlag D. The DEAD box protein eIF4A. 1. A minimal kinetic and thermodynamic framework reveals coupled binding of RNA and nucleotide. *Biochemistry.* 1998; 37:2180–2193. [PubMed: 9485364]
49. Potratz JP, Tijerina P, Russell R, Jankowsky E. Royal Society of Chemistry. Mechanisms of DEAD-box proteins in ATP-dependent processes. RNA helicases. 2010:61–98. *RSC Biomolecular Sciences No. 19.*
50. Banroques J, Doere M, Dreyfus M, Linder P, Tanner NK. Motif III in superfamily 2 “helicases” helps convert the binding energy of ATP into a high-affinity RNA binding site in the yeast DEAD-box protein Ded1. *J Mol Biol.* 2010; 396:949–966. [PubMed: 20026132]
51. Caruthers JM, Johnson ER, McKay DB. Crystal structure of yeast initiation factor 4A, a DEAD-box RNA helicase. *Proc Natl Acad Sci U S A.* 2000; 97:13080–13085. [PubMed: 11087862]
52. Story RM, Li H, Abelson JN. Crystal structure of a DEAD box protein from the hyperthermophile *Methanococcus jannaschii*. *Proc Natl Acad Sci U S A.* 2001; 98:1465–1470. [PubMed: 11171974]
53. Shi H, Cordin O, Minder CM, Linder P, Xu RM. Crystal structure of the human ATP-dependent splicing and export factor UAP56. *Proc Natl Acad Sci U S A.* 2004; 101:17628–17633. [PubMed: 15585580]
54. Cheng Z, Collier J, Parker R, Song H. Crystal structure and functional analysis of DEAD-box protein Dhh1p. *RNA.* 2005; 11:1258–1270. [PubMed: 15987810]
55. Sengoku T, Nureki O, Nakamura A, Kobayashi S, Yokoyama S. Structural basis for RNA unwinding by the DEAD-box protein *Drosophila* Vasa. *Cell.* 2006; 125:287–300. [PubMed: 16630817]
56. Andersen CB, et al. Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA. *Science.* 2006; 313:1968–1972. [PubMed: 16931718]
57. Bono F, Ebert J, Lorentzen E, Conti E. The crystal structure of the exon junction complex reveals how it maintains a stable grip on mRNA. *Cell.* 2006; 126:713–725. [PubMed: 16923391]

58. Hogbom M, et al. Crystal structure of conserved domains 1 and 2 of the human DEAD-box helicase DDX3X in complex with the mononucleotide AMP. *J Mol Biol.* 2007; 372:150–159. [PubMed: 17631897]
59. Del Campo M, Lambowitz AM. Structure of the Yeast DEAD box protein Mss116p reveals two wedges that crimp RNA. *Mol Cell.* 2009; 35:598–609. [PubMed: 19748356]
60. Fan JS, et al. Solution and crystal structures of mRNA exporter Dbp5p and its interaction with nucleotides. *J Mol Biol.* 2009; 388:1–10. [PubMed: 19281819]
61. Collins R, et al. The DEXD/H-box RNA helicase DDX19 is regulated by an α -helical switch. *J Biol Chem.* 2009; 284:10296–10300. [PubMed: 19244245]
62. Nielsen KH, et al. Mechanism of ATP turnover inhibition in the EJC. *RNA.* 2009; 15:67–75. [PubMed: 19033377]
63. Svitkin YV, et al. The requirement for eukaryotic initiation factor 4A (eIF4A) in translation is in direct proportion to the degree of mRNA 5' secondary structure. *RNA.* 2001; 7:382–394. [PubMed: 11333019]
64. Banroques J, Cordin O, Doere M, Linder P, Tanner NK. A conserved phenylalanine of motif IV in superfamily 2 helicases is required for cooperative, ATP-dependent binding of RNA substrates in DEAD-box proteins. *Mol Cell Biol.* 2008; 28:3359–3371. [PubMed: 18332124]
65. Pyle AM. Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu Rev Biophys.* 2008; 37:317–336. [PubMed: 18573084]
66. Buttner K, Nehring S, Hopfner KP. Structural basis for DNA duplex separation by a superfamily-2 helicase. *Nat Struct Mol Biol.* 2007; 14:647–652. [PubMed: 17558417]
67. Rozen F, Pelletier J, Trachsel H, Sonenberg N. A lysine substitution in the ATP-binding site of eucaryotic initiation factor 4A abrogates nucleotide-binding activity. *Mol Cell Biol.* 1989; 9:4061–4063. [PubMed: 2506440]
68. Blum S, et al. ATP hydrolysis by initiation factor 4A is required for translation initiation in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A.* 1992; 89:7664–7668. [PubMed: 1502180]
69. Pause A, Sonenberg N. Mutational analysis of a DEAD box RNA helicase: the mammalian translation initiation factor eIF-4A. *EMBO J.* 1992; 11:2643–2654. [PubMed: 1378397]
70. Pause A, Methot N, Sonenberg N. The HRIGRXXR region of the DEAD box RNA helicase eukaryotic translation initiation factor 4A is required for RNA binding and ATP hydrolysis. *Mol Cell Biol.* 1993; 13:6789–6798. [PubMed: 8413273]
71. Iost I, Dreyfus M, Linder P. Ded1p, a DEAD-box protein required for translation initiation in *Saccharomyces cerevisiae*, is an RNA helicase. *J Biol Chem.* 1999; 274:17677–17683. [PubMed: 10364207]
72. Rocak S, Emery B, Tanner NK, Linder P. Characterization of the ATPase and unwinding activities of the yeast DEAD-box protein Has1p and the analysis of the roles of the conserved motifs. *Nucleic Acids Res.* 2005; 33:999–1009. [PubMed: 15718299]
73. Solem A, Zingler N, Pyle AM. A DEAD protein that activates intron self-splicing without unwinding RNA. *Mol Cell.* 2006; 24:611–617. [PubMed: 17188036]
74. Karow AR, Klostermeier D. A conformational change in the helicase core is necessary but not sufficient for RNA unwinding by the DEAD box helicase YxiN. *Nucleic Acids Res.* 2009; 37:4464–4471. [PubMed: 19474341]
75. Tanner NK, Cordin O, Banroques J, Doere M, Linder P. The Q motif: a newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. *Mol Cell.* 2003; 11:127–138. [PubMed: 12535527]
76. Cordin O, Tanner NK, Doere M, Linder P, Banroques J. The newly discovered Q motif of DEAD-box RNA helicases regulates RNA-binding and helicase activity. *EMBO J.* 2004; 23:2478–2487. [PubMed: 15201868]
77. Lorsch JR, Herschlag D. The DEAD box protein eIF4A. 2. A cycle of nucleotide and RNA-dependent conformational changes. *Biochemistry.* 1998; 37:2194–2206. [PubMed: 9485365]
78. Theissen B, Karow AR, Kohler J, Gubaev A, Klostermeier D. Cooperative binding of ATP and RNA induces a closed conformation in a DEAD box RNA helicase. *Proc Natl Acad Sci U S A.* 2008; 105:548–553. [PubMed: 18184816]

79. Polach KJ, Uhlenbeck OC. Cooperative binding of ATP and RNA substrates to the DEAD/H protein DbpA. *Biochemistry*. 2002; 41:3693–3702. [PubMed: 11888286]
80. Henn A, Cao W, Hackney DD, De La Cruz EM. The ATPase cycle mechanism of the DEAD-box rRNA helicase, DbpA. *J Mol Biol*. 2008; 377:193–205. [PubMed: 18237742]
81. Henn A, et al. Pathway of ATP utilization and duplex rRNA unwinding by the DEAD-box helicase, DbpA. *Proc Natl Acad Sci U S A*. 2010; 107:4046–4050. [PubMed: 20160110]
82. Wang Q, Arnold JJ, Uchida A, Raney KD, Cameron CE. Phosphate release contributes to the rate-limiting step for unwinding by an RNA helicase. *Nucleic Acids Res*. 2010; 38:1312–1324. [PubMed: 19969541]
83. Diges CM, Uhlenbeck OC. *Escherichia coli* DbpA is a 3' → 5' RNA helicase. *Biochemistry*. 2005; 44:7903–7911. [PubMed: 15910005]
84. Karpel RL, et al. Acceleration of RNA renaturation by nucleic acid unwinding proteins. *Brookhaven Symp Biol*. 1975:165–174. [PubMed: 1104090]
85. Karpel RL, Miller NS, Fresco JR. Mechanistic studies of ribonucleic acid renaturation by a helix-destabilizing protein. *Biochemistry*. 1982; 21:2102–2108. [PubMed: 6178431]
86. Rajkowsch L, et al. RNA chaperones, RNA annealers and RNA helicases. *RNA Biol*. 2007; 4:118–130. [PubMed: 18347437]
87. Mohr S, Stryker JM, Lambowitz AM. A DEAD-box protein functions as an ATP-dependent RNA chaperone in group I intron splicing. *Cell*. 2002; 109:769–779. [PubMed: 12086675]
88. Huang HR, et al. The splicing of yeast mitochondrial group I and group II introns requires a DEAD-box protein with RNA chaperone function. *Proc Natl Acad Sci U S A*. 2005; 102:163–168. [PubMed: 15618406]
89. Treiber DK, Williamson JR. Exposing the kinetic traps in RNA folding. *Curr Opin Struct Biol*. 1999; 9:339–345. [PubMed: 10361090]
90. Russell R. RNA misfolding and the action of chaperones. *Front Biosci*. 2008; 13:1–20. [PubMed: 17981525]
91. Russell R, et al. The paradoxical behavior of a highly structured misfolded intermediate in RNA folding. *J Mol Biol*. 2006; 363:531–544. [PubMed: 16963081]
92. Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci*. 2005; 62:670–684. [PubMed: 15770419]
93. Goloubinoff P, De Los Rios P. The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem Sci*. 2007; 32:372–380. [PubMed: 17629485]
94. Bhaskaran H, Russell R. Kinetic redistribution of native and misfolded RNAs by a DEAD-box chaperone. *Nature*. 2007; 449:1014–1018. [PubMed: 17960235]
95. Del Campo M, et al. Unwinding by local strand separation is critical for the function of DEAD-box proteins as RNA chaperones. *J Mol Biol*. 2009; 389:674–693. [PubMed: 19393667]
96. Mohr S, Matsuura M, Perlman PS, Lambowitz AM. A DEAD-box protein alone promotes group II intron splicing and reverse splicing by acting as an RNA chaperone. *Proc Natl Acad Sci U S A*. 2006; 103:3569–3574. [PubMed: 16505350]
97. Del Campo M, et al. Do DEAD-box proteins promote group II intron splicing without unwinding RNA? *Mol Cell*. 2007; 28:159–166. [PubMed: 17936712]
98. Fedorova O, Solem A, Pyle AM. Protein-facilitated folding of group II intron ribozymes. *J Mol Biol*. 2010
99. Staley JP, Guthrie C. An RNA switch at the 5' splice site requires ATP and the DEAD box protein Prp28p. *Mol Cell*. 1999; 3:55–64. [PubMed: 10024879]
100. Linder P. Yeast RNA helicases of the DEAD-box family involved in translation initiation. *Biol Cell*. 2003; 95:157–167. [PubMed: 12867080]
101. Marsden S, Nardelli M, Linder P, McCarthy JE. Unwinding single RNA molecules using helicases involved in eukaryotic translation initiation. *J Mol Biol*. 2006; 361:327–335. [PubMed: 16828800]
102. Zingler N, Solem A, Pyle AM. Dual roles for the Mss116 cofactor during splicing of the ai5 γ group II intron. *Nucleic Acids Res*. 2010

103. Le Hir H, Izaurralde E, Maquat LE, Moore MJ. The spliceosome deposits multiple proteins 20–24 nucleotides upstream of mRNA exon-exon junctions. *EMBO J.* 2000; 19:6860–6869. [PubMed: 11118221]
104. Shibuya T, Tange TO, Sonenberg N, Moore MJ. eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. *Nat Struct Mol Biol.* 2004; 11:346–351. [PubMed: 15034551]
105. Fairman ME, et al. Protein displacement by DEXH/D “RNA helicases” without duplex unwinding. *Science.* 2004; 304:730–734. [PubMed: 15118161]
106. Bowers HA, et al. Discriminatory RNP remodeling by the DEAD-box protein DED1. *RNA.* 2006; 12:903–912. [PubMed: 16556937]
107. Schwer B. A conformational rearrangement in the spliceosome sets the stage for Prp22-dependent mRNA release. *Mol Cell.* 2008; 30:743–754. [PubMed: 18570877]
108. Yang Q, Jankowsky E. ATP- and ADP-dependent modulation of RNA unwinding and strand annealing activities by the DEAD-box protein DED1. *Biochemistry.* 2005; 44:13591–13601. [PubMed: 16216083]
109. Sharpe Elles LM, Sykes MT, Williamson JR, Uhlenbeck OC. A dominant negative mutant of the *E. coli* RNA helicase DbpA blocks assembly of the 50S ribosomal subunit. *Nucleic Acids Res.* 2009; 37:6503–6514. [PubMed: 19734347]
110. Jain C. The *E. coli* RhlE RNA helicase regulates the function of related RNA helicases during ribosome assembly. *RNA.* 2008; 14:381–389. [PubMed: 18083833]
111. Tarn WY, Chang TH. The current understanding of Ded1p/DDX3 homologs from yeast to human. *RNA Biol.* 2009; 6:17–20. [PubMed: 19106629]
112. Gross T, et al. The DEAD-box RNA helicase Dbp5 functions in translation termination. *Science.* 2007; 315:646–649. [PubMed: 17272721]
113. Lehnert V, Jaeger L, Michel F, Westhof E. New loop-loop tertiary interactions in self-splicing introns of subgroup IC and ID: a complete 3D model of the *Tetrahymena thermophila* ribozyme. *Chem Biol.* 1996; 3:993–1009. [PubMed: 9000010]
114. Toor N, Keating KS, Taylor SD, Pyle AM. Crystal structure of a self-spliced group II intron. *Science.* 2008; 320:77–82. [PubMed: 18388288]
115. Toor N, et al. Tertiary architecture of the *Oceanobacillus iheyensis* group II intron. *RNA.* 2010; 16:57–69. [PubMed: 19952115]

Figure 1.

Roles of single-stranded and double-stranded extensions in duplex unwinding. **A**, Strand separation by a processive, 3'-5' helicase begins when the helicase loads on a single-stranded extension with a dangling 3'-end. The helicase then translocates in the 3' to 5' direction into the adjacent double-stranded region. **B**, Single-stranded overhangs enhance strand separation by some DEAD-box proteins, but are not required to have a defined polarity with respect to the adjacent duplex. **C**, Double-stranded extensions can also enhance unwinding for some DEAD-box proteins, and these extensions may be RNA or DNA. **D**, A single-stranded RNA or DNA segment can activate RNA unwinding by DEAD-box proteins even when physically separated from the neighboring duplex, as demonstrated when biotinylated RNA constructs were linked to streptavidin (22).

Figure 2.

Targeting DEAD-box proteins to RNA substrates through specific and non-specific interactions. **A**, Recognition of a specific RNA motif via an ancillary domain. Hairpin 92 from the 23S rRNA, shown by nucleotide letters, is recognized with high specificity by the bacterial proteins DbpA and YxiN. **B**, Electrostatically-driven RNA binding through an ancillary domain or unstructured extension. **C**, Helicase core loading on RNA substrates via dimerization, with a tethering interaction being formed by the helicase core from one monomer and the unwinding being carried out by the other monomer (22). **D**, Targeting through specific interactions between the DEAD-box core and a protein component of the targeted RNP complex.

Figure 3.

DEAD-box protein structure and interactions with ssRNA and ATP. **A**, Schematic depiction of domains and motifs, as present in Mss116p. Cylinders indicate domains that are present in the crystal structure. NTE, N-terminal extension (does not include the mitochondrial targeting sequence); CTE, non-conserved C-terminal extension; BT, basic tail. **B–D**, Structural views of RNA and ATP binding by DEAD-box proteins, shown for Mss116p (59). **B**, Domain structure. Bound U₁₀ RNA and AMP-PNP are magenta and red, respectively. Note the location of the ligand-binding pockets at the interface of the two RecA-like domains. **C**, Interactions and conserved motifs. Conserved sequence motifs are highlighted in the same colors as in panel A. **D**, Stereoview of the RNA binding site. The structure has been rotated by approximately 140° relative to panels B and C. The six RNA nucleotides that are bound in a consistent manner in all available DEAD-box protein:RNA structures are indicated by labels on the first and the last nucleotides (U3 and U8). The conserved helix that necessitates RNA bending is labeled (α 8) and the approximate center of the bend is indicated with an arrow. Amino acids that contact the RNA are shown as stick models.

Figure 4.

Coupling of ATPase cycles to duplex unwinding activity. Pathways for strand separation with or without ATP hydrolysis are indicated by arrows and the numbers 1–3 (see text for further details). Pathway 4 depicts a futile cycle of ATP hydrolysis without complete strand separation. An additional tethering interaction is shown in the model throughout the strand separation process. Modified from ref. (13) with permission.

Figure 5. Representative structures of group I and group II intron RNAs. **A**, Secondary and tertiary structures of the *Tetrahymena thermophila* group I intron. The tertiary structure is a model that is supported by domain structures and biochemical data (113). **B**, Secondary and tertiary structure of the group II intron from *Oceanobacillus iheyensis* (114,115).

Figure 6. Sensitivity of CYT-19 to RNA tertiary structure. Tertiary contacts between the 6-bp P1 duplex (green) and the core of *Tetrahymena* ribozyme (black cylinders) inhibit unwinding of the duplex by CYT-19. However, reversible “undocking” of P1 allows CYT-19 to promote strand separation of the duplex (21).

DEAD-box proteins of *E. coli* and *S. cerevisiae*^{*}[illegible]

[†]Names of proteins that are essential for viability are red.