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RNA Helicase Proteins as Chaperones and Remodelers

Inga Jarmoskaite and Rick Russell

Department of Molecular Biosciences and the Institute for Cellular and Molecular Biology, University of Texas, Austin, Texas 78712; email: i_jarmoskaite@utexas.edu, rick_russell@cm.utexas.edu

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Abstract

Superfamily 2 helicase proteins are ubiquitous in RNA biology and have an extraordinarily broad set of functional roles. Central among these roles are the promotion of rearrangements of structured RNAs and the remodeling of ribonucleoprotein complexes (RNPs), allowing formation of native RNA structure or progression through a functional cycle of structures. Although all superfamily 2 helicases share a conserved helicase core, they are divided evolutionarily into several families, and it is principally proteins from three families, the DEAD-box, DEAH/RHA, and Ski2-like families, that function to manipulate structured RNAs and RNPs. Strikingly, there are emerging differences in the mechanisms of these proteins, both between families and within the largest family (DEAD-box), and these differences appear to be tuned to their RNA or RNP substrates and their specific roles. This review outlines basic mechanistic features of the three families and surveys individual proteins and the current understanding of their biological substrates and mechanisms.

Contents

INTRODUCTION

Structured RNAs function throughout biology in processes such as translation, pre-mRNA splicing, RNA processing, chromosome end maintenance, and the regulation of gene expression. To function, these RNAs must fold to specific three-dimensional structures that include secondary structure (RNA helices) and tertiary structure, and they commonly include proteins to form ribonucleoprotein complexes (RNPs). The recent discovery of pervasive transcription of eukaryotic genomes has revealed further the large number of noncoding, and potentially structured, RNAs produced in cells (1–3).

The underlying physical properties of RNA result in a universal need for proteins to accelerate RNA structural transitions. It has been known for decades that RNAs face substantial challenges in folding efficiently to specific structures and in transitioning between structured conformations (4–6). Local secondary structure can be independently stable and long-lived, such that transitions requiring changes in secondary structure can require assistance in vivo (7). Furthermore, because of the limited diversity of RNA side chains, nonnative base pairs are likely to form during folding, resulting in kinetically trapped structures in vitro and in vivo. Indeed, even mRNAs, which must be unstructured over much of their length to be translated into proteins, are likely to form local structures that require assistance from proteins.

Although many diverse proteins possess RNA chaperone activity and function as chaperones in some instances (8), superfamily 2 (SF2) helicase proteins stand out for their ubiquitous roles in manipulating structured RNAs in vivo (9–11). This expansive collection of proteins comprises multiple families, including many proteins that function on DNA instead of RNA. It also includes proteins that lack conventional helicase activity, despite the helicase designation, which was coined decades ago from sequence similarity with the limited number of known helicases at the time (12). As described below, some SF2 proteins can unwind

RNA locally but do not translocate significantly along RNA, whereas others, not discussed here, translocate along RNA but do not unwind it. For simplicity, we refer to all these proteins as helicases, despite recognizing that not all of them possess helicase activity.

A sizeable subset of these proteins, representing three families, function to manipulate structured RNAs. In parallel with the diversity inherent to structured RNAs and their transitions, SF2 helicases use diverse mechanisms to promote RNA rearrangements. Strikingly, emerging evidence suggests that the mechanisms track to a large extent with the family delineations. In this review, we summarize the mechanistic capabilities of these three protein families, as deduced principally from structures and biochemical studies using simple, defined RNA substrates. We then survey biological processes that require these proteins, with emphasis on the specific RNA and RNP substrates, the conformational transitions, and if known, the mechanisms used to promote these transitions.

HELICASE FAMILIES INVOLVED IN RNA CHAPERONING AND RNP REMODELING

On the basis of comparisons of their sequences and limited structural information, SF2 helicases have been divided into nine families and one smaller "group" (13), refining and extending earlier classification schemes (12, 14). All SF2 proteins share a conserved core that binds a nucleoside triphosphate and a nucleic acid and consists of two domains (D1 and D2), each of which resembles RecA (**Figure 1*a***). The core includes at least 12 identified sequence motifs, which are more highly conserved within than between families. These motifs contact both ligands and play important roles in the processes mediated by these proteins, as has been reviewed elsewhere (13, 15, 16). In addition to the helicase core, many SF2 proteins include ancillary domains. For some families these additional domains are conserved, whereas for others they are highly variable (13).

Most proteins known to function as RNA chaperones or RNP remodelers are found within two families, the DEAD-box and the DEAH/RHA families, and a few are found within the Ski2-like family. We therefore focus below on these three families, and representative schematics and structures are shown in **Figure 1**. Although the DEAD-box and DEAH/RHA families have at times been described collectively as DExD/H-box proteins, a recent analysis showed that these two families are no more closely related to each other than to other SF2 families. Accordingly, they are indicated here as two distinct families (13). This is an important change in viewpoint because the molecular properties and mechanisms of these two families appear to differ substantially from each other.

RNA chaperone: a protein that interacts with RNA to promote RNA folding and/or conformational rearrangements without remaining bound to the folded or rearranged RNA

ATPase ACTIVITY AND RNA UNWINDING

As a step toward defining their mechanisms in RNA chaperoning and remodeling, the capabilities of individual SF2 helicases have been probed using simple, defined RNA substrates. Although these studies have revealed differences between families, some central properties are also shared by proteins in these families, in other SF2 families, and even in the more distantly related SF1 helicases. A hallmark of these proteins is DNA- and/or RNA-dependent nucleotide hydrolysis activity, which is derived from energetic coupling between these ligands. As illustrated most dramatically by structures of DEAD-box proteins, the two core domains have substantial flexibility relative to each other (17–19). Upon binding of the nucleotide, principally to D1, the domains have increased affinity for each other and are found in more intimate contact, at least in the presence of bound nucleic acid. Nucleotide hydrolysis and product release reset the protein to the open complex. The consequences of the cycle vary, as outlined below.

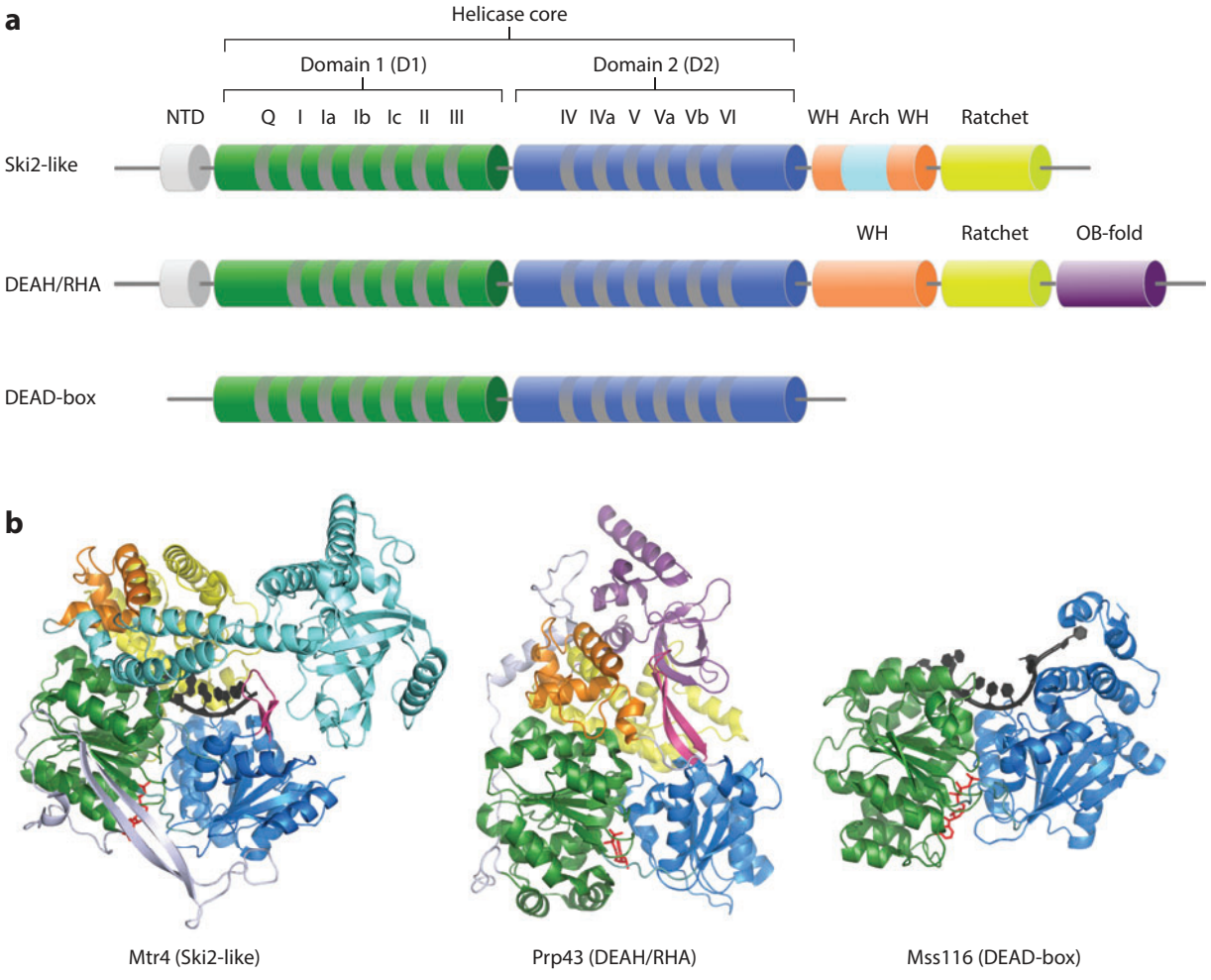

Figure 1

Superfamily 2 (SF2) families involved in RNA chaperoning and RNP remodeling. (*a*) Arrangement of conserved structural domains. Conserved motifs of the helicase core are shown in dark gray. The domain arrangements of DEAH/RHA and Ski2-like families shown are based on Mtr4 and Prp43 structures, respectively (domains not to scale). The winged helix (WH) and ratchet domains are conserved in DEAH/RHA and Ski2-like helicases, and the oligonucleotide/oligosaccharide-binding fold (OB-fold) domain is conserved in DEAH/RHA helicases, whereas the arch domain is present in only a subset of Ski2-like helicases. The N-terminal domains (NTD), shown for Mtr4 and Prp43, are variable. Some individual proteins from all three families include other, nonconserved domains that are not shown here. (*b*) Crystal structures of Ski2-like (Mtr4) (28), DEAH/RHA (Prp43) (30), and DEAD-box (Mss116) (59) helicases. Domains are colored as in panel *a*. The nucleotide (ADP for Mtr4 and Prp43 and AMP-PNP for Mss116) is shown in red, and cocrystallized single-stranded RNA (ssRNA) bound to Mtr4 and Mss116 is shown in black. The β hairpin within D2 in Ski2-like and DEAH/RHA helicases that is thought to function as a pin during RNA unwinding is highlighted in pink. Abbreviations: RHA, RNA helicase A; RNP, ribonucleoprotein complex.

Directional RNA Translocation and Unwinding by DEAH/RHA and Ski2-Like Proteins

Conventional helicase mechanisms include translocation, namely directional movement, of the protein along one of the nucleic acid strands. This movement is achieved for SF1 and SF2 helicases at the level of a protein monomer and is directly linked to unwinding because movement along one strand results

in displacement of the complementary strand. The mechanistic steps involved in translocation have been described in detail for the SF1A DNA helicases PcrA and Rep (14). Crystallographic and biochemical approaches have provided high-resolution views of intermediates and revealed dynamics in the translocation process. When bound to a DNA that includes a 3′ extension, these proteins form extensive contacts to single-stranded (ss) and double-stranded (ds) regions of the DNA, essentially encircling the ssDNA region. This encapsulation is made possible by domain insertions in D1 and D2 that provide additional DNA-binding surfaces. Translocation occurs as ATP binding induces closure of the two core domains (D1 and D2), resulting in movement of D1 while D2 remains stationary on the DNA. ATP hydrolysis and product release allow a transient loosening of D2 from contacts with both ssDNA and D1, resulting in domain opening and movement of D2 by 1 nt in the direction of translocation, 3′–5′ for these proteins. Reformation of contacts by D2 then resets the core for ATP binding and another turn of the cycle.

A similar reaction cycle appears to operate in some SF2 helicases. The viral helicases NS3 and NPH-II (from hepatitis C and vaccinia virus, respectively), which form their own group within SF2, unwind RNA with moderate processivity by translocating from 3′ to 5′ (20–22). Although structural and biochemical data suggest some differences in the detailed mechanism of these proteins relative to the well-studied SF1 proteins, such as differences in the identities of amino acids that contact the nucleobases of unwound RNA and possible differences in chemical or kinetic step sizes, the basic features of translocation and unwinding are maintained. Thus, SF2 RNA helicases are capable of functioning much like SF1 DNA helicases. However, these viral helicases evolved to function in the context of viral genomes, where processivity and directionality are probably required for function. In contrast, helicase proteins that manipulate cellular RNAs are much less likely to encounter long, continuous helices, and one could envision that their mechanisms would be correspondingly different.

Compared with the SF1 and SF2 families discussed above, there is less structural and mechanistic information for DEAH/RHA and Ski2-like proteins. However, such information is now accumulating at a rapid rate. The first crystal structure of a Ski2-like protein was Hel308, an archaeal DNA helicase (23, 24), and structures have subsequently been obtained for the *Saccharomyces cerevisiae* RNA helicases Brr2, Mtr4, and Ski2 (**Figure 1*b***) (25–29). The first crystal structure of a DEAH/RHA helicase was the *S. cerevisiae* protein Prp43 (30, 31), which functions to remodel the spliceosome and the preribosome. Although not recognized by sequence analysis, the structures revealed that these protein families share two domains outside the core, referred to as a winged-helix domain and a ratchet domain. These domains make key contacts with substrate RNA and are proposed to play key roles in RNA unwinding (32).

DEAH/RHA and Ski2-like proteins are thought to unwind RNA helices by a mechanism that is fundamentally similar to SF1 and viral SF2 helicases. Unwinding by Mtr4 of a 20-bp duplex requires a 3′-ssRNA extension, indicating that Mtr4 binds to the extension and unwinds RNA by translocating from 3′ to 5′ (33), and Mtr4 can unwind a helix as long as 36 bp when in complex with partner proteins of the TRAMP complex (34). 3′ overhangs also enhance unwinding by Brr2 (25). Similarly, directional unwinding is indicated for Prp22, a DEAH/RHA protein that functions in pre-mRNA splicing (35). The unwinding efficiencies appear to decline with increasing helix length, suggesting that the level of processivity is much lower than for SF1 helicases and lower than for the viral SF2 proteins noted above. However, the lower processivity probably does not reflect a difference in mechanism, but rather simply a change in the rate constant for further translocation relative to that for dissociation from the helix, which defines processivity (36). The mechanistic features of DEAH/RHA and Ski2-like helicases suggest a

general requirement for a 3′ single-stranded extension to allow for helicase loading, which most likely generates important constraints on their physiological substrates and specific functions.

Nonprocessive RNA Unwinding by DEAD-Box Proteins

DEAD-box proteins make up the largest family of SF2 helicases, and they function throughout biology to promote RNA rearrangements and RNP remodeling. Although DEAD-box proteins display RNA unwinding activity, they use a nonconventional mechanism. Early work using mammalian eIF4A protein showed that DEAD-box proteins can unwind RNA helices up to 15 bp but with efficiencies that decrease with increases in helix length or stability (37), suggesting very low processivity. In addition, early studies showed that DEAD-box proteins do not require either 5′ or 3′ extensions, implying that they can interact directly with a duplex region and at least initiate unwinding without translocating (38–41). This point was underscored by the finding that, although DEAD-box proteins do not perform ATP-dependent unwinding of DNA helices, the *S. cerevisiae* protein Ded1 can unwind a DNA helix that is substituted in one strand with a small number of internal RNA nucleotides, implying that the unwinding is initiated and centered on the RNA segment (42). The lack of translocation was highlighted by findings showing that complete unwinding of RNA helices can occur in a single cycle of ATP binding and hydrolysis (43–45). Furthermore, complete unwinding is possible with the ATP analog ADP-BeF_3 (46), indicating that unwinding can precede ATP hydrolysis and the release of the hydrolysis products. These steps then promote release of the tightly bound RNA strand after unwinding, and they may also contribute to unwinding of longer or more stable helices (47, 48).

Fluorescence studies and crystallography of the *S. cerevisiae* protein Mss116 have led to a detailed physical model for nonprocessive RNA unwinding by DEAD-box proteins (49). This work showed that D2 alone can bind dsRNA without unwinding it, and it extended previous results indicating that D1 can bind ATP independently (50–53). Thus, in this model the two ligands bind initially to the separated domains, consistent with earlier structures indicating that the two RecA-like domains are flexible relative to each other in the absence of ligands (17–19, 54–56). Simultaneous binding of both ligands leads to closure of the domains (18, 19, 49, 57–60), providing a physical model for energetic coupling between binding of adenosine nucleotide and RNA (45, 61–66). The domain closure results in the exclusion of one strand of dsRNA, bending of the other strand, and consequent local unwinding of the helix. Domain closure also promotes ATP hydrolysis, which allows release of the tightly bound RNA strand after helix unwinding and resetting of the DEAD-box protein. Unlike the families described above, DEAD-box proteins lack conserved domains that surround the ssRNA as well as a "pin" element that may function as a wedge in unwinding (**Figure 1*b***) (13). Either or both of these differences likely contribute to their lack of processivity.

In the context of this model, there may be intermediates in which the domains are in contact but unwinding is not achieved, and these intermediates may accumulate under conditions that do not promote unwinding. For example, binding of a 154-mer RNA and AMP-PNP to the *Bacillus subtilis* protein YxiN leads to favorable domain closure, as monitored by smFRET, but AMP-PNP does not produce detectable unwinding of a model RNA duplex (66). Analogously, binding of an ATPase-deficient mutant of YxiN to a closely related RNA and AMP-PNP or ATP gives domain closure but not RNA unwinding (67). Alternatively, this domain closure may reflect binding of YxiN to a single-stranded segment within the large RNA, whereas domain closure may not be achieved upon binding of dsRNA.

In addition to accelerating local unwinding of RNA, some DEAD-box proteins can

accelerate the reverse reaction, that is, the annealing of two strands to form a dsRNA helix (41, 68–70). Strand annealing activity does not depend on ATP (41, 69) and may reflect binding of the separated strands to the dsRNA binding site of D2 (49). A recent analysis suggested that DEAD-box proteins can be divided into two broad groups based on their levels of ATPase activity and corresponding RNA unwinding activity (71). Interestingly, Rok1, one of the proteins in the "low" ATPase/unwinding group, is suggested to function by promoting an RNA annealing reaction in ribosome biogenesis (see below).

Unstructured, Basic "Tails" of DEAD-Box Proteins as Tethers

Despite their ability to interact directly with a duplex to initiate unwinding, several DEAD-box proteins are activated for unwinding by ssRNA extensions, which can be either 5′ or 3′ extensions (40, 41, 71–74). Given the puzzling ability of DEAD-box proteins to use extensions of either polarity, researchers described them as bidirectional. However, it is now clear that the extensions do not increase unwinding efficiency by providing starting points for translocation, but instead provide binding sites for additional monomers or ancillary regions of the same monomer, which can tether the helicase core in proximity to a substrate helix and remain bound during the local RNA unwinding reaction (16, 75).

Several lines of evidence support this conclusion. The extension does not have to be contiguous with the duplex to be unwound: For Ded1, the extension can even be connected by a bridging streptavidin protein as long as it allows proximal binding of an additional protein molecule (40). For other DEAD-box proteins, the extension does not have to be single stranded, as a dsRNA or DNA extension is at least as effective as ssRNA for the *Neurospora crassa* protein CYT-19 (76). Deletion of an unstructured C-terminal region of CYT-19 leaves the unwinding activity of the helicase core intact but abrogates the stimulation by DNA or RNA extensions, indicating that this "C-tail" is responsible for binding the extension and tethering the core for unwinding of an adjacent helix (77, 78).

There is significant variation in the properties of these tethering interactions. Some DEAD-box proteins function as general RNA chaperones, and interactions of the C-tail are correspondingly versatile (75). There is no evidence for RNA sequence or structure specificity, and the C-tail is very flexible relative to the core, accounting for the enhanced RNA unwinding from extensions regardless of their polarity (78). By contrast, some yeast DEAD-box proteins have C-terminal basic regions that may function similarly but with less flexibility between the tail and the core, such that a preference for polarity is observed (71). The *Thermus thermophilus* protein Hera includes a bipartite extension with an RNA recognition motif (RRM) domain that binds ssRNA with a preference for guanosine and a short unstructured region that binds dsRNA (79, 80). Other DEAD-box proteins are targeted to specific substrates by interactions with ancillary domains (81, 82) or surfaces within the helicase core (83, 84).

Remodeling of RNP Complexes

Although much is known about the abilities of SF2 RNA helicases to unwind duplexes, the physiological substrates for many of these proteins include assembled proteins. Thus, a few studies have probed the abilities of certain SF2 helicases to remodel simple, defined RNP complexes. The viral helicase NPH-II can remove several protein complexes from ssRNA or dsRNA in an ATP-dependent manner (85, 86), most likely by translocating from 3′ to 5′ along one RNA strand and displacing the protein(s) as well as the complementary RNA strand, if present (87). Ded1 is also able to remove proteins from RNA (86, 88), despite unwinding RNA nonprocessively as described above. Interestingly, Ded1 displays selectivity, accelerating dissociation of some protein complexes but relying on spontaneous dissociation of the tight-binding U1A protein before unwinding

the two RNA strands that form the U1A binding site (88). The physical origins of this selectivity remain to be elucidated (87).

RIBOSOME BIOGENESIS

Here and in the sections that follow, we outline the biological functions of SF2 helicase proteins in rearranging RNAs and remodeling RNPs. Ribosome biogenesis is an elaborate process that involves regulated cleavage, modification, and folding of several large ribosomal RNAs, as well as extensive assembly with proteins and changes in RNP constitution throughout the process (89, 90). Large fractions of the DEAD-box proteins of *Escherichia coli* and *S. cerevisiae* function in ribosome biogenesis (4 of 5 and 15 of 26, respectively), and in *S. cerevisiae* several DEAH/RHA helicases and a Ski2-like helicase also participate. In bacteria, the RNA helicases seem to be required only at low temperatures, presumably owing to increased lifetimes of non-native RNA structures (91, 92). However, in eukaryotes, most of the helicases involved in ribosome biogenesis are essential, likely reflecting the increased complexity of the eukaryotic ribosome and a greater need for regulation.

Ribosome Biogenesis in *Escherichia coli*

Ribosome biogenesis in *E. coli* involves four DEAD-box helicases: DbpA, SrmB, CsdA, and RhlE. DbpA (YxiN in *B. subtilis*) was originally implicated on the basis of the specific stimulation of its ATPase activity by 23S rRNA, which later was narrowed down to hairpin 92 (hp92) of the peptidyltransferase center (93, 94). The recognition of hp92 is mediated by a C-terminal RRM domain, which is flexibly linked to the helicase core and enhances unwinding of helices positioned either 3′ or 5′ from hp92 (81, 82, 95, 96). Functional and structural evidence supports a model in which DbpA rearranges RNA structure around hp92 in the 50S precursor while remaining tethered via the RRM domain. Despite this specific hp92 recognition, ΔdbpA mutants show no obvious defects, and defective ribosome assembly is observed only with a dominant negative DbpA mutant that lacks ATP-dependent unwinding activity but retains RNA binding (97, 98). Thus, in vivo, DbpA-mediated rearrangements appear to be redundant with other pathways but can be blocked by bound DbpA (98).

Recent evidence also suggests specific sites for SrmB. The absence of functional SrmB leads to accumulation of 40S intermediates that contain immature 23S rRNA and reduced amounts of some ribosomal proteins (99). Tandem affinity purification revealed SrmB interactions with two ribosomal proteins, L4 and L24, which bind a 5′ region of 23S rRNA (100). However, rRNA mutations that suppress the requirement of SrmB map to domain II of 23S rRNA, which is distant in the mature ribosome. Some suppressors map to a section of 5S rRNA with a base complementary to domain II of 23S rRNA, suggesting that SrmB may prevent 5S rRNA annealing and promote native 23S rRNA folding and protein assembly within domain II (101).

CsdA, also known as DeaD, is induced at low growth temperatures, and several lines of evidence indicate that it functions as a general RNA chaperone in ribosome biogenesis and other RNA-related processes, including mRNA decay and translation (102, 103). CsdA functions at multiple stages of ribosome biogenesis and can suppress defects in ΔsrmB strains, suggesting functional overlap (104). Interestingly, mutations in CsdA can be suppressed by overexpression of another cold-shock protein and general chaperone, CspA—a small (70 amino acids), unrelated protein that can destabilize RNA helices and functions in transcription antitermination (105–107). CsdA contains a predicted RRM in its C terminus that may contribute to general RNA recognition, possibly by recognizing a commonly occurring structural element (39).

RhlE appears to be largely but not completely redundant with CsdA. Although growth defects are not observed in ΔrhlE cells, removal of RhlE and CsdA enhances the cold-sensitive phenotype of ΔcsdA cells and increases the accumulation of 50S precursors (108). However,

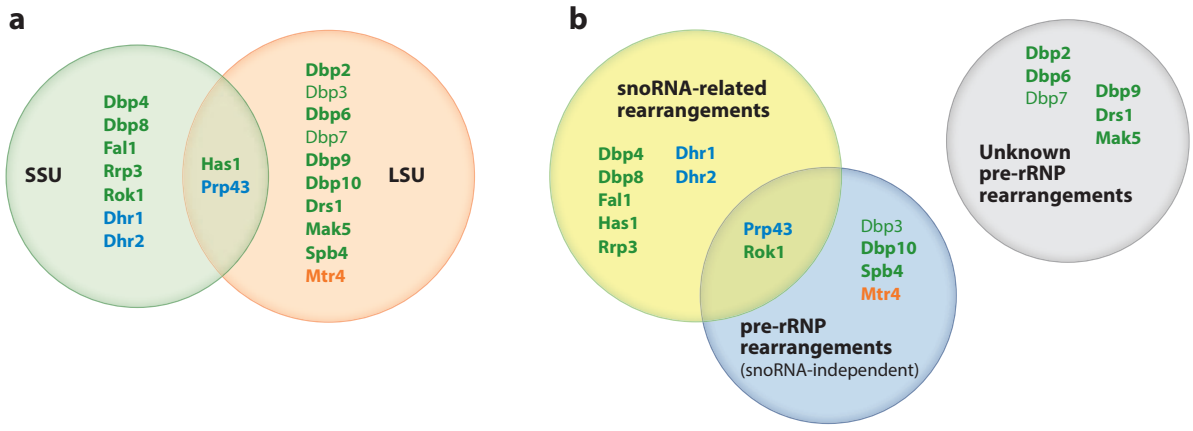

Figure 2

Helicases that function in eukaryotic ribosome biogenesis. (*a*) *Saccharomyces cerevisiae* helicases that function in biogenesis of the small ribosomal subunit (SSU) and large ribosomal subunit (LSU). Essential helicases are in bold. DEAD-box, DEAH/RHA, and Ski2-like helicases are shown in green, blue, and orange, respectively. (*b*) Known and proposed roles of RNA helicases in ribosome biogenesis. Small nucleolar RNA (snoRNA)-related rearrangements include promoting binding and dissociation of snoRNAs as well as rearrangements associated with snoRNA-guided cleavage events. Helicases indicated as promoting snoRNA-independent rearrangements facilitate pre-rRNA cleavage at specific snoRNA-independent processing sites.

unlike CsdA, RhlE cannot substitute for SrmB, and its overexpression even increases the accumulation of 50S precursors observed in ΔsrmB cells (108). Together, the results suggest a complex network of functions for SrmB, CsdA, and RhlE, with extensive but incomplete overlap among them.

Ribosome Biogenesis in *Saccharomyces cerevisiae*

Nineteen yeast helicases have been implicated in ribosome biogenesis. Seventeen of them are essential, and several have been linked to distinct steps in small- or large-subunit biogenesis on the basis of the sizes and compositions of ribosome intermediates that accumulate upon mutation or depletion of the helicase protein (**Figure 2**) (109). Although it can be difficult to separate direct from indirect effects, as some of the blocked steps may be downstream from those mediated directly by the helicase (89), these studies have provided important insights and constraints on the helicase functions in ribosome biogenesis. A distribution of the proposed roles of *S. cerevisiae* helicases in ribosome biogenesis is shown in **Figure 2*b***. Below we discuss examples of proteins implicated in each of these roles, illustrating the mechanistic diversity of SF2 helicases, particularly within the DEAD-box family.

Several helicases have been suggested to regulate nucleolytic processing of pre-rRNA into mature rRNA by remodeling structures adjacent to cleavage sites. The DEAD-box protein Dbp3 promotes site-A3 cleavage by RNase MRP during 25S rRNA maturation and may disrupt an adjacent hairpin to increase access of the RNase (110). The DEAH/RHA protein Prp43 (see below) is recruited near the site of Nob1-mediated 3′ cleavage of 18S pre-rRNA by the cofactor Pfa1, which binds the OB-fold (oligonucleotide/oligosaccharide-binding fold) domain of Prp43 and stimulates its helicase activity (31, 111–113). 3′ end processing of 5.8S rRNA requires the Ski2-like helicase Mtr4 (114), which functions as part of the TRAMP complex (see below) and is also involved in general quality control during ribosome biogenesis (89).

The possibility of an interesting divergence of DEAD-box protein functions has been

introduced by recent studies of Rok1 in small subunit biogenesis. Rok1 promotes a structural change to facilitate cleavage at site A2 by the Nob1 nuclease. Interestingly, in vitro experiments suggest that, rather than disrupting structure, Rok1 specifically promotes the temporary formation of a helix that allows correct processing of pre-18S rRNA (115, 116). The specificity for this "pre-A2" helix is conferred by cofactor Rrp5, which stimulates Rok1-mediated annealing of pre-A2 by ~10-fold relative to other helices. Studies using model helices, noted above, showed that Rok1 has low ATPase and RNA unwinding activities relative to other DEAD-box proteins (71), suggesting an intriguing link between the capabilities and functions of Rok1. Nevertheless, Rok1 does retain a basal level of nucleotide-dependent RNA unwinding activity (71), and it may also function in snoRNP dissociation (see below).

Several helicases function in rearrangements involving small nucleolar RNAs (snoRNAs). snoRNAs are small structured RNAs that base-pair with prerRNAs to guide site-specific cleavage, methylation, and pseudouridylation, and they can direct pre-rRNA folding (117). There are 75 snoRNAs in yeast from structurally distinct box-C/D and H/ACA classes: Three are essential (U3, U14, snR30) and guide cleavage events, and 72 are nonessential and specify nucleotide modifications (117). Three DEAD-box proteins are implicated in dissociation of certain snoRNAs. Has1 is required primarily for dissociation of the essential U3 and U14 snoRNAs, although several other snoRNAs are also affected upon Has1 depletion (118). By contrast, snR30 dissociation requires Rok1 (119). Interestingly, dissociation of U14 requires Dbp4 in addition to Has1 (120), indicating that removal of a given snoRNA can require direct or indirect activities of more than one helicase. Several helicases may function in U3 snoRNP-guided cleavage and rearrangements of pre-rRNA. The DEAH/RHA helicases Dhr1 and Dhr2 and DEAD-box proteins Dbp8, Rrp3, and Rok1 coprecipitate U3 snoRNA and are required for U3-guided cleavages (121). One or more of these helicases may also function in U3-assisted formation of the central pseudoknot in the 18S rRNA (122).

Of the helicases in eukaryotic ribosome biogenesis, the DEAH/RHA protein Prp43p is particularly versatile, playing multiple roles while also functioning in pre-mRNA splicing (see below). In addition to promoting Nob1-mediated cleavage of 18S rRNA, as noted above, Prp43 coprecipitates with snoRNAs (primarily box-C/D) and rRNA sequences near snoRNA binding sites. It is also required for binding or release of snoRNAs from the preribosome (112, 123–125). The broad spectrum of RNA substrates indicates that Prp43 recognizes general features of structured RNAs, either intrinsically or through cofactors such as Pfa1 (113). Surprisingly, in vitro studies showed that Prp43 does not require a 3′ extension for RNA unwinding, which would seem to be at odds with the 3′–5′ directionality observed for other DEAH/RHA helicases (126, 127). Together, these results have led to the suggestion that Prp43 could function similarly to DEAD-box proteins by forming tethering interactions with structured RNA via a cofactor protein or an ancillary domain and by disrupting RNA structure locally (128), which would be an important exception from the general differences in functional mechanisms between DEAH/RHA and DEAD-box proteins.

SPLICEOSOME ASSEMBLY AND FUNCTION

After ribosome biogenesis, eukaryotic pre-mRNA splicing is the process that requires the most RNA helicases (reviewed in References 129 and 130). Eight helicases function in splicing in *S. cerevisiae*, including three DEAD-box, one Ski2-like, and four DEAH/RHA proteins. These helicases have been implicated in RNA secondary-structure rearrangements and protein displacement, allowing the spliceosome to assemble, rearrange, and dissociate in a controlled manner (**Figure 3**). Splicing helicases also function in proofreading, antagonizing the forward progression of slowly reacting pre-mRNAs and intermediates and promoting

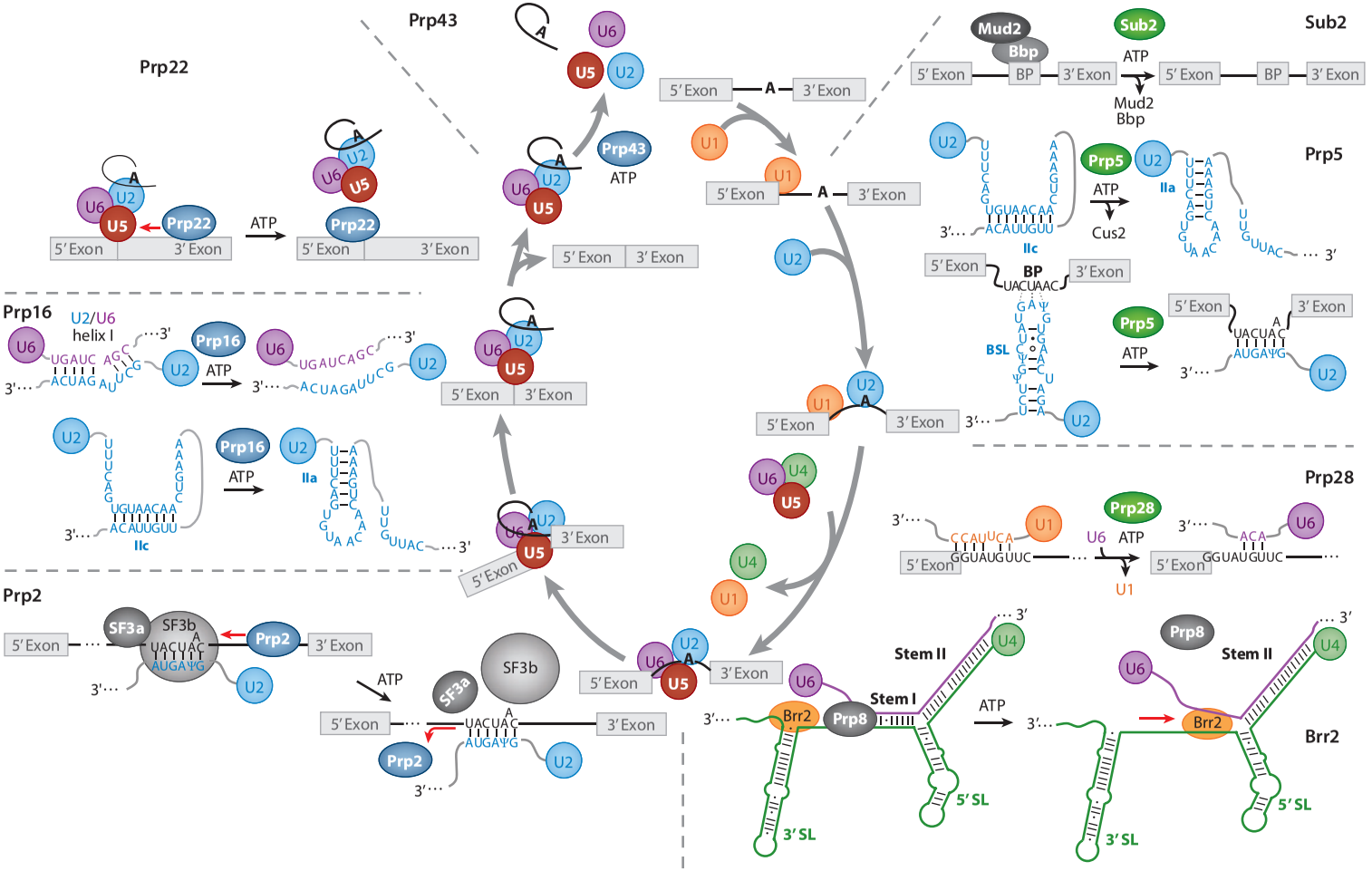

Figure 3

Helicase-mediated rearrangements in splicing. DEAD-box proteins are shown in green, DEAH/RHA proteins in blue, and Ski2-like proteins in orange. Abbreviations: BP, branchpoint (shown as an A in the cycle); Bbp, BP-binding protein (also known as Msl5); BSL, branchpoint stemloop; SL, stemloop. The direction of translocation by Prp2, Prp22, and Brr2 is indicated by red arrows.

their dissociation (131). Below, we summarize the current knowledge of helicase-mediated rearrangements of the *S. cerevisiae* spliceosome and discuss how the properties of each helicase family correspond to their roles in pre-mRNA splicing.

DEAD-Box Helicases: Chaperoning Spliceosome Assembly

Two DEAD-box helicases, Sub2 and Prp5, are required for branchpoint (BP) recognition by U2 snRNP and promote rearrangements that allow base-pairing of the BP with U2 snRNA (132, 133). Genetic studies have implicated Sub2 in dissociation of the Mud2/Bbp heterodimer from the BP sequence, and consistent with a role in protein displacement, studies of the human homolog UAP56 suggest that ATPase activity, but not RNA unwinding, is required for BP exposure (133, 134). Prp5 promotes a transition between alternative conformations of the U2 snRNP, which involves direct or indirect disruption of the 8-bp helix IIc, formation of alternative helix IIa, and ejection of the Cus2 protein (**Figure 3**) (135–137). This transition increases the accessibility of the BP-recognition sequence of U2 snRNA, located in the loop of a conserved branchpoint interaction stemloop (BSL) (135, 138, 139). Upon initial recognition of the BP sequence by the BSL, Prp5 may also unwind the BSL stem to allow stable association of U2 snRNP with the BP (**Figure 3**) (139).

Following stable U2 snRNP binding, the Prp28 helicase is required for unwinding of the 6-bp helix between U1 snRNP and the 5′ splice site (5′-ss), facilitating release of U1 snRNP and exposing the 5′-ss for base pairing with the U6 snRNP (**Figure 3**) (140). Although Prp28 and Prp5 have been described as lacking unwinding activity (135, 141), the reported experiments have used helices longer than the 10–15 bp that are efficiently unwound by other DEAD-box proteins. Overall, the localized rearrangements by all three DEAD-box helicases are consistent with the general capabilities of DEAD-box proteins and in contrast with the generally more extensive remodeling events mediated by the helicases described below.

DEAH/RHA Helicases: Activation for Catalysis, Product Release, and snRNP Recycling

The four spliceosomal DEAH/RHA-family helicases catalyze rearrangements that accompany or follow the chemical steps of splicing, beginning with activation of the spliceosome for the first step of splicing. Strikingly, these rearrangements generally involve more extensive base-pairing interactions and/or displacement of proteins than the changes promoted by DEAD-box proteins. Current data also suggest a strong correspondence of the functional roles of these proteins with the capabilities of the protein family as a whole, as established from crystallographic and biochemical studies described above.

Prp2 promotes dissociation of proteins from the BP region, exposing the BP adenosine for attack on the 5′-ss (142, 143). Prp2 requires 23–33 nt of pre-mRNA in the 3′ direction from the BP, suggesting 3′–5′ translocation (144). Along the way, Prp2 is thought to dislodge the BP-binding SF3a and SF3b proteins and a number of others, contributing to catalytic activation of the spliceosome (**Figure 3**) (142, 143, 145). Following lariat formation, Prp16 promotes rearrangement of the spliceosome for the second catalytic step. The rearrangement involves refolding of U2 snRNP to the IIa state, destabilization of U2/U6 helix 1 (**Figure 3**), and displacement of several proteins (137, 146–149). Following splicing, Prp22 releases the mRNA by disrupting base pairs between the U5 snRNP and the mRNA and may destabilize protein–RNA contacts between the U5 snRNP and the exon–exon junction (**Figure 3**) (150, 151). Prp22 initiates translocation ~23 nt downstream from the splice junction, suggesting 3′–5′ translocation (35, 152). Finally, Prp43 mediates the extensive rearrangements that allow release of the intron lariat and recycling of the U2, U5, and U6 snRNPs (153–155). The

activity of Prp43 in spliceosome disassembly is stimulated by cofactor Ntr1, which also enhances the in vitro unwinding activity of the helicase (127, 155).

Ski2-Like Helicase Brr2: Remodeling U4/U6 snRNP

Similar to the DEAH/RHA proteins, the Ski2-like helicase Brr2 promotes a large-scale rearrangement, acting early in the splicing cycle to disrupt an extensive base-pairing network between the U4 and U6 snRNAs (**Figure 3**) (156, 157). The disruption releases the 5′-ss-recognition sequence within U6 snRNA and allows it to form catalytically important intra-U6 and U2/U6 interactions. The mechanism of U4/U6 snRNP dissociation is not fully understood, but recent data suggest that Brr2 binds the 3′ end of the U4 snRNA and translocates from 3′ to 5′ to unwind U4/U6 stem I and, presumably, stem II (158–160). This activity is tightly regulated by U5 snRNP components Prp8 and Snu114, and recent studies have provided insights into the structural basis of Prp8-dependent inhibition of premature unwinding activity by Brr2 (160, 161). Although data indicate that Brr2 readily unwinds protein-free U4/U6 snRNA in vitro, how proteins that coat the RNA in the U4/U6 snRNP affect unwinding remains unknown (156, 158).

Proofreading by Spliceosomal Helicases

In analogy with GTPases in translation, several splicing helicases use ATP to enhance fidelity by proofreading the splicing process (131, 162). Productive steps are in kinetic competition with a nonproductive branch in which a helicase uses ATP to reject the pre-mRNA substrate (163, 164). For a substrate with a nonconsensus sequence, the reduced rate of the forward step increases flux through the nonproductive branch, leading to rejection and eventual discarding of the unspliced substrate or a splicing intermediate. In addition, the helicases may act preferentially on stalled splicing complexes because of their decreased stability and enhanced dynamics, analogous to DEAD-box proteins that act as chaperones by resolving misfolded RNA structures (131, 165) (see the section titled Self-Splicing Introns, below).

As expected from this mechanism, the proofreading checkpoints largely overlap with the step(s) mediated by a given helicase in the productive splicing cycle. Thus, Prp5 rejects pre-mRNAs with mutations in the branch site (166), Prp28 and Prp16 suppress splicing of 5′-ss mutants (167–170), and Prp22 primarily proofreads the 3′-ss (171). Some of the helicases may also act with some promiscuity on structures that become exposed owing to their lower stability or to a lack of bound proteins. Thus, Prp43 can reject or discard stalled intermediates in at least two steps of the spliceosome cycle, in addition to disassembling the spliceosome to complete the native cycle (172, 173). By contrast, Prp22 can reject BP and 5′-ss mutants, although less efficiently than 3′-ss mutants (171).

SELF-SPLICING INTRONS: DEAD-BOX PROTEINS AS GENERAL RNA CHAPERONES

Group I and group II introns are autocatalytic RNAs that are broadly distributed in nature and function by splicing themselves out of precursor RNAs, with some retaining ancestral roles as mobile genetic elements (174). Several mitochondrial group I introns in *Neurospora crassa* require the DEAD-box protein CYT-19 for efficient folding (175), and all of the *S. cerevisiae* mitochondrial group I and group II introns depend on Mss116, a CYT-19 homolog (176, 177). Group II introns in plant mitochondria and chloroplasts also require DEAD-box proteins, suggesting a widespread role (178, 179). The versatility of these DEAD-box proteins indicates that they are general RNA chaperones (175, 177), and biochemical studies of these proteins and introns have been instrumental in elucidating general features of the mechanisms by which DEAD-box proteins manipulate structured RNAs.

Model group I introns have been used to probe how DEAD-box proteins promote transitions away from misfolded structures. A ribozyme derivative of the *Tetrahymena thermophila* group I intron (from the large-subunit rRNA) misfolds to a single, dominant conformation that is compact and includes extensive native structure but is thought to have a nonnative topology (180, 181). This misfolded conformation undergoes a slow "refolding" rearrangement to the native state that is rate limited by extensive unfolding (181, 182). CYT-19 accelerates refolding in an ATP-dependent manner, indicating that it can use ATP to accelerate unfolding of the misfolded ribozyme (76). The function of CYT-19 as a general chaperone, along with the noncognate nature of the *Tetrahymena* ribozyme, suggests that CYT-19 accelerates this refolding reaction without recognizing specific features of the misfolded ribozyme, and indeed CYT-19 can also unfold the native ribozyme (165). CYT-19 also accelerates refolding of a misfolded group I intron from the bacterium *Azoarcus* (183), and it promotes splicing of its cognate introns in vitro, most likely by accelerating refolding transitions (175). These refolding processes are known or suggested to require unfolding of tertiary structure, but DEAD-box proteins have not been shown to disrupt RNA tertiary contacts. Recent work indicates that CYT-19 disrupts tertiary structure in the *Tetrahymena* ribozyme by using a "helix capture" mechanism by trapping helical elements after they lose tertiary contacts spontaneously and then using RNA unwinding activity to separate the strands of the trapped helix (C. Pan, J.P. Potratz, B. Cannon, Z.B. Simpson, J.L. Ziehr, P. Tijerina & R. Russell, manuscript submitted).

Group I introns have also been valuable for probing how directionality in folding can be conferred upon the inherently nonspecific activity of these DEAD-box proteins. For the *Tetrahymena* ribozyme, there is a correlation between global RNA stability and the efficiency of unfolding by CYT-19, given that less stable conformations are unfolded more efficiently. The effect is that net folding proceeds from the less stable misfolded conformation to the more stable native state despite the absence of specific recognition by CYT-19 (165). If the native structure is destabilized by mutations or by lowering Mg^{2+} concentration, it is unfolded efficiently by CYT-19. Thus, instead of the native ribozyme accumulating, the misfolded conformation or unfolded conformations accumulate (165). Mss116, which has greater RNA unwinding activity than does CYT-19, can readily unfold the native structure under a broader range of conditions (184, 185). Mss116 can also function as a chaperone to promote conformational transitions between or following the two steps of splicing for a model intron from yeast (186), most likely by disrupting structure nonspecifically, with directionality conferred by the functionally irreversible dissociation of the intron from the ligated exons.

Group II introns also undergo slow folding transitions to the native state, and these transitions can be accelerated in vitro by DEAD-box proteins (187). Two yeast mitochondrial introns have been used most extensively in these studies. CYT-19 promotes native folding of the bI1 intron in an ATP-dependent manner and is dispensable after the native state is reached, establishing that it chaperones folding of the RNA (188). A second intron (aI5γ) and its ribozyme derivative (D135) fold slowly under near-physiological conditions (184, 189), with D135 folding through multiple pathways on timescales from minutes to hours (190, 191). Mss116 accelerates native folding of both the intron and the D135 ribozyme (184, 189, 191, 192), and further work has shown that Mss116 accelerates two transitions, only one of which requires ATP (191, 193). The ATP-dependent step is thought to reflect disruption of nonnative structure, which includes or is stabilized by exonic structure, analogous to the roles of DEAD-box proteins in folding of other group I and II introns (193, 194). This step may also include nucleotide-dependent dissociation of Mss116 from a folding intermediate (193). The ATP-independent step occurs earlier in folding and may include compaction of the largest domain of the intron, a transition that can limit

folding for a subpopulation of the ribozyme (190, 195).

GENERAL RNA CHAPERONES IN TRANSLATION

Eukaryotic translation requires multiple helicases, largely for rearrangements of the 5′ untranslated region (5′-UTR) in cap-dependent initiation. In this process, the preinitiation complex (PIC) is recruited to the 5′ cap through interactions with the eIF4F complex, followed by 5′ to 3′ scanning along the 5′-UTR in search of the start codon (196). Both PIC loading and scanning, with the latter process involving up to thousands of nucleotides, require disruption of RNA structures within the 5′-UTR. Three helicases, eIF4A, Ded1, and DHX29, probably clear the way for the PIC by resolving these structures.

The DEAD-box protein eIF4A is a component of the eIF4F complex, which also includes eIF4G and the cap-binding protein eIF4E. Owing to its localization to the cap, eIF4A may promote PIC loading by disrupting RNA secondary structures in the vicinity of the 5′ terminus (196, 197). A poor helicase on its own, eIF4A is enhanced for RNA unwinding by interactions with other initiation factors (198). eIF4G binds and modulates the orientation of the eIF4A core domains, and yeast eIF4G can bias eIF4A to unwind helices with 5′ overhangs, potentially generating an overall 5′–3′ directionality for eIF4A-mediated rearrangements of the 5′-UTR (199–202). eIF4B binds to D2 of eIF4A and can interact with ssRNA via its RRM, potentially trapping ssRNA produced by eIF4A (196, 200, 203). eIF4B may also promote RNA unwinding by tethering eIF4A to RNA, analogous to the ancillary RNA-binding domains and extensions found in other helicases (203).

Following PIC loading, eIF4A may also disrupt downstream 5′-UTR structures to facilitate scanning. Consistent with this model, eIF4A and eIF4B cross-link to the mRNA up to 52 nt downstream from the cap (204). eIF4A has a much higher intracellular concentration than do the other initiation factors and ribosomes, suggesting that eIF4A may carry out some functions independently and that multiple eIF4A monomers can act per mRNA (196, 205). However, Ded1p and DHX29 are also involved in translation and are more potent helicases, suggesting that eIF4A may not be the primary helicase during scanning (196). In addition to cap-dependent translation initiation, eIF4G and 4A are required for initiation at Type 1 and Type 2 IRES elements in viral mRNAs, where they induce conformational rearrangements necessary for PIC loading and initiation (206, 207).

Preinitiation complex (PIC): a complex consisting of the 40S ribosome, eIF2-GTP with initiator tRNA, and eIFs 1, 1A, 3, and 5

Both the helicase and RNP remodeling activities of Ded1 are likely to play roles in translation initiation. Ded1 interacts with eIF4G, and this interaction can both stimulate and repress translation initiation (208). Whereas the role of Ded1 in repression is ATP independent, ATP is required for Ded1-mediated release of mRNAs from stress granules, which reverses repression (208). As a helicase, Ded1 may disrupt 5′-UTR helices to promote PIC loading and scanning of the 5′-UTR, although these functions remain to be established experimentally (196).

The DEAH/RHA helicase DHX29 is required for efficient translation of mRNAs with highly structured 5′-UTRs and is also important for overall translation (209, 210). DHX29 interacts with the 40S ribosome in the PIC near the mRNA entry channel and promotes disruption of double-stranded segments, allowing these sequences to enter the ribosome (210). DHX29 may unwind these mRNA structures directly, or it may act indirectly by remodeling the entry channel of the ribosome (210).

Another helicase that promotes translation of specific groups of mRNAs is the DEAH/RHA helicase RNA helicase A (RHA), which interacts with mRNAs containing the 150-nt posttranscriptional control element (211, 212). RHA also interacts with other structures in 5′-UTRs, including G quadruplexes (see the sidebar, Disruption of G-Quadruplex Structures), and it presumably destabilizes these structures to give the observed increases in polysome loading and translation. Finally, Vasa (DDX4) promotes translation of mRNAs

DISRUPTION OF G-QUADRUPLEX STRUCTURES

G quadruplexes (G4s) are four-stranded structures composed of stacked G quartets, in which four guanosines interact by Hoogsteen interactions. G-rich sequences that adopt G4s in vitro are abundant in cellular RNAs, and G4s may play regulatory roles throughout RNA metabolism (240). G4 structures are extremely stable owing to their extensive network of H bonds and π–π interactions, as well as cation coordination in the G4 interior. Thus, it is not surprising that helicases have been implicated in G4 remodeling.

The human DEAH/RHA helicases RHAU and RHA efficiently disrupt RNA G4s in vitro, and both are likely to unwind G4s in the cell. RHAU may disrupt a G4 within the telomerase RNA to promote its native folding (241, 242). In addition, RHAU interacts with many mRNAs that contain G4 motifs (242). G4 recognition is linked to an N-terminal accessory domain of RHAU, but the mechanism of disruption is unknown (241–243). The second helicase, RHA, may unwind DNA and RNA G4s during transcription and can resolve other noncanonical structures (244). It remains to be explored whether G4 unwinding activity is common among RNA helicases and how many of them disrupt G4s physiologically.

encoding proteins that are involved in embryogenesis and germline development in animals (213). Vasa interacts with the initiation factor eIF5B, which mediates ribosomal subunit joining (214, 215), and the role of the helicase appears to be to promote eIF5B loading to the PIC, perhaps by removing repressors from the eIF5B binding site (214, 215).

Although helicase functions have been best characterized in translation initiation, a handful of RNA helicases function at later steps. The Ski2-like protein Slh1 associates with translating ribosomes and is important for translation (216). The DEAD-box protein Dbp5 and its cofactors Gle1 and IP6 function in translation termination (217, 218). On the basis of genetic data, Dbp5 is suggested to facilitate binding of release factors eRF1 and 3, likely by promoting rearrangements of the ribosome (219). The translating ribosome may also disrupt mRNA structures directly during elongation, analogous to the prokaryotic ribosome (220, 221), limiting the requirement for helicases past the initiation stage.

Exosome: a barrel-shaped multiprotein complex that functions in RNA processing and decay in eukaryotes and Archaea

RNA DECAY

Stable RNA structures and bound proteins can inhibit enzymatic RNA degradation, and unsurprisingly, RNases are often found to recruit RNA helicases (222). The DEAD-box helicase RhlB is a component of the *E. coli* degradosome and promotes degradation of RNAs with stable secondary structures by the exonuclease PNPase (223, 224). Interactions with the degradosome have also been reported for other DEAD-box proteins in *E. coli*, and DEAD-box components are found in the degradosomes of other bacteria (103, 222).

The eukaryotic exosome primarily utilizes helicases from two regulatory complexes: the Ski2 helicase of the cytoplasmic Ski complex and the closely related Ski2-like helicase Mtr4 of the nuclear complex TRAMP (225). Both helicases appear to unwind structured RNAs and channel ssRNA into the exosome for degradation (34, 226). Directional 3′ to 5′ unwinding by these Ski2-like helicases is thought to promote coupling of unwinding and degradation, which also proceeds from 3′ to 5′. An interesting interplay has been observed between Mtr4 and the poly(A) polymerase component of TRAMP. The polymerase promotes Mtr4 loading on RNAs by adding oligo(A) tails, and once bound, Mtr4 inhibits the polymerase, thus controlling the length of the tail (34, 227). The RHAU helicase can also transiently interact with the exosome and promotes degradation of AU-rich element–containing mRNAs, likely by disrupting RNA structures or protein binding (228).

OTHER RNA CHAPERONE AND REMODELING ACTIVITIES

Transcription

Researchers have long known that the DEAD-box proteins p68 and p72 function in

transcription by recruiting transcription regulators to RNA polymerase II, including the tumor suppressor p53 (229). This function appears to be carried out at the level of DNA, but recent results suggest a general RNA chaperone function for the *S. cerevisiae* homolog Dbp2. Dbp2 promotes clearance of cryptic RNA transcripts from chromatin, apparently by remodeling RNA cotranscriptionally and promoting binding of decay factors (230). Dbp2 probably remodels the RNA indiscriminately, as it also promotes loading of export factors to native transcripts (231). Whether corresponding roles are played by p68 and/or p72 remains unknown, but there is evidence for their localization to regions of elongation in transcription (229).

Nuclear Export

Several DEAD-box proteins have been implicated in the RNP remodeling events that occur as mRNA is exported through the nuclear pore complex. Dbp5 is localized to the cytoplasmic face of the nuclear pore complex and is required for dissociation of several export factors, contributing to the directionality of mRNA export (232, 233). The ATPase activity of Dbp5, which is required for protein displacement, is regulated by the activator Gle1 and coactivator IP6, whereas the nuclear pore component Nup159 promotes recycling of nucleotide-free Dbp5 after ATP hydrolysis (83). Inside the nucleus, UAP56 (Sub2) plays an incompletely understood, ATP-dependent role in mRNA export as a component of the TREX complex (234, 235). Furthermore, multiple human helicases are used by viruses during export of viral RNAs, including DEAD-box proteins DDX3 (the human homolog of Ded1) and DDX1, as well as the DEAH/RHA helicase RHA (236).

Editing

Illustrating the diversity of SF2 helicase–dependent processes, RNA helicases have been implicated in small RNA–guided pre-mRNA editing that occurs in the mitochondria of some protozoa (237). During editing, the 5′ region of a short guide RNA (gRNA) pairs with pre-mRNA, whereas the partially complementary 3′ region serves as a template for uridine insertions and deletions by the editosome. Editing creates new complementarity between the pre-mRNA and the 5′ region of another gRNA, resulting in a 3′–5′ cascade of editing events. In *Trypanosoma brucei*, the sequential binding and dissociation of gRNAs are promoted by the DEAD-box helicase REH1, which appears to dissociate gRNAs after editing (238). A second helicase, REH2 (related to DEAH/RHA and Ski2-like families), associates with the editosome and may promote RNA rearrangements during editing (239).

CONCLUSIONS

It is now clear that SF2 helicase proteins function throughout RNA biology, playing a diverse set of roles and using a diverse set of mechanisms. The list of helicases that function to rearrange or remodel RNAs or RNPs is large and growing. Still, despite remarkable progress in recent years, we have very limited knowledge of the interaction sites for helicases on their biological RNA and RNP substrates, and we know little about the structural rearrangements that they promote. Mechanistically, the available data suggest a general model in which DEAD-box proteins act locally, disrupting or in some cases promoting RNA structure without significant translocation. By contrast, DEAH/RHA and Ski2-like proteins load onto ssRNA and translocate from 3′ to 5′, thereby disrupting RNA structures and displacing proteins as they translocate. In the coming years, it will be fascinating to continue to learn about the structures and dynamics of the RNA and RNP substrates and the mechanisms used by helicases to manipulate them.

SUMMARY POINTS

1. Helicase proteins from three families within SF2—the DEAD-box, DEAH/RHA, and Ski2-like families—function as RNA chaperones and RNP remodelers and act on a wide variety of physiological substrates.
2. The available data indicate that DEAH/RHA and Ski2-like helicases unwind model RNA substrates with a mechanism that includes 3′–5′ translocation and a modest amount of processivity. DEAD-box proteins initiate RNA unwinding internally and do not translocate significantly.
3. Intriguingly, the differences in mechanistic properties between families show a good correspondence with differences in their in vivo roles. Thus, DEAH/RHA and Ski2-like proteins require 3′ overhangs for helicase loading and then unwind RNA and/or displace proteins directionally, whereas DEAD-box proteins initiate limited RNA unwinding internally.
4. Large, structured RNPs such as the ribosome and the spliceosome rely heavily on the activities of RNA helicase proteins for assembly and function. In prokaryotes, DEAD-box proteins play extensively overlapping roles in ribosome assembly, whereas in eukaryotes, larger numbers of helicase proteins play more specific roles, in the contexts of both the ribosome and the spliceosome.
5. The biological roles of SF2 helicases in RNA and RNP rearrangements represent a remarkably diverse set of activities, including unwinding of dsRNA, annealing of ssRNA, displacement of proteins, and disruption of highly stable noncanonical RNA structures.

FUTURE ISSUES

1. Exceptions to Summary Point 3, above, are highly likely to arise. That is, there will be proteins that do not interact with and unwind RNA using the mechanism expected for their SF2 family. A current candidate is Prp43, which is a DEAH/RHA family protein but displays some behaviors that resemble those of DEAD-box proteins.
2. It is not at all clear how DEAD-box helicases displace proteins from RNPs. DEAH/RHA and Ski2-like proteins, as well as proteins from other SF2 families, most likely displace proteins by translocating along the RNA substrate. However, DEAD-box proteins function extensively as RNP remodelers, removing proteins while promoting RNA rearrangements, yet there is no evidence for significant translocation by any DEAD-box proteins.
3. For most SF2 RNA helicases, the exact RNA and RNP rearrangements they promote in vivo remain unclear. A combination of in vivo and in vitro approaches will be required to enhance the understanding of the biological roles of these proteins.
4. Some physiological roles and physical properties for RNA helicase proteins likely have yet to be discovered, especially given the continuing discoveries of vast new functional classes of noncoding RNAs.

DISCLOSURE STATEMENT

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