Review

RNA helicases at work: binding and rearranging

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RNA helicases are ubiquitous, highly conserved enzymes that participate in nearly all aspects of RNA metabolism. These proteins bind or remodel RNA or RNA–protein complexes in an ATP-dependent fashion. How RNA helicases physically perform their cellular tasks has been a longstanding question, but in recent years, intriguing models have started to link structure, mechanism and biological function for some RNA helicases. This review outlines our current view on major structural and mechanistic themes of RNA helicase function, and on emerging physical models for cellular roles of these enzymes.

RNA helicases: ubiquitous and central players in RNA metabolism

RNA helicases are highly conserved enzymes that use ATP to bind or remodel RNA or ribonucleoprotein complexes (RNPs) [1]. One of the largest protein classes in RNA metabolism, RNA helicases are found in all kingdoms of life [2]. In eukaryotes these enzymes participate in nearly all aspects of RNA metabolism [1]. RNA helicases have received significant attention, ever since their identification in the 1980s. Many RNA helicases are essential for viability, and a growing number of these enzymes are known to play major regulatory roles in cells [1,3]. However, despite important insights into structural, mechanistic and cellular aspects of their function, it has remained enigmatic how these enzymes physically perform their cellular tasks. The last few years have now seen a notable increase in the number of cell biological, genetic, molecular biological, biochemical-biophysical and structural studies on RNA helicases. Although much remains to be learned, intriguing models are emerging that start to link structure, mechanism and biological function for some RNA helicases. In this review, I outline our current view on major structural and mechanistic aspects of RNA helicase function, and how these translate into cellular roles for these enzymes. For space reasons, I will focus mainly on the eukaryotic proteins.

RNA helicase basics: superfamilies, families and structural themes

RNA helicases are closely related to DNA helicases [4]. Both DNA and RNA helicases fall into two categories, those that form oligomeric (mostly hexameric) rings, and those that do not [5]. Based on sequence and comparative structural and functional analyses, all helicases are classified into six superfamilies (SFs) [5,6]. The ring-forming helicases comprise SFs 3 to 6, and the non-ring forming ones comprise SFs 1 and 2 [5]. All eukaryotic RNA helicases belong to SFs 1 and 2 (Figure 1). Ring-shaped RNA helicases are found in bacteria (e.g. Rho [7]) and viruses (e.g. Φ29 P4 [8]). Although these enzymes will not be discussed here, excellent recent reviews on these proteins are available [7,9].

Sequence comparisons revealed that both SF1 and 2 consist of well-defined helicase families with distinct structural and functional signatures (for a detailed description of the SF1 and SF2 families, see Ref. [4]). RNA helicases are found in six of these families; the remaining families consist of DNA helicases (Figure 1). Several helicase families contain both RNA and DNA helicases, and some enzymes, including proteins from the viral NS3/NPH-II group, RNA helicase A (DHX9) and Upf1-like helicases work on both DNA and RNA [10–12]. The lack of clear correlation between the helicase families and specificity for RNA or DNA suggests that discrimination between RNA and DNA might not have been a predominant evolutionary force for the differentiation of the helicase families [4].

Helicases of SFs 1 and 2 contain a structurally conserved helicase core, formed by two highly similar helicase domains arranged in tandem (Figure 2). Both SF1 and SF2 helicases contain at least 12 characteristic sequence motifs at defined positions in the helicase core (Figure 2A,B). However, not all motifs are present in each helicase family [4]. The level of sequence conservation in these motifs is high within each family, but decreases between different families [4]. Only limited sequence conservation remains across both superfamilies (Figure 2C).

In addition to the family-typical sequence domains, the Ski2-like, DEAH/RHA and NS3/NPH-II families have a prominent β-hairpin between motifs Va and VI [13–16]. This feature is not seen in other RNA helicase families [4]. The SF1 Upf1-like family and in the retinoic-acid-inducible gene I (RIG-I)-like family have inserts within or between the helicase core domains [4] (Figure 2A). The inserts are occasionally large and adopt independent folds, but generally have only minor or no effects on the fold of the helicase core domains [17–19]. A small number of individual proteins in other helicase families also feature inserts, such as the DEAD-box protein DDX1 [20], but these inserts are not typical for the families.

In essentially all SF1 and SF2 helicases, the structurally conserved helicase core is surrounded by C- and N-terminal domains, which are often larger than the helicase core and frequently contain one or more specific functionalities

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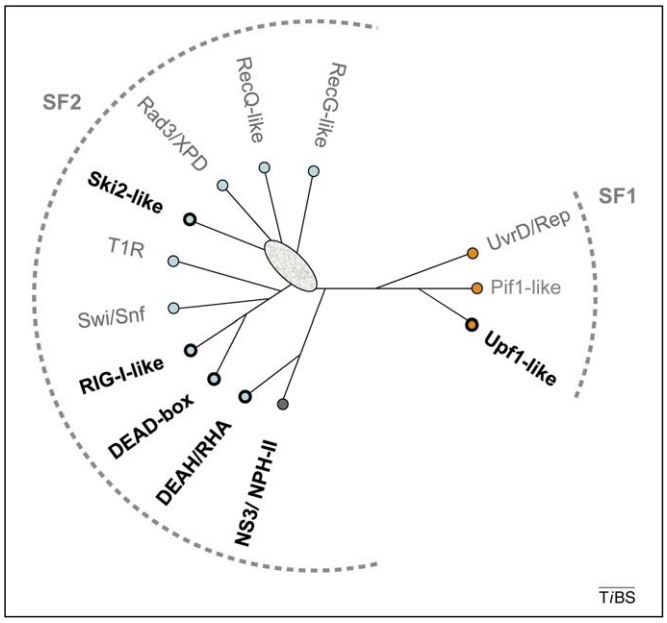

Figure 1. SF1 and SF2 helicase families. Unrooted cladogram showing the families of the SF1 (right), and the SF2 (left) according to Ref. [4]. Branch lengths are not to scale. The oval indicates significant uncertainty in cladogram topology in this region. Boldfaced names show families harboring RNA helicases (non-standard abbreviations: T1R, type 1 restriction enzymes; RHA, RNA helicase A).

including nucleases, RNA- or DNA-binding domains [10,14,21]), protein-binding domains (e.g. CARD domains [22]), or oligomerization modules [23]. C- and N-terminal domains are thought to be crucial for the cellular specificity of helicases by facilitating recruitment of the proteins to specific complexes, either through interactions with other proteins, or as seen in the bacterial DEAD-box protein DbpA or the DEAH/RHA protein RHAU (DHX36), by facilitating recognition of specific nucleic acid regions [24,25]. Except for the C-terminus of the spliceosomal DEAH proteins, sequences of C- and N-terminal domains are generally not conserved within or between families [4]. However, recent work shows structural conservation in the C-terminal domains of Ski2-like and DEAH/RHA proteins [14,26].

RNA helicase mechanisms: more than unwinding

Paradoxically, helicases as defined by characteristic sequence motifs are not always helicases as defined by enzymatic function, the ATP-dependent unwinding of nucleic acid duplexes [27,28]. For example, proteins of the Swi/Snf family and the ATP-dependent restriction endonucleases (T1R, Figure 1) generally display no unwinding activity, even though they hydrolyze ATP in a DNA-dependent fashion, possess all of the helicase motifs, and are built around a helicase core structure [29].

RNA helicases generally unwind RNA duplexes *in vitro*, provided appropriate substrates are used [30]. However, RNA helicase activity *in vitro* does not imply that a given enzyme necessarily unwinds duplexes in the cell. Yet, even for RNA helicases that perform other tasks, RNA helicase activity is an excellent proxy for measuring the ability of the enzymes to remodel RNA structures in an ATP-dependent fashion.

At least two distinct types of RNA helicase activity have been identified: canonical duplex unwinding (Box 1), and unwinding by local strand separation (Box 2). Canonical duplex unwinding refers to the mechanism displayed by many DNA helicases and by several viral RNA helicases of the NS3/NPH-II group [5,31]. The helicase binds to a single-stranded region adjacent to the duplex and then translocates along the bound strand with defined directionality, either 3′ to 5′ or 5′ to 3′, thereby displacing the complementary strand (Box 1). As a consequence, most canonically operating helicases require substrates with single-stranded regions in a defined orientation (polarity) with respect to the duplex (Box 1). RNA helicases of the Ski2-like, the RIG-I-like, the DEAH/RHA, and the Upf1-like families also display preferred unwinding polarities

Box 1. Translocation-based duplex unwinding by canonical DNA and RNA helicases

The helicase binds to the single stranded region and in multiple, ATP-dependent consecutive steps translocates towards the opposite end (Figure I). In the process, the complementary strand is removed. There are monomeric and oligomeric canonical helicases [27]. Each translocation step consists of multiple processes including ATP binding and hydrolysis, a power stroke to produce the forward movement, and dissociation of the products of the ATP hydrolysis. For detailed reviews and discussions on canonical unwinding mechanism, see Refs [5,27,31,46].

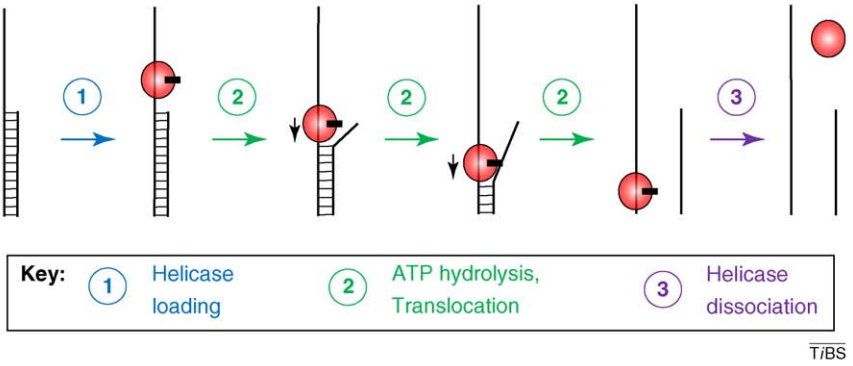

Figure I. Schematic view of the main steps of translocation-based duplex unwinding. Lines represent RNA strands, the oval marks the helicase and the black rectangle indicates the ATP. Only a monomeric enzyme is displayed, but canonical helicases have also been shown to function as oligomers [27]. Only selected, main intermediates are shown.

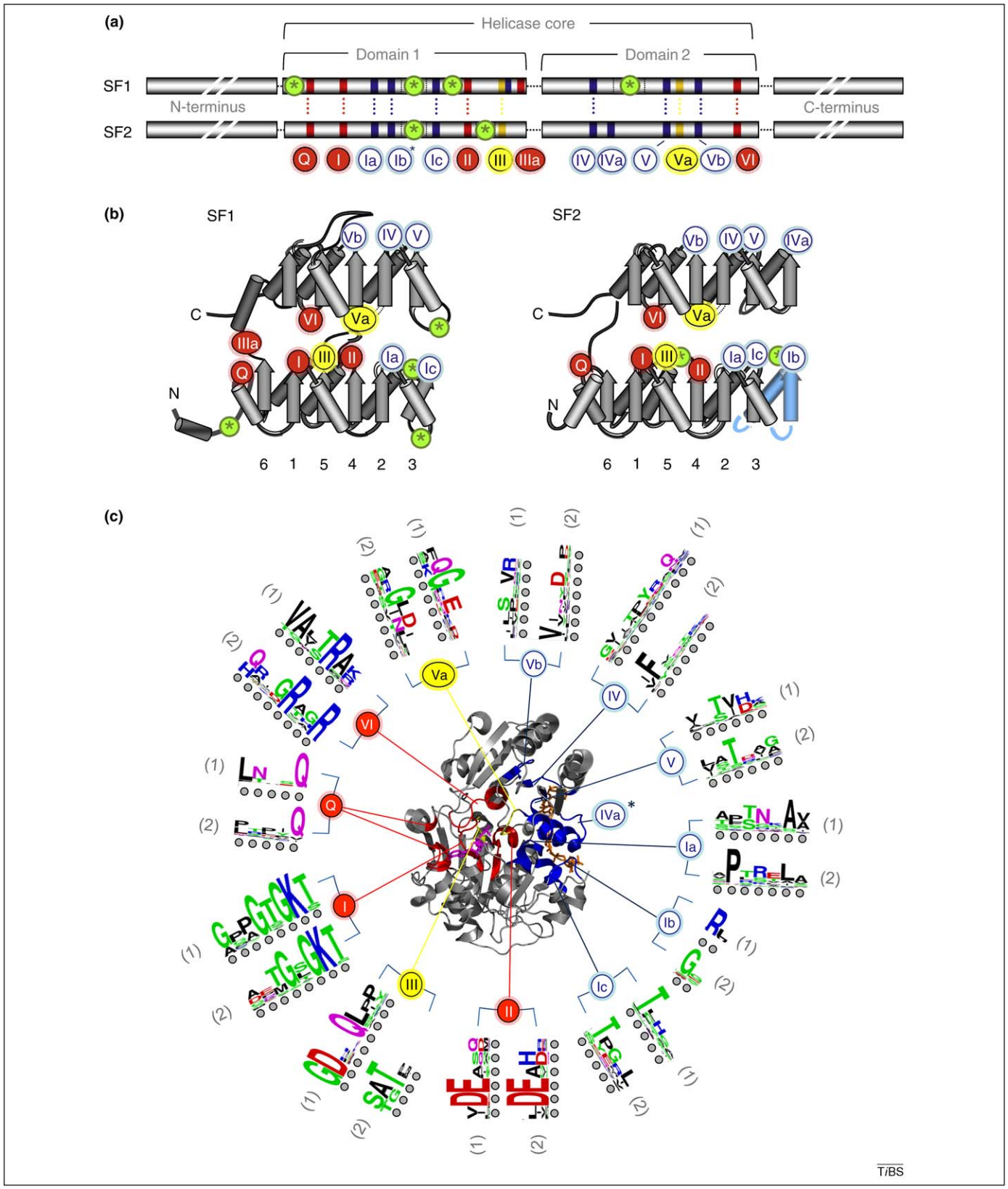

Figure 2. The helicase core of SF1 and SF2 proteins. **(a)** Characteristic sequence motifs of SF1 and SF2 proteins in the helicase core [4]. The motifs are colored according to their predominant biochemical function: red, ATP binding and hydrolysis; yellow, coordination between nucleic acid and NTP binding sites; blue, nucleic acid binding. Motif Ib (asterisk) is not present in all SF1 and SF2 families. Green circles with asterisks designate insertions of additional domains. Distance between the conserved motifs is not to scale. **(b)** Location of the helicase motifs in the helicase core fold (arrows: β-strands, cylinders: α-helices). Numbers under the diagrams show connectivity of the β-strands of the first RecA-like domain. Helicase motifs are marked by circles (coloring and numbering as in panel A). Domain insertions are marked by green circles with asterisks. The rightmost β-strand and α-helix in the SF2 (blue) is not present in all SF2 families [4]. **(c)** Location of the characteristic motifs in three-dimensional structure of the helicase core, as represented by SF2 DEAD-box helicase Vasa [49]. The bound ATP analog is colored magenta, the RNA wheat. Helicase motifs are colored as in panel A. Corresponding sequence logos indicate conservation within the helicase motifs in SF1 (1) and SF2 (2). Colors mark properties of the amino acids as: green, polar; blue, basic; red, acidic; and black, hydrophobic. Circles under the letters are visual guides.

Box 2. Duplex unwinding by local strand separation

This unwinding mode is employed by DEAD-box helicases [77]. The helicase is loaded directly on the duplex region, aided by single stranded or structured nucleic acid regions (Figure I, step 1). These regions have to be proximal, but no covalent connection to the duplex is necessary. Therefore, DEAD-box proteins unwind tailed substrates without apparent polarity, but often require unpaired regions for efficient unwinding. Duplex loading can involve multiple protomers, but can also be mediated by accessory protein domains [77]. Although the exact mechanisms of loading processes are not yet understood, it is known that the loading can occur at any place in the duplex, at an end or internally, and on either strand. Duplex loading is accompanied by ATP binding [34]. Upon loading, the DEAD-box protein locally opens the duplex strands (Figure I, step 1). This step requires ATP, but not ATP hydrolysis, suggesting that ATP binding suffices [39–41]. The local helix opening reduces the number of basepairs in the duplex, and the remaining basepairs dissociate without further action from the enzyme (Figure I, step 2). Unwinding rate constants decrease with duplex length and stability, because more and more stable basepairs dissociate slower. Although ATP hydrolysis is dispensable for duplex unwinding, it is crucial for efficient release of the DEAD-box protein from the RNA (Figure I, step 6), and thus for enzyme recycling [40]. Not every ATP-driven local helix opening will lead to complete strand separation. ATP hydrolysis can occur after the helix has been opened by ATP binding, but before the strands have separated (Figure I, step 4). Although ATP hydrolysis promotes enzyme dissociation, strand separation can take place before the enzyme dissociates (Figure I, step 5), or the enzyme dissociates before complete helix separation (Figure I, step 7). In this case, the strands quickly re-anneal (Figure I, step 8). Such 'non-productive' ATP hydrolysis events are more prevalent for longer and more stable duplexes, because unwinding events occur less frequently. Therefore, unwinding of longer or more stable helices involves greater numbers of hydrolyzed ATPs per duplex separated [36,39].

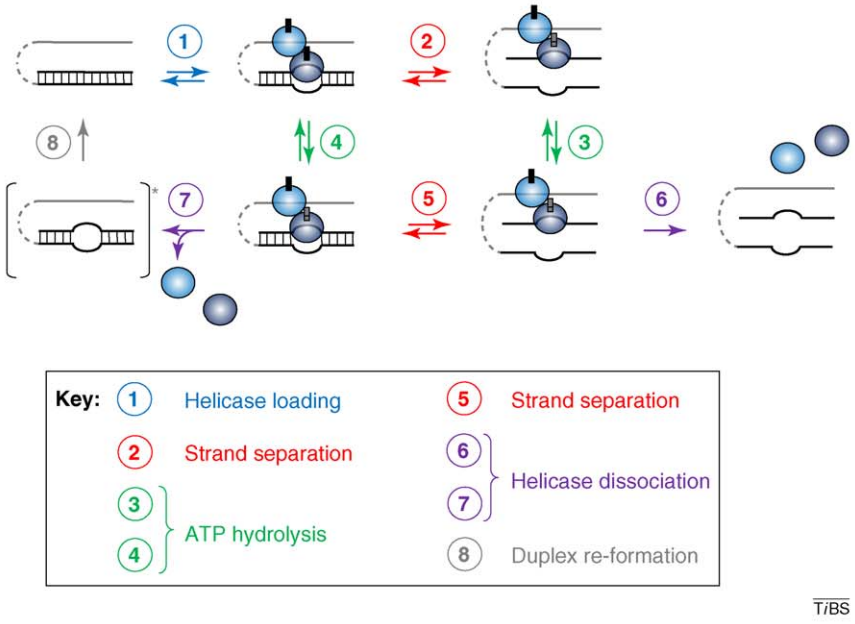

Figure I. Schematic view of the steps of unwinding by local strand separation. Lines represent RNA strands, the ovals mark the helicase and the small rectangles indicate ATP and ADP. The different colors of the helicase protomers emphasize their distinct roles in the unwinding process. The asterisk after step 7 highlights the transient nature of the RNA species with a partially opened helix. Adapted, with permission, from Ref. [40]).

[10,12,32,33]. Based solely on this observation, it is occasionally concluded that these RNA helicases unwind duplexes in the canonical fashion. However, to my knowledge, no eukaryotic RNA helicase has yet been directly shown to unwind duplexes based on directional translocation, that is, in multiple consecutive unwinding steps. Alternative unwinding scenarios thus remain possible for these helicases.

RNA helicases of the DEAD-box family unwind duplexes by local strand separation (Box 2). This distinct unwinding mechanism is not based on translocation [34–37]. Instead, DEAD-box proteins directly load to the duplex region and then pry the strands apart in an ATP-dependent fashion (Box 2) [34–37]. As a result, unwinding occurs without defined polarity, even though single stranded, or in some cases structured RNA extensions, stimulate strand separation by most DEAD-box proteins [35,37]. Unwinding can be accomplished with a single round of ATP binding/hydrolysis, and for several DEAD-box proteins, strand separation does not require ATP hydrolysis, but only ATP binding [38–41]. However, ATP hydrolysis is necessary for efficient release of the DEAD-box helicase from the RNA and thus for multiple substrate turnovers [40] (Box 2). Unwinding efficiency greatly decreases with increasing length and stability of the duplex, and most DEAD-box proteins only unwind duplexes containing less than 10–12 basepairs with appreciable activity [36,39,42]. However, RNAs in eukaryotic cells form few, if any, uninterrupted duplexes exceeding this length [43]. The distinct unwinding mode of DEAD-box proteins thus appears uniquely suited for the localized separation of short duplexes in the cell [35].

For both canonical and non-canonical RNA helicases, RNA binding and unwinding involves ATP-dependent, coordinated changes in the orientation of the two helicase domains [5,27,31,43]. Without ATP, the cleft between the two domains opens, although to varying degrees in different helicase families [43]. ATP binding generally promotes

closing of the two domains [5,27,31,43]. The nucleic acid is bound by all helicases in the same orientation (Figure 2C). The conserved helicase domains involved in nucleic acid binding make similar contacts in all, canonical and non-canonical RNA helicases; and nearly all contacts are with the sugar-phosphate backbone [5].

For canonically operating helicases (e.g. hepatitis C virus NS3), additional contacts from accessory domains are established with bases [16]. These contacts ensure the translocation of the helicase along the RNA/DNA by 1 nt per ATP consumed [44]. Structural models now exist for translocation by several canonical RNA and DNA helicases, and all of these models suggest movement by 1 nt per ATP [5,16,31]. However, the directional movement is accomplished through different base contacts by different enzymes [5,16,31]. Many translocating helicases appear to move in bursts of several 1 nt steps before repeating a rate limiting step [44–46]. The distance translocated during these bursts is often measured as the kinetic step size, reaching up to 18 nt for certain helicases [47,48]. The exact structural bases for the kinetic step sizes are not clear.

Structures with bound RNA are available for only a few DEAD-box proteins [49–53]. In all of these structures, the DEAD-box proteins establish contacts to the RNA almost exclusively to the backbone, consistent with their distinct unwinding mode. The conformation of the bound RNA strand in the presence of ATP analogs is characterized by pronounced bends in the backbone, a marked difference to RNA or DNA conformations in most canonical helicases [16,53]. The bends make the bound RNA incompatible with double helical architecture, and probably represent the RNA conformation following strand separation [53]. How exactly DEAD-box proteins perform the act of strand separation is not yet understood on a structural level.

In addition to duplex unwinding, RNA helicases display an array of additional activities. Most prominently, several RNA helicases have been directly shown to displace other proteins from RNA in an active, ATP-dependent fashion [54]. Protein displacement or RNP remodeling is thought to be central to the physiological function of RNA helicases, because RNAs are generally bound to other proteins *in vivo* [55]. Protein displacement is not necessarily coupled to duplex unwinding, and has also been seen for DEAD-box proteins, indicating that protein removal is not restricted to RNA helicases that unwind duplexes in the translocation-based, canonical fashion [56–58]. Nevertheless, *in vitro* some helicases can only remove a certain spectrum of proteins (e.g. proteins with small RNA binding sites), whereas other helicases displace a more diverse set of proteins [56]. The inability of certain helicases to remove a given protein from RNA might spatially regulate helicase activities in larger RNP assemblies [54].

The RNA helicase RIG-I recently was shown to translocate on double-stranded RNA in an ATP-dependent fashion, without unwinding the duplex [59]. This activity resembles the translocation of type 1 restriction enzymes and some Swi/Snf proteins on dsDNA [29]. RIG-I functions in the innate immune system as a pattern recognition receptor for the identification of viral RNAs in the cytoplasm, and the translocation is thought to aid the detection of viral RNAs, which can form long dsRNA during viral replication [59]. However, translocation on dsRNA appears unlikely to be prevalent among eukaryotic RNA helicases that function on cellular RNAs, given that these RNAs are known to contain only short helical regions.

In addition to the activities listed above, a growing number of RNA helicases are known to facilitate strand annealing, or its intramolecular version, RNA folding [43]. Interestingly, pronounced strand annealing activity has also been seen for DNA helicases of the RecQ family [60]. Many RNA helicases display a basal annealing activity that enhances the second order rate constant for duplex formation by a factor of 3 to 10 [61]. However, several DEAD-box helicases including Ded1p (DDX3) and Mss116p are among the strongest known strand annealers. These proteins enhance the second order rate constant for duplex formation by several orders of magnitude up to the diffusion limit, the physically possible ceiling [61,62]. Although most RNA helicases tested do not require ATP to promote strand annealing, some do; however, it is not clear whether ATP hydrolysis is involved [63]. Strand annealing activity, in conjunction with duplex unwinding or protein displacement is thought to enable RNA helicases to catalyze RNA or RNP structure conversions that involve both disruption and formation of RNA/RNP structures [64]. Indeed, several RNA helicases promote such RNA structure conversions on model RNAs or on physiological substrates [64,65].

The spectrum of different activities by RNA helicases raised the question of which mechanistic features underlie the different activities. To date, ATP-dependent or ATP-modulated RNA binding appears to be the smallest common denominator for all RNA helicases. Interestingly, the cellular function of at least one RNA helicase, the DEAD-box protein eIF4A-III (DDX48), is based on ATP-dependent RNA binding [66].

RNA helicases in the cell: specific roles for non-specific enzymes

In vitro, the vast majority of RNA helicases do not display sequence or structural preferences, besides the polarity requirements of some enzymes for unwinding RNA duplexes (Box 1). In the cell, however, most RNA helicases function in specific processes such as ribosome biogenesis, pre-mRNA splicing and translation (Figure 3). Many RNA helicases appear to participate solely in one process. Several enzymes including Prp43p (DHX15), RNA helicase A (DXH9), eIF4A-III (DDX48) and p68 (DDX5) have been implicated in several processes (Figure 3). Of these proteins, the enzymes functioning in mRNA metabolism are thought to remain bound to a given set of mRNAs, thereby affecting multiple mRNA processing steps [67]. Some of these helicases (e.g. DDX3, DHX9, p68) have emerged as key players in the regulation of biological processes, including tumorigenesis [10,67,68]. Several of these proteins (e.g. DHX9, DDX3) are also inactivated or co-opted by viruses to enable viral replication [67,69].

Many RNA helicases have been assigned to specific reaction steps in multi-step processes. For example, it is known for several DEAD-box proteins in which pre-rRNA processing step they participate, and it is comparably well

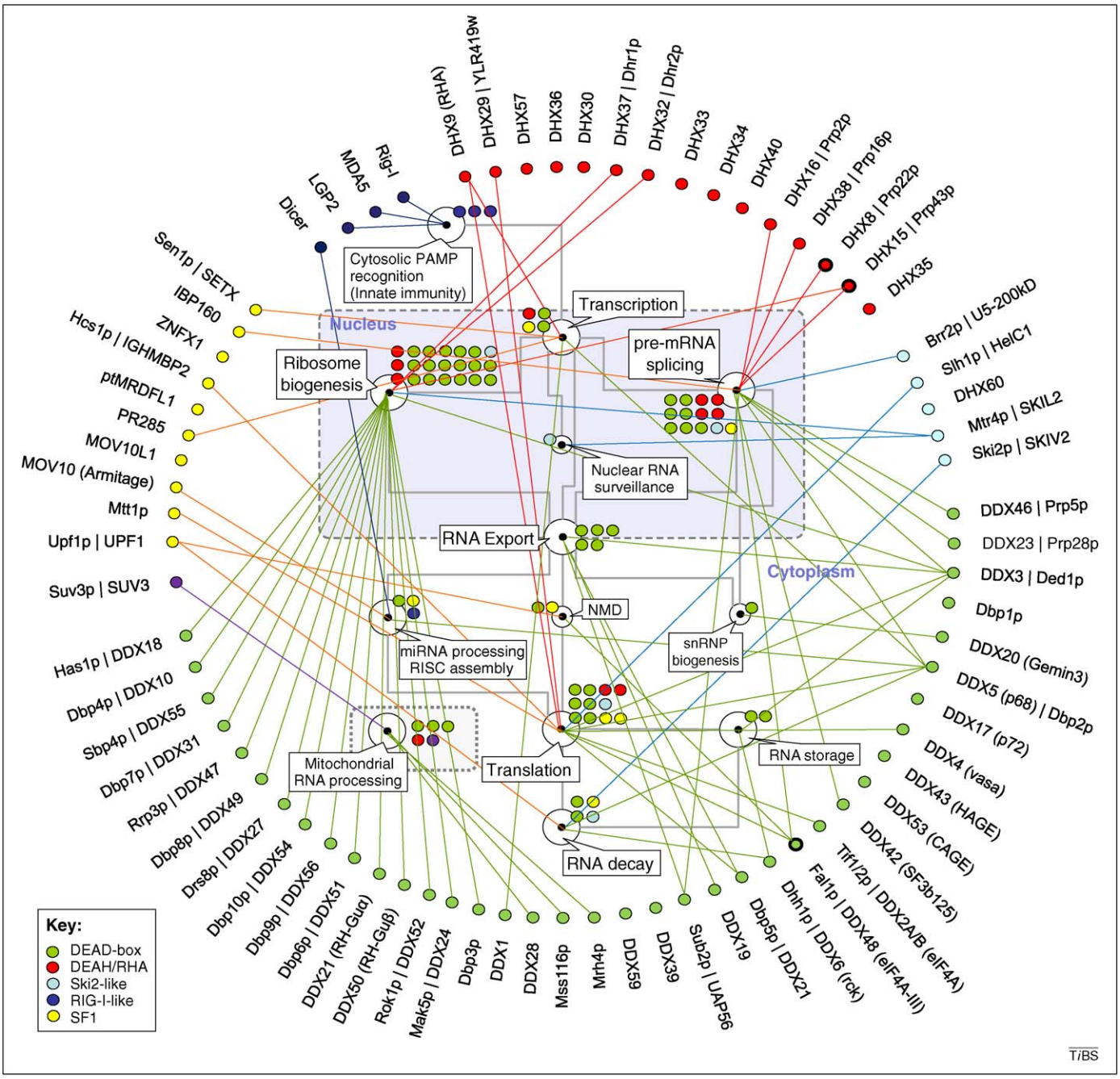

Figure 3. Cellular roles of eukaryotic RNA helicases. Selected, basic processes of eukaryotic RNA metabolism are represented by the white circles, as indicated by the callouts (NMD, nonsense mediated decay). The grey lines mark connections between processes. The colored circles represent the number of individual RNA helicases involved in a given process. RNA helicases (yeast and human orthologs) are grouped and color-coded according to their families (see legend at left lower corner). Connectors indicate involvement in one or more processes of RNA metabolism. Clear assignment of SUV3 to either SF is not possible, even though the protein is highly conserved throughout evolution [4]. Circles with bold lines emphasize the three RNA helicases (Prp22p, Prp43p, eIF4A-III) for which specific binding site information is available.

understood at which step in pre-mRNA splicing many spliceosomal RNA helicases function [70,71]. Further roles for the DEAD-box proteins in ribosome biogenesis steps are known for only a few examples, most of which promote the release of small nucleolar RNA (snoRNA) (e.g. refs. [72–74]). Many of the spliceosomal RNA helicases appear to provide directionality for inherently reversible transitions during the splicing reaction, most probably by coupling an essentially irreversible ATP turnover to a transition, thereby suppressing reversible steps in the reaction cascade (Box 3).

From a process point of view, the role of the spliceosomal helicases is similar to the role of RNA helicases that facilitate RNA folding in fungal mitochondria [75,76] (Box 3). These RNA helicases (Mss116p, CYT-19) function as RNA chaperones that guide RNAs to their native conformation through a series of folding steps [77]. The RNA helicases promote forward steps, slow reverse steps or, through remodeling of RNA, open new reactions paths that facilitate correct folding [64,77] (Box 3). Studies of pre-mRNA splicing also revealed that at least three RNA helicases function in proofreading events at various stages

Box 3. Kinetic schemes of RNA helicase functions in RNA metabolic processes

RNA helicases have been implicated in proofreading processes and in conferring a direction to series of inherently reversible reactions (Figure I). Without further stimuli (no helicase), a process involving series of inherently reversible reactions would equilibrate, possibly resulting in the continued presence of multiple intermediate species (Figure Ia). Helicases can promote a forward step or inhibit a reverse step, thereby providing direction to the entire process [70]. Alternatively, helicases can alter the topology of the reaction mechanism, for example by addition of new reaction pathways. This can be accomplished through remodeling of RNA structures or simply by interactions of the helicase with the RNA [64,75]. Topological alterations of the mechanism changes equilibrium concentrations of final and intermediate species [64]. During proofreading, RNA helicases promote the processing pathway for correct substrates or govern the activation of a discard pathway for incorrect substrates, or both [78] (Figure Ib).

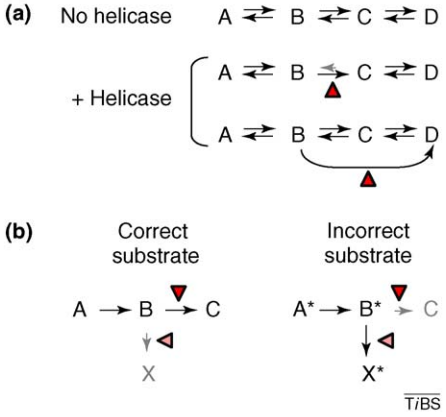

Figure I. Schemes for RNA helicase functions in the cell. **(a)** A series of inherently reversible reactions. The red triangle marks the helicase. **(b)** Proofreading by RNA helicases. The left panel shows processing of the correct substrate, the right panel processing of the incorrect substrate. The triangles mark possible action points of the helicase.

of the splicing reaction [78]. As proofreaders, the RNA helicases distinguish between correct and incorrect substrates, and promote further processing of correct substrates while facilitating the discarding of incorrect ones (Box 3).

For the vast majority of RNA helicases, it is not clear which exact physical functions they perform in the cell. Devising physical models for the cellular functions of RNA helicases is the current frontier, but detailed molecular models are only available for very few enzymes. Not surprisingly, attempts to analyze cellular functions of RNA helicases on a detailed molecular level face formidable challenges. Most importantly, RNA targets or RNA binding sites are unknown for the vast majority of RNA helicases, and the lack of sequence or structure specificity greatly complicates target identification. However, without knowing where a helicase binds its substrate, it is essentially impossible to establish physical models for the function of these proteins. Moreover, most, if not all RNA helicases work in the context of large multi-component assemblies and thus interact with many other proteins [43]. Because it is known in only few cases whether and how other proteins modulate RNA helicase activities, it is difficult to assess how helicase activities seen *in vitro* translate into cellular function.

The perhaps most detailed physical models for RNA helicase function in the cell are available for the DEAH/RHA protein Prp22p (DHX8) and the DEAD-box protein eIF4A-III [66,79] (Figure 4). Prp22p promotes release of spliced mRNA from the spliceosome and also participates in the exon ligation step [79]. During its function, Prp22p changes its position on the RNA several times (Figure 4A). One of these changes requires ATP and most probably involves directional movement of Prp22p 3′ to 5′ on the mature mRNA, consistent with the unwinding polarity of the protein [79]. During this movement, Prp22 breaks several RNA–RNA and RNA–protein contacts, thereby causing the dissociation of the mRNA from the spliceosome [79] (Figure 4A).

eIF4A-III functions as part of the exon junction complex (EJC), a multiprotein complex that is deposited roughly 20 nucleotides upstream of exon–exon junctions during pre-mRNA splicing in higher eukaryotes [66,80] (Figure 4B). The EJC remains stably bound to the mRNA after export into the cytoplasm, where it affects several downstream steps of mRNA metabolism including nonsense-mediated decay, translation, and RNA localization [66,80]. Although eIF4A-III can unwind RNA duplexes *in vitro*, in the cell it functions as an adaptor for the other EJC components that ensures their extremely stable association to the RNA [81]. Structural studies have shown that the stable binding is accomplished through arrest of the ATP hydrolysis cycle and prevention of hydrolysis product dissociation from the active site of eIF4A-III [50,51]. Trapping of the ATP hydrolysis products is caused by the EJC components Magoh and Y14 [82] (Figure 4B).

The models for both Prp22p and eIF4A-III function provide rationales for their inherent lack of sequence specificity. Both enzymes interact with many different substrates at specific positions. Sequences at these binding sites are probably diverse and pronounced sequence preferences would be detrimental for association of the helicases to such sites.

Binding to various different sites on pre-ribosomal RNA also has been revealed for the DEAH/RHA protein Prp43p [83]. Using cross-linking combined with deep sequencing, an approach similar to the crosslinking and immunoprecipitation of RNA–protein complexes (CLIP) technique, binding sites for Prp43p were identified in a genome-wide, unbiased fashion [83]. Given the increasing availability of deep sequencing capacity, this approach should become instrumental in locating target sites for RNA helicases. For Prp43p, binding sites on pre-rRNA are now known, and the next challenge will be elucidation of Prp43p function at each position. Suggested roles include snoRNA dissociation, promotion of snoRNA binding, and facilitation of nuclease processing [83].

How Prp43p, Prp22p, eIF4A-III, and most other RNA helicases find their binding sites is not clear. Experimental evidence suggests that several RNA helicases bind cofactors that aid recruitment to complexes such as the spliceosome or to P-bodies [15,84,85]. Other RNA helicases identify targets on their own, according to a complex code of features on the RNAs. A prominent example is RIG-I,

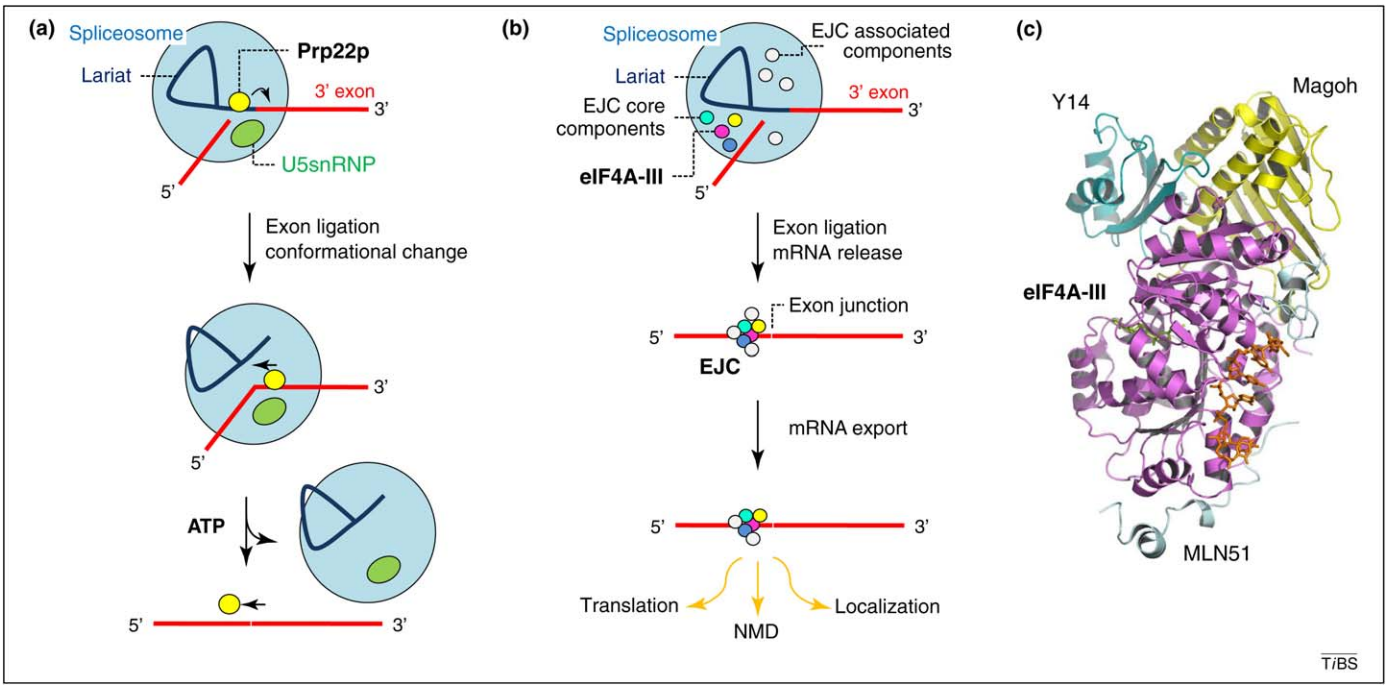

Figure 4. Cellular functions of the DEAH/RHA helicase Prp22p and the DEAD-box protein eIF4A-III. **(a)** Prp22p promotes mRNA release from the spliceosome [79]. The mRNA exons are marked red; U5snRNP aids in configuring the active site for the second splicing step. Prp22p binds the intron close to the 3′ splice site. Upon exon ligation, Prp22p changes it position and binds 3′ to the exon junction. Subsequently, Prp22p hydrolyzes ATP and moves towards the 5′ end of the spliced mRNA, thereby removing the bound spliceosome. The figure is adapted from Ref. [79]. **(b)** eIF4A-III acts as RNA adaptor for the other components of the EJC. The EJC is assembled during mRNA splicing and core components remain stably bound to eIF4A-III. Associated components dissociate at various stages of mRNA metabolism and further associated proteins bind throughout the travel of the EJC bound mRNA [66]. The number of associated components shown is not representative. **(c)** Crystal structure of the core EJC bound to RNA [51]. Proteins are labeled and the bound RNA is shown in orange.

which as part of the innate immune system discriminates cellular from viral RNAs [86]. To accomplish this, RIG-I uses a 5′-terminal triphosphate on the viral RNAs, together with RNA secondary structure in the vicinity of the 5′-triphosphate [86].

Although binding to specific sites is probably crucial for the majority of cellular RNA helicases, seemingly indiscriminate action on RNAs by certain RNA helicases might also be important. The DEAD-box helicases Mss116p and CYT-19, RNA chaperones that aid the folding of mitochondrial RNAs in fungi [62,87,88], perform their function at least in part by indiscriminate disruption of improperly formed RNA secondary structure that slows RNA folding [64,89]. Sequence and presumably site specificity of these RNA helicases would be incompatible with function on a variety of differently folded substrates [64,76].

RNA helicases interact constantly with other proteins in the context of larger multi-component complexes [43]. An increasing number of studies have focused on identifying interacting proteins (co-factors) and on elucidating their effects on RNA helicase activities. Frequently, co-factors increase unwinding activities or RNA-stimulated ATPase activity of the helicases (e.g. Refs [85,90–92]). These stimulations often reflect the ability of the co-factor to increase the RNA affinity of the helicase, which might be a straightforward strategy for recruitment of helicases with inherently poor RNA affinity. A remarkable version of co-factor-mediated stimulation of an RNA helicase was shown for Dbp5p (DDX19/21), a DEAD-box protein that functions in mRNA export. Dbp5p is activated by Gle1p bound to the small molecule inositol hexakisphosphate [93,94]. Gle1p accumulates at the cytosplasmic side of the nuclear pore and thus activates Dbp5p in a localized fashion [93,94].

Instead of stimulating activities of helicases, co-factors also can inhibit or essentially arrest the ATPase cycle (e.g. eIF4A-III [66]). In addition, co-factors can interfere with binding of RNA or prevent association of other proteins. Binding of the cytoplasmic nucleoporin NUP214 blocks the RNA binding site of Dbp5p, and thus its RNA-related functions [52]. The tumor suppressor protein programmed cell death 4 (PDCD4), which inhibits translation, binds to the translation initiation factor eIF4A (DDX2), a DEAD-box protein distinct from the EJC component eIF4A-III [95,96]. PDCD4 prevents association of the translation initiation factor eIF4G [97], which is crucial for translation initiation and stimulates eIF4A activities [98]. Finally, co-factors can bind to RNA helicases without notable effects on activities. This scenario has been observed for the interaction of the DEAD-box protein UAP56 with Aly [99].

Most studies of co-factor–helicase interactions have concentrated on effects on the helicase. Comparably little work has focused on the effect of RNA helicases on other proteins. This aspect has been examined on the interaction between the helicase and the RNA polymerase of hepatitis C virus, and on RNA helicases in the bacterial and the mitochondrial degradosomes [100–102]. In all of these cases, functional crosstalk between helicase and other proteins has been observed.

Concluding remarks and future perspectives

This short overview on current topics of research on RNA helicases highlights remarkable progress over the last

years. Yet, much remains to be learned about structure, mechanism and physiological function of RNA helicases, before molecular models of cellular functions of these enzymes can be established. On the structural front, the next challenges include obtaining more structures of full length proteins, bound to RNA and ATP analogs, to establish detailed structural models of helicase activities for enzymes from different helicase families. On the mechanistic side, focused structure–function studies are needed to verify and complement structural models with dynamic information. In addition, the study of RNA helicases in authentic complexes will probably become a more central theme. With regards to the cellular function of RNA helicases, focus will undoubtedly be on identification of RNA targets, on elucidating means by which the enzymes are recruited to their sites of action, and on devising physical models for RNA helicase function in their physiological environment, building on information from structural and mechanistic studies. Finally, it will be important to examine effects of posttranslational modifications in RNA helicases [103].

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