RNA Biology 10:1, 56–70; January 2013; © 2013 Landes Bioscience

Rarely at rest

RNA helicases and their busy contributions to RNA degradation, regulation and quality control

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Keywords: RNA helicase, DExD/H box helicase, RNA-protein interactions, RNA degradosome, exosome, post-transcriptional regulation, RNA turnover, non-coding RNA, ribonuclease, RNA surveillance

RNA helicases are compact, machine-like proteins that can harness the energy of nucleoside triphosphate binding and hydrolysis to dynamically remodel RNA structures and protein-RNA complexes. Through such activities, helicases participate in virtually every process associated with the expression of genetic information. Often found as components of multi-enzyme assemblies, RNA helicases facilitate the processivity of RNA degradation, the remodelling of protein interactions during maturation of structured RNA precursors, and fidelity checks of RNA quality. In turn, the assemblies modulate and guide the activities of the helicases. We describe the roles of RNA helicases with a conserved "DExD/H box" sequence motif in representative examples of such machineries from bacteria, archaea and eukaryotes. The recurrent occurrence of such helicases in complex assemblies throughout the course of evolution suggests a common requirement for their activities to meet cellular demands for the dynamic control of RNA metabolism.

Introduction

Life and its myriad biochemical processes are seldom at standstill. A salient illustration is the dynamic nature of the control of gene expression; consider for instance the cellular RNA transcript pool, which is in continuous flux and responds sensitively to changes in the balance of synthesis and degradation rates. In all kingdoms of life the regulation of transcript degradation is a crucial mechanism of cellular development and response to varying environmental conditions. Key to these processes are numerous enzymes, such as RNA helicases, which manipulate RNA structure and remodel ribonucleoprotein complexes, and a variety of ribonucleases (RNases). The RNases encompass different catalytic mechanisms and substrate preferences, and they often play dual roles in RNA metabolism through their capacity not only to degrade RNA, but also to specifically process precursors of structured RNA into their mature forms.[1,2] RNases may also function in conjunction with *trans*-acting small RNAs to rapidly modulate the expression of specific transcripts, often as part of stress responses.[3] While some RNases are capable of working autonomously, many require protein partners, such as the RNA helicases, for full functionality.

This review will explore the interplay of RNases and RNA helicases (particularly the members of the DExD/H box family) in RNA degradation and processing. This interplay is best understood currently in the context of multi-component assemblies, and we discuss how these assemblies affect the functional and allosteric regulation of the helicases, and how the helicases modulate RNA processing and decay.

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Submitted: 07/19/12; Revised: 09/16/12; Accepted: 09/17/12
http://dx.doi.org/10.4161/rna.22270

Nucleic Acid Helicases in Broad Perspective

DNA and RNA helicases are ubiquitous enzymes involved in the manipulation of nucleic acids and their complexes with proteins. Since their identification in the 1980s, it has become increasingly apparent that helicases participate in virtually every cellular process involving nucleic acids, including DNA replication, transcription, RNA-folding and ribosome genesis.[4-7] Helicases have been widely understood to be nucleoside triphosphate-dependent enzymes that can interact with nucleic acids and unwind duplex substrates. Unexpectedly, they were also found to displace bound proteins to remodel nucleic acid-protein complexes.[5,8]

Helicases may be classified into six superfamilies (SF1 to SF6) based on conserved sequence and structural elements,[7,9] with all family members possessing Walker A and B motifs that are common to many NTPase enzymes.[10] Broadly, helicases can be grouped into two structural categories based on whether they form oligomeric (mostly hexameric) rings or not (www.rnahelicase.org/index.html). The ring-forming helicases comprise SFs 3 to 6, and are represented by proteins such as the bacterial Rho transcription termination factor and bacteriophage P4.[11]

The largest family, SF2, is formed by the DEAD-box and related DEAH, DExH and DExD RNA helicases. These proteins share at least 12 characteristic sequence motifs, some of which are conserved across the SF2 family, while others vary between the sub groups.[12] DEAD family members are defined at the simplest level by the presence of an Asp-Glu-Ala-Asp (DEAD) motif (and DExD/H variations thereof) within the

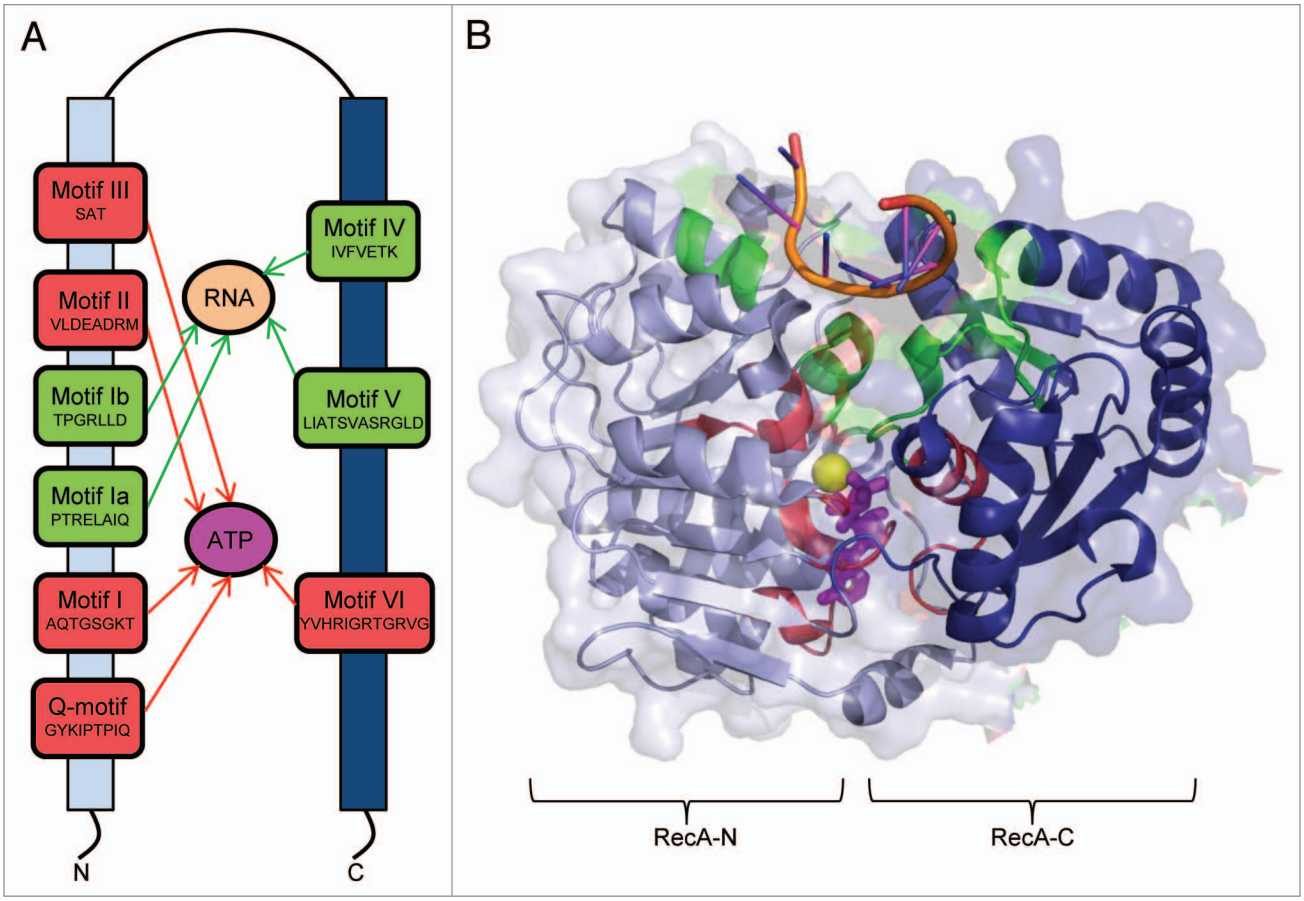


Figure 1. The structure of a representative DEAD box helicase engaged with RNA and ATP, and a schematic of the principal helicase superfamily 2 (SF2) motifs. (**A**) A schematic to show the conserved DEAD box motif and affiliated sequence motifs. The N-terminal RecA domain is shown as a light blue bar and the C-terminal RecA domain is shown as a dark blue bar. Sequence motifs involved with binding to ATP/Mg^{2+} are highlighted in red, and motifs involved with the interaction with RNA are highlighted in green. (**B**) The crystal structure of the Drosophila Vasa helicase in complex with single strand RNA and non-hydrolysable ATP. The secondary structural elements of the helicase are represented in cartoon style, and the protein surface is semi-transparent. The conserved RNA binding motifs are shown in green and the RNA is shown in orange. Motifs involved in ATP binding and hydrolysis are colored red, non-hydrolyzable ATP is shown as magenta sticks, and the magnesium ion is shown as a yellow sphere.

ATPase motif II. The conserved sequence motifs are highlighted in **Figure 1A** from the structure of the representative DEAD-box helicase Vasa of Drosophila. (We will return to this structure, shown in **Fig. 1B**, in greater detail in the next section). The SF2 proteins are ubiquitous to all kingdoms of life, and multiple paralogues are encoded by the genomes of many organisms.[5,13,14] The NTPase activities of these helicases are stimulated by RNA, and while the DEAD-box proteins use ATP as substrate, DEAH proteins have been found to use other NTPs.[15] The DEAD family and its close DExD/H relatives will be the main helicases discussed in this review.

It is becoming increasingly apparent that some DExD/H RNA helicases do not in fact have any unwinding activities per se; instead they perform other functions such as strand annealing,[16] protein displacement[17] and single-strand RNA-displacement.[12] Some RNA helicases nucleate ribonucleoprotein assembly by acting as "clamps" on the RNA substrate.[12] Often helicases function in multicomponent assemblies to aid RNA degradation and processing, and to act as motors for these macromolecular machines.[18]

Structure and Mechanism of the DExD/H-Box Motif Helicases

Helicases of superfamilies 1 and 2 contain a conserved core composed of a repeat of a domain that is a structural homolog of the bacterial enzyme for DNA recombination, RecA. The first structural view of how these domains in DEAD-box helicases engage RNA and ATP was provided by the crystal structure of the Drosophila Vasa in complex with single stranded RNA and non-hydrolyzable ATP (AMP-PNP) (**Fig. 1B**).[19] This and subsequent crystal structures of DExD/H-box proteins, together with supporting biophysical data, indicate that the two RecA-like domains of these enzymes do not have a defined relative orientation in the apo form, and are tethered together by a flexible linker. However, with RNA and ATP binding the RecA domains come together to form an intra-domain cleft that clamps down upon the nucleotide (see the magenta stick representation of the AMP-PNP in **Fig. 1B**). Within this cleft, the ester linkage to the γ-phosphate of the ATP is presented for hydrolytic attack.[19-24]

The orientation of the domains in the ATP-bound state also generates an RNA-binding surface distal to the ATP binding site, and RNA bound here bridges the two RecA-like domains (top of **Fig. 1B**). The RecA domains contact mostly the phosphodiester backbone of the RNA, and interactions are mediated by a structural motif whose evolutionary origin was likely to have been an ancient anion-binding module.[25] The phosphodiester backbone of the RNA has a kinked conformation that is incompatible with duplex geometry, suggesting that RNA binding by DExD/H-box family members might induce distortions that disrupt RNA secondary structure to favor unwinding. The RNA unwinding activity of the DExD/H helicases is typically restricted to less than two full turns, so it seems that the enzymes can only be weakly processive at best. This behavior might be rationalized by the crystal structure of Vasa, which suggests that the site of interaction with the RNA is not extensive. Another interesting feature of the helicases is that they don't appear to unwind with strict polarity (i.e., 5' → 3', or the reverse direction),[26] and this behavior might also be accounted for by the limited extent of the RNA binding surface, which might only permit the helicase to opportunistically grasp exposed single-stranded regions.

The RecA domain interactions with the RNA backbone provide no direct base recognition and therefore do not confer sequence specificity. Nonetheless the different DExD/H-box helicases have an apparent high degree of discriminating specificity for particular RNAs and play well defined roles in vivo. For instance, several yeast DEAD-box proteins have specific functions such as nuclear mRNA splicing or ribosome biogenesis, but these specificities are not recapitulated in vitro with the purified enzymes.[12] This puzzling aspect of helicase specificity is most likely explained in part by the variety of domains fused to the conserved RecA core at either or both termini, which can contribute to recognition of sequence or defined structure in the RNA targets. An example of an appended domain that helps to recognize specific RNA targets is the evolutionarily conserved RRM RNA-binding motif, which confers specific recognition of 23S rRNA in bacteria through interaction with a specific base within the context of a defined RNA fold.[27,28]

Other appended domains, and the RecA core itself, can in some cases help recruit partner proteins that may influence specificity (**Fig. 2**). The auxiliary domains may form compact folds or more variable polypeptide tails that are predicted to be natively unstructured but nonetheless assist RNA-binding and strand displacement.[29-31] These protein-protein interactions, in the context of multi-component assemblies such as the bacterial RNA degradosome, or eukaryotic exon-junction complex (discussed below), may control the activity of the helicases in vivo so that they do not hydrolyse NTPs in futile cycles.[32,33]

Assemblies with Helicases: The Bacterial RNA Degradosome

In many bacterial species, a multi-enzyme assembly known as the RNA degradosome serves as a central machinery of RNA decay and processing. The components of the degradosome cooperate to degrade most RNA transcripts and to process precursors of structured RNAs.[34] In *Escherichia coli* and several other bacteria, the core of the complex is formed by the conserved endoribonuclease RNase E. The protein composition of the degradosome assemblies differs between evolutionarily divergent bacterial species, and some degradosome assemblies include enzymes such as exoribonucleases and metabolic enzymes, but they often include a DEAD-box helicase that directly associates to an endoribonuclease (**Fig. 3**). An RNA helicase has been found to be part of the RNA degradosome in several species, including *E. coli*, *Caulobacter crescentus* and *Pseudoalteromonas haloplanktis* where a DEAD-box protein was shown to interact with RNase E by co-immunoprecipitation.[35,36] In *Pseudomonas syringae* and *Rhodobacter capsulatus* RNA helicases were shown to interact with RNase E by co-sedimentation and co-immunopurification[37,38] and in *Vibrio angustum* a helicase has been shown to bind to RNase E by bacterial two-hybrid analysis and co-immunoprecipitation.[39]

Numerous bacterial species lack RNase E, such as the Gram-positive model organism *Bacillus subtilis*, but in these organisms an RNA degradosome assembly is proposed to be organized through the endoribonuclease RNase Y, which has an entirely different fold and evolutionary ancestry from RNase E.[40-42] A subject of debate is whether an RNase Y based degradosome assembly recruits the exo/endoribonucleases RNase J1/J2, with the interaction between RNase Y and RNase J1/J2 in *B. subtilis* being shown by Strep-protein interaction and bacterial two-hybrid experiments,[40,42] but not replicated in separate yeast two-hybrid experiments[43] and difficult to reconcile with observations that the subcellular localization of RNase Y and RNase J1/J2 differ in vivo (44; note that RNase Y = YmdA and RNase J1 = YkqC in this reference), although it cannot be ruled out that the fluorescent tag used has not interfered with protein-protein interactions. A putative degradosome assembly in *Staphylococcus aureus* based on RNase Y has been identified by two-hybrid analysis,[45] but a direct interaction between RNase Y and RNase J1/J2 has not been shown.

The degradosome of *Escherichia coli*. The vast body of functional data on the RNA-degradosome is derived from studies on the *E. coli* assembly, for which the canonical components and their interaction sites are represented schematically in **Figure 4**. RNase E has a globular N-terminal catalytic domain[46] and a natively disordered C-terminal domain that recruits the other key degradosome proteins: the DEAD-box helicase RhlB, enolase and the exoribonuclease polynucleotide phosphorylase (PNPase). The recognition sites for these partner proteins are small segments of 20 to 40 amino acids found within the C-terminal domain of RNase E, and structures are available that show how these recognize enolase[47] and PNPase.[48] The degradosome is directed to the cytoplasmic membrane by a small amphipathic helix in RNase E[49] and possibly the catalytic domain itself.[50] This localization is likely to affect the organization and activity of the degradosome. The binding site for RhlB has been mapped by limited protease digestion[30] to a segment that encompasses a conserved 13-residue core motif in RNase E that is essential for the interaction with that helicase[36] and which likely interacts with the C-terminal RecA domain of RhlB.[30,51] The interaction between RhlB and

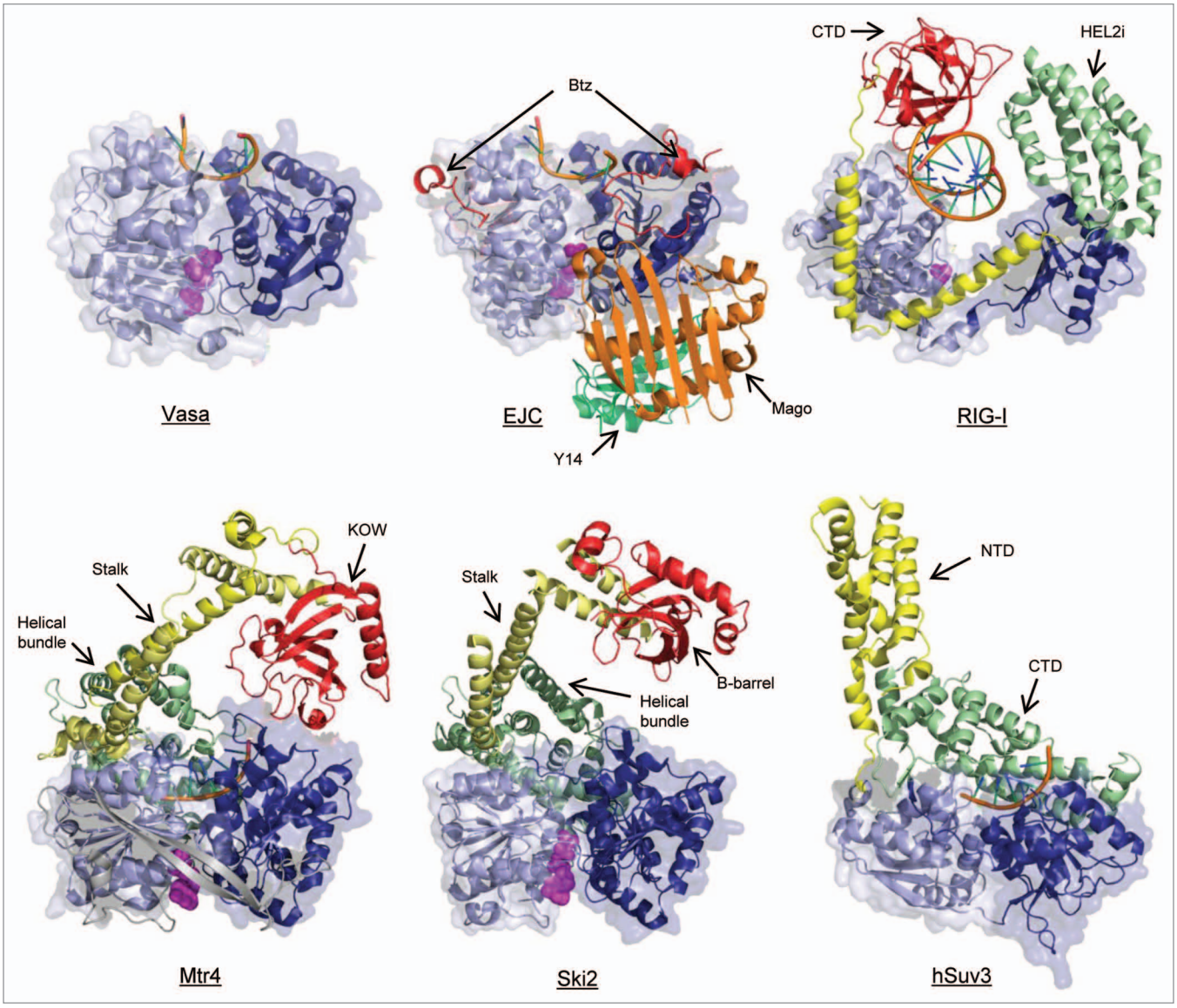


Figure 2. The structure of SF2 family helicases discussed in this review (Vasa, exon-junction complex (EJC), RIG-I, Mtr4, Ski2, hSuv3). For all helicases the two structurally conserved RecA core domains are shown in the same orientation as light blue (N-terminal) and dark blue (C-terminal) cartoons with semi-transparent surfaces. Where present in the crystal structures RNA is shown as an orange cartoon, and ATP/Mg^{2+} is shown as purple spheres. The figure highlights how auxiliary domains or protein partners can interact with the conserved RecA core of SF2 family members. Additional domains appended to or associating with the helicases are shown as cartoon representation and labeled.

RNase E stimulates the helicase activity of RhlB,[30,52] but there are currently no structural data for the complex that help to explain how this boost occurs.

The identification of the RNA helicase RhlB as part of the *E. coli* RNA degradosome, nearly two decades ago, led to the proposal that its role is to aid the processing of RNA substrates within the assembly (ref. 53; see also ref. 54). As RNase E specifically cleaves single stranded substrates, and the channel for RNA in PNPase is only sufficiently wide to accommodate single-stranded RNA (as we will describe further below), it seems reasonable that the unwinding or remodelling activities of RhlB are required to improve the efficiency of mRNA degradation. Indeed, the ATPase activity of RhlB has been shown to be required for the degradation by PNPase of structured RNA transcripts with a repetitive element that forms stable stem-loop structures.[30,55] The binding site for RhlB in RNase E is flanked by RNA-binding domains (**Fig. 4**; termed RBD for "RNA binding domain" and AR2 for "arginine rich region 2"). The cooperation of RhlB with those two domains is crucial to stimulate ATP-dependent RNA degradation. Additionally the arginine rich tail on the C-terminus of RhlB, which is predicted to be structurally disordered, contributes to the interaction between RNA substrates and this helicase.[30]

The potential role of RhlB in aiding the degradation of RNA by the RNases of the degradosome assembly is summarized schematically in **Figure 5**. The model proposes that RNA elements

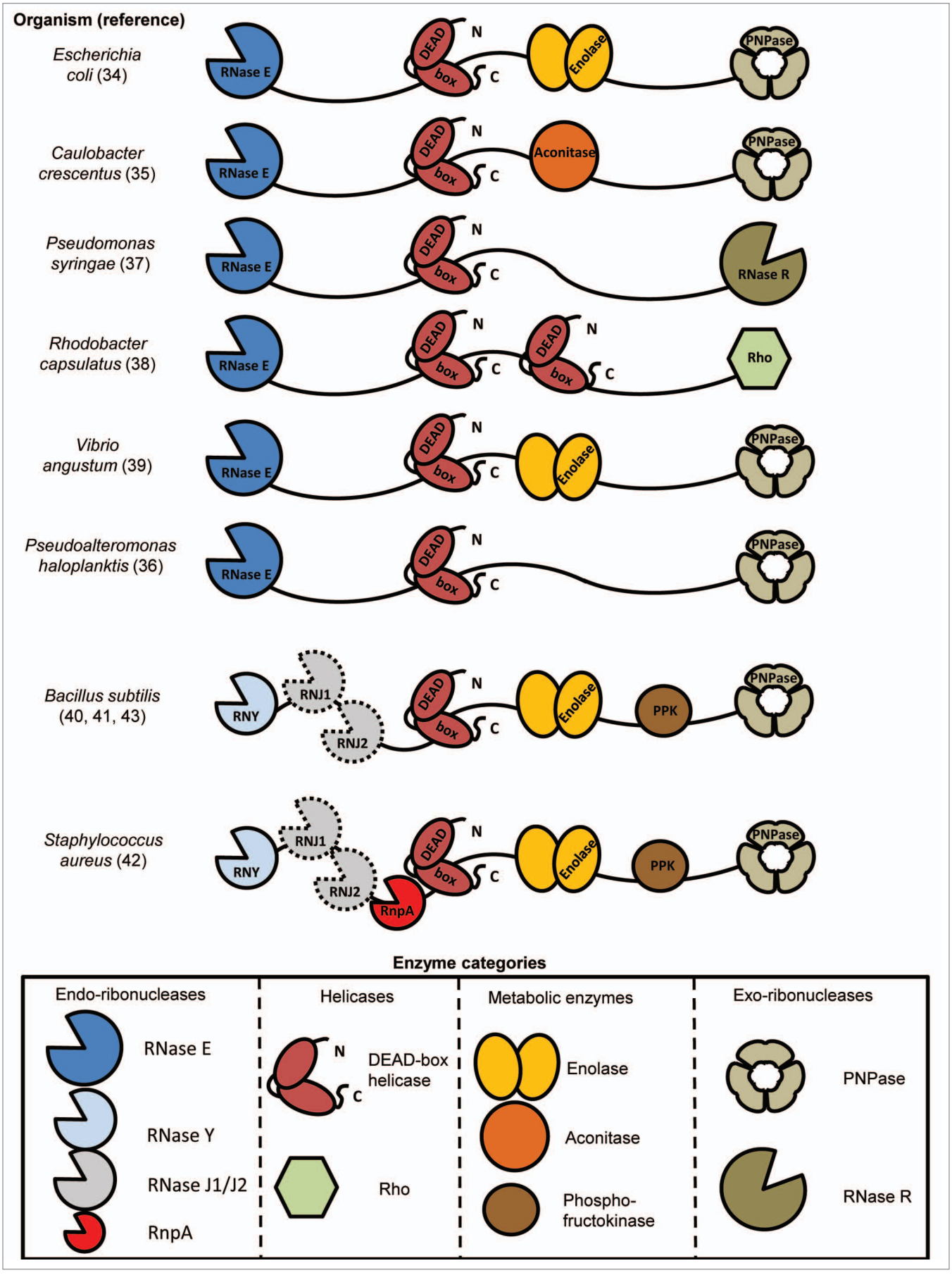


Figure 3 (See opposite page). RNA helicases are recurrent components of diverse bacterial RNA degradosomes. The cartoon provides a schematic of the components of various bacterial RNA degradosomes where RNA helicases are proposed to form part of the complex. A degradosome has also been suggested for *Bacillus subtilis*, but reports conflict on whether RNase J1/J2 form part of this assembly[43] and with data for different subcellular localization of RNase J1 and RNase Y.[44] RNase J1/J2 are bordered with a dashed line to represent the uncertainty of whether these proteins form part of the *B. subtilis* degradosome. The evidence for the *Staphylococcus aureus* degradosome is based on yeast two-hybrid screens and awaits validation.

with regions of secondary structure are bound to the RNA degradosome cooperatively through the combined contributions of the RBD, AR2 and RhlB. Based on studies of other DEAD box helicases, ATP-binding by RhlB may be necessary and sufficient for RNA binding or unwinding of the RNA, but hydrolysis of the ATP is the license for product release for the next cycle of RNA unwinding.[12] Dissociation of phosphate or ADP favors release of the RNA from the helicase, which is required for continuing the cycle. It is unlikely that the process of RNA unwinding will be a futile cycle in which the RNA abruptly snaps back into its native conformation; instead it is likely that the newly unwound RNA substrate will be efficiently cleaved by both RNase E and PNPase.

Under stress conditions, some of the four other DEAD box helicases present in *E. coli* may substitute for RhlB in the degradosome. For instance, CsdA has been shown to associate with RNase E under cold shock conditions.[56] Additionally, RhlE has been shown to be able to interact with RNase E in vitro and this helicase can also aid the degradation of mRNA by PNPase.[57] Swapping helicases perhaps modifies the degradosome activities or substrate preferences.

Helicases as Threading Machines: Cooperation with the Exoribonuclease Polynucleotide Phosphorylase and the RNA Exosome

The degradosome's exoribonuclease PNPase cannot by itself degrade structured RNA, and as mentioned above the DEAD-box helicase RhlB aids the ribonuclease in the degradation of such species.[53] RhlB facilitates formation of single stranded RNA, which PNPase processively cleaves in the 3' to 5' direction by phosphorolysis. PNPase plays many roles in RNA metabolism, including contribution to the decay of bulk RNA, quality control of rRNA, cold shock response, and the stability of small regulatory RNA,[2,58-63] and some of these activities are likely to require partnership with the helicase.

PNPase assembles into a stable homotrimer with a ring-like quaternary architecture that forms a narrow central processing channel through which the substrate threads to reach the three active sites (ref. 64; see **Fig. 4**). Another, more flexible ring is formed over the entrance to the channel by the arrangement of RNA binding S1 and KH domains; these help to capture and guide the RNA into the channel.[65] PNPase is found in all bacterial species examined thus far, and additionally in some eukaryotic organelles including chloroplasts of some plants, where the enzyme is implicated in maintaining phosphate homeostasis[66] and mammalian mitochondria, where PNPase is thought to have a role in transporting RNA into the organelle.[67] Structurally homologous to PNPase are the archaeal and eukaryotic exosomes, which also share the chamber-like quaternary architecture. The structurally conserved organization of RNase PH domains capped with KH and S1 domains in PNPase and exosome complexes is summarized schematically in **Figure 6**.

The eukaryotic exosome was discovered during a search for yeast factors that play roles in RNA processing and degradation.[68,69] Its chamber is formed from nine distinct protein subunits that share homology with the corresponding domains of PNPase. In the eukaryotic nucleus, the exosome is required for the processing of rRNAs, small nuclear RNAs, small nucleolar RNAs, and long non-coding RNAs (lncRNAs).[70] In the cytoplasm, the exosome participates in bulk mRNA turnover and the degradation of mRNAs with a premature stop codon.

While bacterial PNPase and the archaeal exosome harbour phosphorolytic exo-ribonuclease activity, the eukaryotic exosome has lost this catalytic capacity. Instead, ribonuclease activity is conferred by additional protein components that interact with the nine-subunit exosome core. In both the nuclear and the cytoplasmic forms, the exosome's endo- and exo-ribonuclease activities are largely attributable to the enzyme Rrp44 that binds to one face of the exo-9 channel (namely, the opposite face to which the S1 and KH like domains associate; see **Fig. 7B**). Additionally, in the nucleus Rrp6, a homolog of bacterial distributive exo-ribonuclease RNase D, associates with the exosome. Recently, it has been shown that despite lacking ribonuclease activity, the exo-9 channel is still the entry point for RNA substrate, and that the exo-9 channel itself modulates the RNase activities of both Rrp44 and Rrp6.[71]

Predicted to occur in numerous species,[72] the archaeal exosome, like its eukaryotic counterpart, has a nine subunit chamber-like architecture. The archaeal assembly is constructed of three hetero-dimers of Rrp41-Rrp42 and a trimeric cap of Rrp4 or Csl4.[73] Both Rrp4 and Csl4 have an N-terminal all β-domain and a central S1-RNA binding domain. Additionally, Rrp4 has a C-terminal KH domain, while Csl4 has a C-terminal zinc-ribbon domain.[74] Although the majority of studies on the archaeal exosome have focused on assemblies with a homotrimeric Rrp4 or Csl4 cap, it has recently been shown that hetero-trimeric caps are found in vivo, and it has been suggested that such caps endow the exosomes with capacity to bind different RNA substrates.[75]

In both PNPase and the exosome, the entrance to the central channel narrows to form an aperture just wide enough for single-stranded RNA substrate to feed through (**Fig. 7**).[64,76,77] The narrow shape suggests a necessity for structured RNA substrates to be un-wound prior to processing by the degradative machinery. As discussed in the previous section, PNPase as part of the RNA degradosome is in proximity to the DEAD-box helicase RhlB. Like the protein complexes formed by PNPase, both the cytoplasmic and nuclear exosomes are known to associate with peripheral proteins assemblies containing RNA helicases. These exosome-associated complexes act as adaptors to bring RNAs to the exosome for processing or degradation.

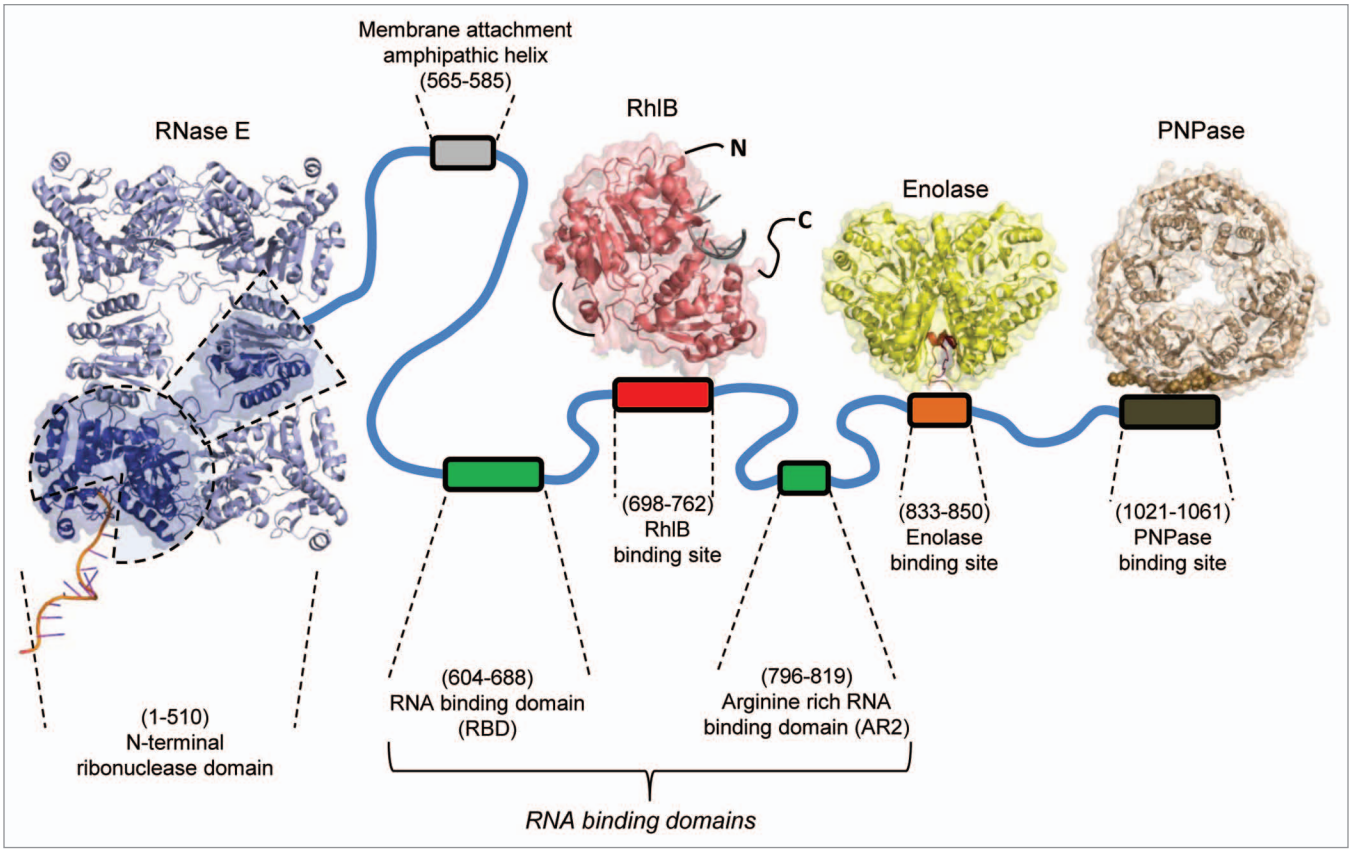
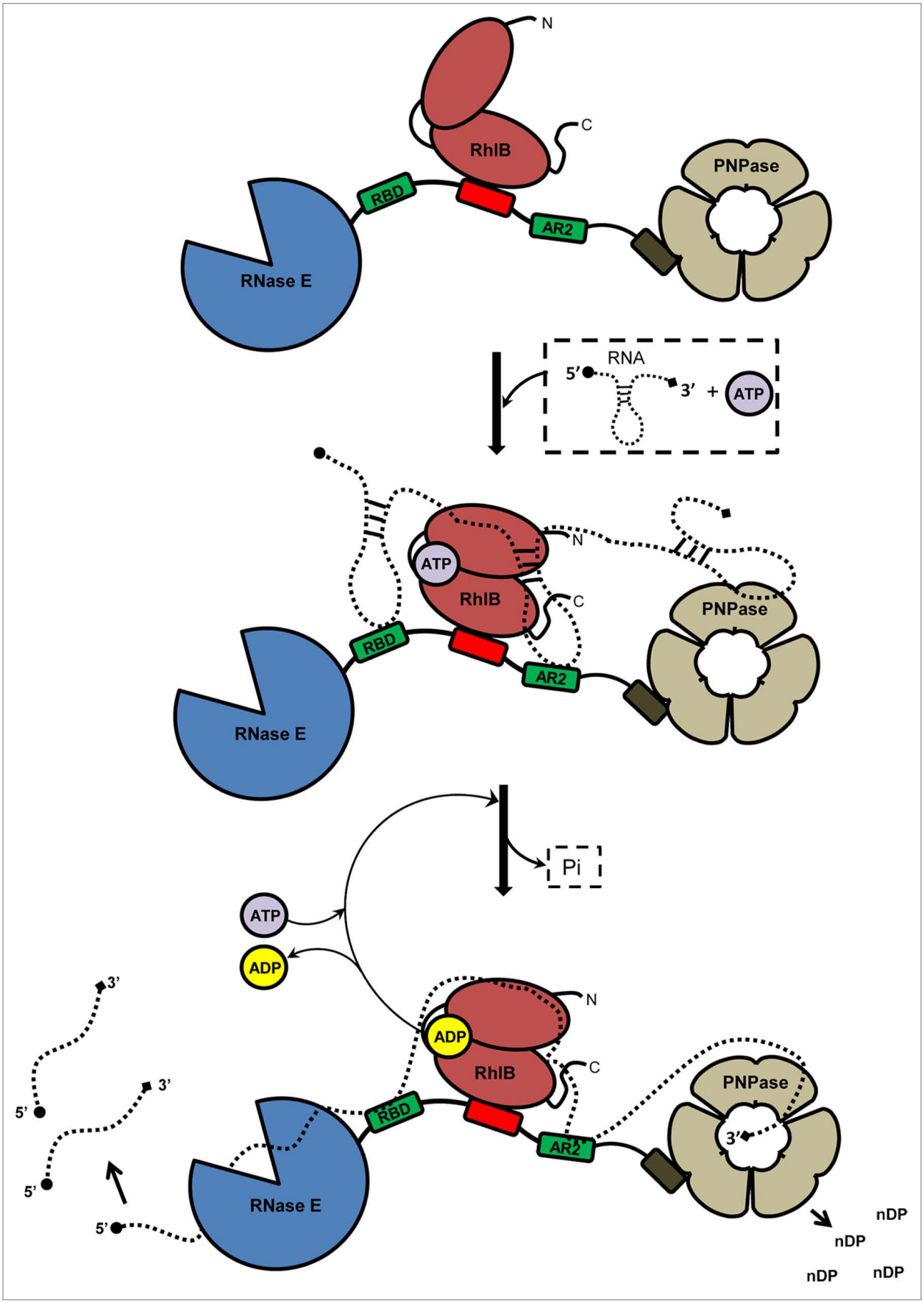


Figure 4. Domain structure of RNase E with the binding sites for RNA, helicase, enolase and PNPase. One putative protomer of the degradosome assembly is shown, and four such protomers might form the core of the canonical degradosome.[34] The disordered C-terminal domain of RNase E is depicted as the thick, blue curvy line. The recognition sites for monomeric RhlB, dimeric Enolase and trimeric PNPase are small segments of 20 to 40 amino acids represented as solid blocks on the C-terminus or RNase E. The catalytic N-terminal domain of RNase E is a tetramer (blue), and one protomer is highlighted with a dotted outline. The large domain harbours the catalytic site and is within the dashed circle, with the missing wedge engaging the RNA substrate; the small domain that organizes the dimer-of-dimer interfaces is outlined with the trapezoid. PDB codes for the structrures presented: RNase E, 2BX2; Vasa, the Drosophila homolog of *E. coli* RhlB, 2DB3; enolase, 3H8A; PNPase, 3GME.

In the cytoplasm the exosome interacts with the Ski complex,[78] comprising the proteins Ski2 (helicase), Ski3 (tetratrico-peptide repeat protein -TPR) and Ski8 (WD40 protein). The Ski complex physically interacts with the exosome via the protein Ski7.[79] In the nucleus, the exosome interacts with the TRAMP complex (*Trf*4/*Air*2/*Mtr*4p *P*olyadenylation complex) comprising poly(A) polymerase (Trf4/Trf5), a zinc finger protein (Air1/Air2), and the DExH helicase Mtr4. This assembly contributes to RNA quality control by recognizing aberrant transcripts, tagging them with polyA tails, and directing them to the exosome. The helicase Mtr4 is required for nearly all of the functions of the exosome in the yeast nucleus,[80] including ribosome biogenesis.[81] The human homolog of Mtr4 is a component of two distinct complexes that interact with the exosome.[82] The potential role of helicases to unwind structured RNA substrates prior to channelling into the narrow processing channels of PNPase and the exosome is summarized schematically in **Figure 7**.

The crystal structures of the helicase components of the cytoplasmic Ski complex (Ski2) and the nuclear TRAMP complex (Mtr4) have been recently solved.[83-85] Both proteins have a classical DEAD-box helicase core, but additionally both proteins have a structurally similar insert within a helical bundle domain appended to the helicase core (**Fig. 2**). The insertion forms an elongated structure protruding from the helicase core, with a β-barrel domain sitting atop two pairs of anti-parallel α-helices, and it has a role in RNA binding, but not formation of the Ski/TRAMP complexes. In addition, the barrel like KOW domain appended to the elongated helical structure in Mtr4 has been shown to be important for activation of exosome function in vitro.[31]

Figure 5 (See opposite page). A model for the interplay of RNA helicases and ribonucleases in the RNA degradosome. (Top) The degradosome (enolase is not depicted for clarity) primed for RNA substrate capture. (Middle) Structured RNA is captured by the RNA binding elements of RNase E (RBD and AR2) and the C-terminal arginine rich extension of RhlB. ATP binding and hydrolysis promotes cycles of RNA unwinding and remodelling the RNA interactions with RBD and AR2 by RhlB. (Bottom) As RNA unwinding by RhlB proceeds the RNA substrate can be efficiently cleaved by RNase E and/or PNPase. The model is based on inferences from experimental data.[12,32]



There are other links between RNA helicases and components of exosome/PNPase like machineries. In human mitochondria the partner of PNPase is likely to be DExH/D helicase hSuv3.[86,87] The crystal structure of the human helicase hSuv3p, thought to interact with human PNPase has also recently been solved and is shown in **Figure 2**.[88] The hSuv3 protein has a similar duplicated RecA-like core with additional strands and auxiliary α-helical domains at both termini, and the C-terminal domain helps to cradle the single stranded RNA on the surface of the RecA-like domains. Much like the Ski2 and Mtr4 helicases, a prominent feature of this structure is an extended helical protrusion emanating from the N-terminal RecA-like domain. Unlike the exosome associating helicases this protrusion is not capped with a barrel like domain, but the conservation of the helical protrusion may suggest a role for this domain in the association with PNPase.

In the yeast *Saccharomyces cerevisiae* the nuclear exosome component Rrp6 has recently been shown to be genetically linked to the DEAD-box helicase Dbp2, and a double deletion of these two genes results in a synthetic lethal phenotype.[89] Dbp2 is the yeast ortholog of the mammalian helicase p68, which has well defined roles in ribosome biogenesis, and transcriptional and co-transcriptional processes with RNA polymerase II.[90] Also, the *S. cerevisiae* Suv3 homolog forms a degradosome complex with the 3' → 5' exoribonuclease Dss1 (related to bacterial RNase II).[91,92] The mitochondria of *Trypanosoma bruceix* also has a degradosome like assembly, with an RNase II-related exoribonuclease associated with an RNA helicase.[93]

Helicases in Eukaryotic mRNA Degradation Pathways

In eukaryotes, one mRNA decay pathway involves deadenylation of the 3' end of the transcript, followed by de-protection of the 5' end of the RNA by removing the 7-methylguanosine cap, which licenses 5' → 3' exoribonuclease degradation. In this pathway, the RNA helicase Dhh1/Rck/DDX6 is a cofactor that promotes the decapping process, and recent evidence suggests that this results from the action of the helicase to slow ribosome transit rates on the transcript, making them more vulnerable to decapping.[94] The Dhh1 homolog in trypanosomes affects post-transcriptional regulation of many developmentally important transcripts in an ATPase dependent manner,[95] and the mechanism, hypothetically, might involve ribosome transit. In an interesting parallel, the RhlB helicase of the degradosome has been found to help recruit the degradosome to polysomes in *E. coli*, and it is proposed that this interaction may help sRNA mediated silencing of target transcripts.[96]

The human DEAH helicase RHAU (DHX36, G4R1) is involved in AU-rich element mediated mRNA decay.[97] The AU-rich element is a degradation signal in the 3' untranslated region and is found in many unstable mRNAs. RHAU interacts with deadenylases and the human exosome, and it enhances mRNA decay in an ATP-dependent fashion. Curiously, this helicase can resolve RNA G-quadruplexes,[98] which are unusually stable nucleic acid structures; it therefore seems likely that the helices must employ a special unwinding mechanism to cope with such a substrate.

Helicase Activity in Surveillance: The Case of Non-Sense Mediated Decay

The intricate machinery of non-sense mediated decay (NMD) illustrates the contributions of DExD/H helicases to RNA surveillance and to their actions in nucleating and remodelling ribonucleoprotein complexes.

The NMD decay pathway was originally discovered as an evolutionary conserved eukaryotic mechanism to detect and degrade mRNAs with a premature stop codon. Subsequently, NMD has been shown to be responsible for modulating the steady-state level of mRNAs generally, and it triggers mRNA decay when translation is inefficient. NMD is promoted by long 3' untranslated regions or when a stop codon is present at least 50–54 nucleotides upstream of an exon-to-exon splice junction in metazoa. The components of the NMD machinery include kinases, an endonuclease (Smg6) and the superfamily 1 helicase Upf1. The helicase Upf1 is the central and required component of the NMD machinery. An mRNA tagged by the NMD machinery is cleaved by the endonuclease Smg6, followed by disassembly of the NMD complex by Upf1 in an ATP-dependent process.[99]

NMD can be triggered by the inappropriate location of the exon-junction complex (EJC), which forms at exon-exon splice junctions and includes a DEAD-box helicase. The EJC is not strictly required for NMD, but enhances NMD when positioned downstream of the terminating ribosome, and it represents an evolutionary tinkering of the NMD pathway. The EJC comprises four proteins [MAGO, Y14, Barentz and the DEAD-box Helicase eIF4AIII (eukaryotic initiation factor 4AIII)] (**Fig. 2**). The eIF4AIII engages mRNA, and hydrolysis of ATP by the helicase is controlled by interactions with partner proteins.[33] In effect, the helicase acts as an ATP-dependent clamp that nucleates assembly of a ribonucleoprotein complex.[12] The EJC forms a complex with the proteins Upf2 and Upf3 on the mRNA. A model for surveillance complex formation during non-sense mediated decay proposes the formation of a complex of the EJC with the UPF assembly.[100] Premature translation termination recruits to the ribosome Upf1, a SF1 RNA helicase. If recruitment occurs at a spacing of less than 30 nucleotides from a downstream Upf2-Upf3-EJC complex, then a kinase is recruited that phosphorylates the helicase, triggering binding of Upf2-Upf3-EJC complex. The mRNP is remodelled by the activity of Upf1, which unwinds RNA in a cofactor dependent fashion and is followed by endonucleolytic cleavage of the mRNA by the endonuclease Smg6. Allosteric RNA clasping domains of Upf1 can open and shut to engage and disengage the RNA substrate and thereby control the helicase activity. Upf2 acts as the activating switch for the process.[101]

Helicases in Riboregulation

RNA interference (RNAi) is the mechanism by which short double stranded RNAs trigger silencing of cognate genes.[102] The process involves cleavage of the short duplex by the RNase III enzyme Dicer to generate small RNAs that are 21–23 nucleotides in length.[103] These RNA species are incorporated into the multi-enzyme RNA induced silencing complexes (RISC) where

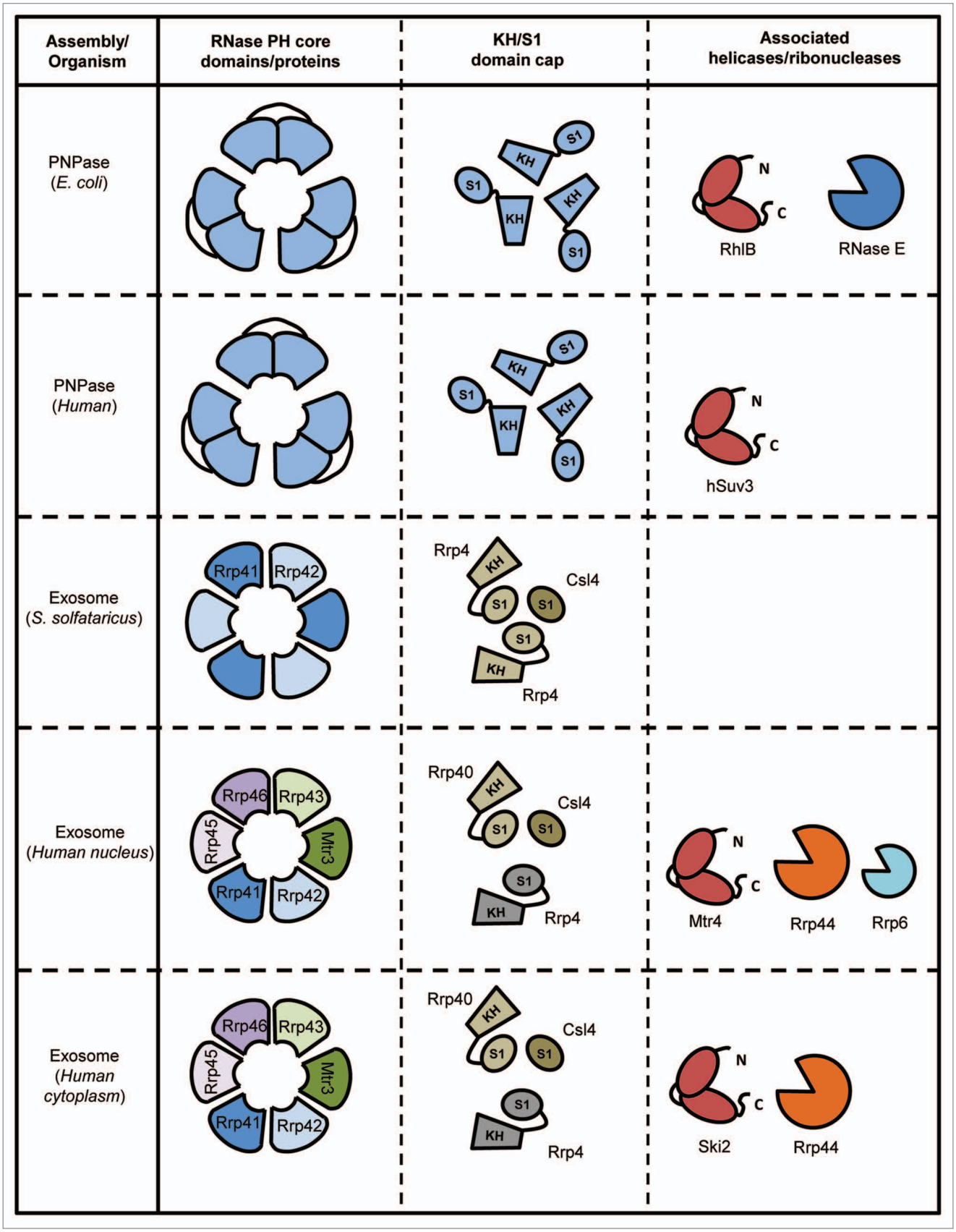


Figure 6. A schematic representation of the domain architecture of PNPase and exosome ring-like assemblies. Fused domains or separate proteins forming the ring-like core are shown in the left column, the arrangement of S1 and KH domain containing proteins forming the "cap" is shown the second column. Additional RNases and/or helicases known to associate with the assembly are shown in the final column.

Table 1. A selection of representative DExD/H RNA helicases associated with RNA degradation

Helicase	Roles	Reference
Upf1	Non-sense mediated decay in yeast and metazoans.	99–101
eIF4AIII	Component of the EJC, involved in non-sense mediated decay in vertebrates; see **Figure 2.**	33
Mtr4	Component of the adaptor for feeding RNA to the nuclear exosome; see **Figures 2, 6 and 7**.	31, 80–84
Ski2	Component of the adaptor for feeding RNA to the cytoplasmic exosome; see **Figures 2 and 6**.	78, 79, 85
hSuv3	Potential adaptor for human PNPase; see **Figures 2 and 6**.	86–88
Dhh1/Rck/DDX6	Activation of transcript decapping in metazoans, acting as a general translation repressor.	94
RHAU	AU-rich element mediated mRNA decay in metazoans; may unwind guanine tetraplex RNA structures.	97, 98
RhlB	Component of the degradosome of gamma-proteobacteria; see **Figures 3, 4, 5 and 7.**	34–39
CshA	Component of the degradosome of gram-positive bacteria; see **Figure 3**	40–42

they guide the gene silencing machinery to target transcripts. The human Dicer protein contains an N-terminal helicase domain that belongs to the RIG-I family of DEAD-box helicases (**Fig. 2**), that includes pattern recognition receptor of the innate immune system (104; see also www.rnahelicase.org/rig.htm). Several functions have been proposed for this helicase domain, including substrate binding in humans,[105] recognition of precursors of microRNA in *Drosophila melanogaster*,[106] and catalysis of translocation on long double stranded RNA substrates in *C. elegans* and *D. melanogaster*.[107,108] Despite the different roles suggested for Dicer helicase in diverse species, the overall domain architecture of Dicer is conserved, and it has been proposed that binding of RNA substrate by Dicer results in a marked conformational change where the helicase clamps down onto the double stranded RNA in a manner similar to RIG-I[109] (as shown in the top right panel, **Fig. 2**).

Riboregulation is also an important aspect of gene regulation in bacteria, especially in their multifaceted responses to stress. This mode of regulation is mediated by small non-coding RNAs of 50 to 300 nucleotides that share imperfect sequence complementarity with the target RNAs. Although there is no definitive evidence that helicases are involved in this process, indirect evidence suggests that they could play a role. In this regard, it is interesting to note that truncations of RNase E that lose the degradosome scaffolding domain, including the helicase binding site, are deficient for some small RNA mediated responses. Deletion of this portion of *Salmonella* RNase E (residues 702 to 1061) weakens the repression of the mRNA encoding the outer membrane protein ompD by the sRNA MicC.[110] Other studies show that similar deletions decrease the degradation rate of sRNAs,[62] and diminish the effectiveness for target gene silencing.[111,112] Some portion of the C-terminal domain of RNase E may be important for mediating the repression effects of sRNAs, and one model proposes that these domains may help to recruit the complex of sRNA with the RNA chaperone Hfq.[113,114] Binding data suggest that RNA can bridge between Hfq and the RNA-binding domains that are located in the C-terminal half of RNase E.[115] The interaction of these RNA-binding domains may help to present the seed region of sRNA, and also assist the delivery of the target to the catalytic domain of RNase E for cleavage. We envisage a mechanism for this action in which sRNA activates the catalytic domain of RNase E, while other components of the degradosome assembly including RhlB might interact with the target site to aid presentation to the active site.[116] As mentioned above, RhlB has been found to help recruit the degradosome to polysomes in *E. coli*, where it may help sRNA mediated silencing of target transcripts.[96]

Summary and Perspective

RNA helicases have been shown to contribute to RNA decay and processing in diverse biological systems. Representative examples of the interplay between RNA helicases and RNA processing enzymes have been discussed here, and are summarized in **Table 1**. We have described how the RhlB helicase of the *E. coli* degradosome contributes to the degradation of structured RNA intermediates by unwinding and/or remodelling, so that such species become suitable single-stranded substrates for RNase E and PNPase. Similarly, a helicase may play a similar role to favor substrate feeding to the eukaryotic exosome (**Fig. 7**).

RNase E of the *E. coli* degradosome is also involved in the processing of precursors of structured RNAs, such as tRNAs, rRNA and 6S RNA. The question naturally arises as to why the latter structured substrates are processed, while the former are degraded. In these cases, and more generally in cases where a helicase participates in quality control checks of RNA, the mechanism might involve a test of the extent of exposed single-stranded region. For instance, if the substrate has a footprint of fewer than 8 nucleotides on the helicase, then it may lead to displacement, while those with a greater footprint cannot be displaced.[16,117,118] Perhaps instead they might be unfolded elsewhere—the unfolded structure for instance would have such footprints on the RBD and AR2 sites in the context of the *E. coli* RNA degradosome (**Fig. 4**).

It seems likely that the fold of the RNA is a likely determinant of the pathway it will follow in the presence of helicase. One striking feature of RNA folds is the complexity of its three dimensional structure. In terms of intricacy and compact nature, the three-dimensional structures of folded RNAs rival those of proteins. RNA folds are stabilized by numerous types of tertiary interactions, and curiously even random sequence RNA seems to have greater propensity for forming compact objects compared with proteins. The fold of an RNA can be strongly influenced by its interactions with proteins and from base-pairing interactions with trans-acting RNA species, and this presents some knotty puzzles for understanding how they might be recognized with specificity. For instance, how are folded RNAs recognized and

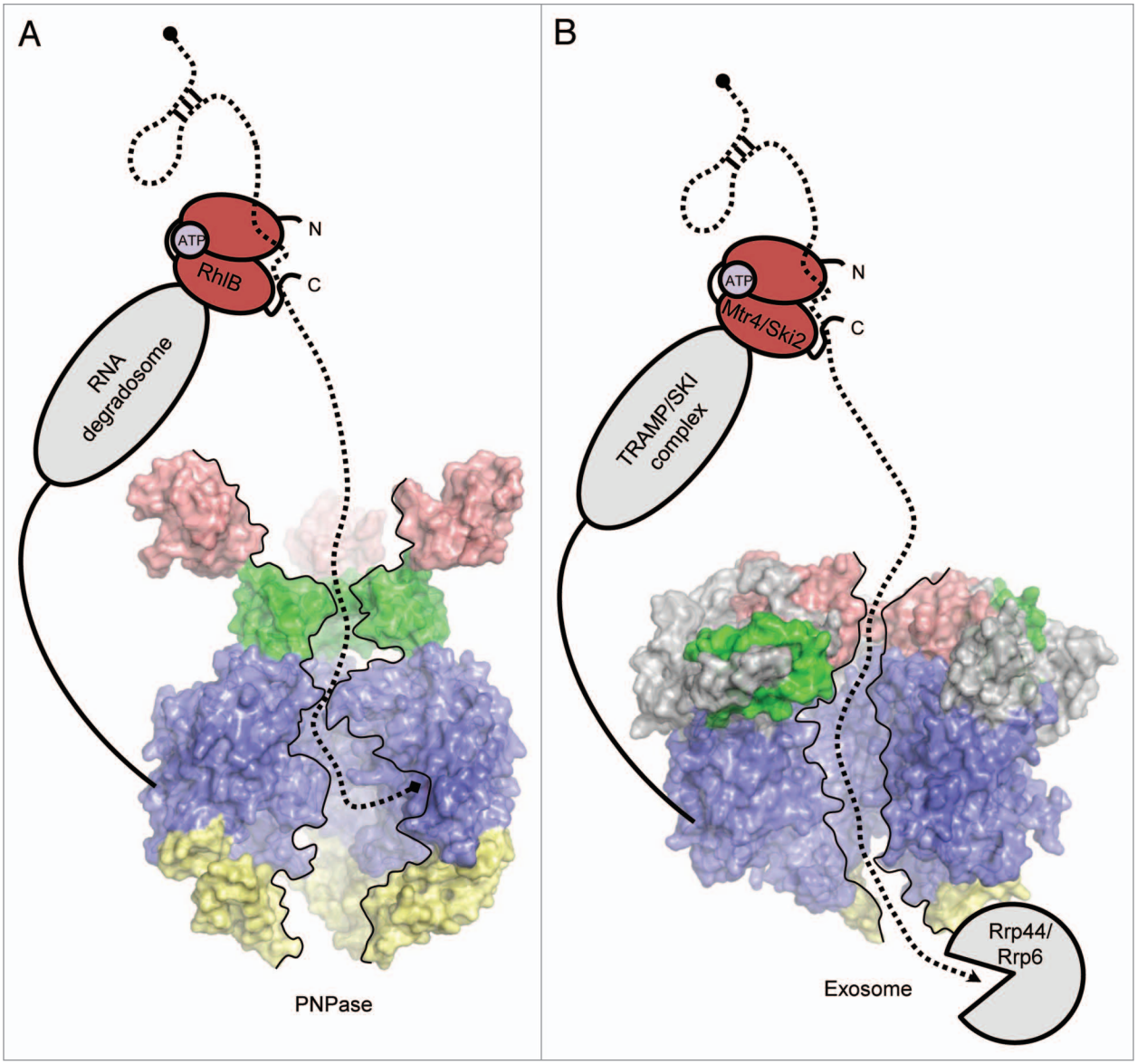


Figure 7. The potential cooperation of helicases with processing chambers. The helicase in the RNA degradosome (RhlB) and the TRAMP (***T***rf4/***A***ir2/Mtr4p Polyadenylation) complex,[70] may help to generate single stranded RNA that can be fed into the narrow central channels of PNPase and the exosome, respectively. The crystal structures are shown for the trimeric polynucleotide phosphorylase of *C. crescentus* [PDB 4AIM (**A**)] and the hetero-hexameric eukaryotic exosome [PDB 2NN6 (**B**)]. The core of the two enzymes, shown in blue, is formed by RNase PH-like subunit that forms the central channel. The KH domains are shown in green and the S1 domains are in salmon. The path of the single stranded RNA is inferred from structural data for the bacterial PNPase[65] and the exosome.[73,75]

distinguished from improperly folded species? Moreover, targets of non-coding RNAs are recognized through cognate base-pairing, but these can be influenced by tertiary interactions or association with protein partners. The subtlety of the recognition has been highlighted with the recent finding that a small RNA can lose its capacity to regulate translation by the substitution of a single canonical A:U base pair with a G:U wobble base pair within a duplex region of 12 complementary base pairs.[119] This corresponds to small discrimination energy that cannot account for the differences in activity of the cognate and near-cognate sRNAs. We suggest that a similar situation is likely to occur in the discrimination of RNAs for quality control mechanisms. One solution to this puzzle of specificity is for recognition to occur out of equilibrium, with energy dependent proofreading steps.[80] This would bear some analogy to the processes of kinetic proofreading that is at play for translational fidelity.[120] RNA helicases are well suited for this role, and may drive systems out of equilibrium, so that the RNA substrates of the helicases may be directed toward degradative pathways if they fail fidelity tests. This hypothesis awaits experimental validation.

The conservation of DExD/H-box helicases in assemblies from phylogenetically diverse lineages implicates a biological importance of their contributions to these machineries. It is becoming clear that regulation of genetic information in organisms from

all phyla involves complex, densely interwoven networks of transcription factors as well as riboregulators. In the course of evolution of such networks, there must have arisen a demand for precision and accuracy in recognition and action of nucleic acid mediated processes, including the specific processing and degradation of RNAs. The DExD/H-box helicases may have met this need in numerous ways. The fusion of auxiliary protein domains to the N or C terminus of the RecA core, and their recruitment in complex RNA processing and degradative machineries may have conferred these ancient proteins with increased specificity during the evolution of regulatory complexity. As we move to understand the role of helicases in complex machinery, there will likely be a rich harvest of insight into how biological systems achieve exquisite and rapid responses in the face of diverse and varying stimuli.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors are supported by the Wellcome Trust. We thank Martyn Symmons, Gadi Schuster, A.J. Carpousis, Mark Carrington and Patrick Linder for invaluable comments and stimulating discussions. We thank the anonymous reviewers for helpful comments. Resources: the RNA helicase database (www.rnahelicase.org/index.html).

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