Critical Reviews and Perspectives

Prp43/DHX15 exemplify RNA helicase multifunctionality in the gene expression network

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ABSTRACT

Dynamic regulation of RNA folding and structure is critical for the biogenesis and function of RNAs and ribonucleoprotein (RNP) complexes. Through their nucleotide triphosphate-dependent remodelling functions, RNA helicases are key modulators of RNA/RNP structure. While some RNA helicases are dedicated to a specific target RNA, others are multifunctional and engage numerous substrate RNAs in different aspects of RNA metabolism. The discovery of such multitasking RNA helicases raises the intriguing question of how these enzymes can act on diverse RNAs but also maintain specificity for their particular targets within the RNA-dense cellular environment. Furthermore, the identification of RNA helicases that sit at the nexus between different aspects of RNA metabolism raises the possibility that they mediate cross-regulation of different cellular processes. Prominent and extensively characterized multifunctional DEAH/RHA-box RNA helicases are DHX15 and its *Saccharomyces cerevisiae* (yeast) homologue Prp43. Due to their central roles in key cellular processes, these enzymes have also served as prototypes for mechanistic studies elucidating the mode of action of this type of enzyme. Here, we summarize the current knowledge on the structure, regulation and cellular functions of Prp43/DHX15, and discuss the general concept and implications of RNA helicase multifunctionality.

INTRODUCTION

Gene expression is a fundamental cellular process involving diverse coding and noncoding RNAs. Messenger RNAs (mRNAs) are transcribed and processed copies of genes that are decoded on ribosomes for synthesis of the cellular proteome. In contrast, noncoding RNAs fulfil regulatory functions by base pairing with target RNAs or serving as structural scaffolds and/or catalytic components within ribonucleoprotein (RNP) machineries, such as ribosomes or spliceosomes. RNA has remarkable capabilities to form complex secondary, tertiary and quaternary structures (1), and attaining correct structure is critical for its stability, assembly into RNPs and function. However, accurate RNA folding represents a major challenge due to the high potential for misfolding (2). Important strategies for ensuring production of functional RNAs/RNPs are co-transcriptional folding/RNP assembly, chaperoning by RNAs and protein, and resolving aberrantly folded RNAs allowing them to re-fold correctly [see e.g. (3–12)]. Beyond this, RNAs and RNPs involved in gene expression are very dynamic; some RNAs undergo significant structural transitions during their biogenesis or function, and RNP complexes, such as the spliceosome, require disassembly after fulfilling their functions (13). RNA helicases have emerged as important chaperones of RNA folding and major regulators of RNA/RNP remodelling events (5,14–16).

RNA helicases are a large family of proteins that employ their nucleotide triphosphate (NTP)-dependent activities to remodel RNA structures and RNP complexes in many different cellular processes (5,14–16). More than 70 RNA helicases have been identified so far in human cells, most of which are DExD- or DEAH/RHA-box proteins (17). While initially characterized as RNA duplex

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unwinders, the repertoire of functions attributed to RNA helicase family members also extends to include roles in annealing RNA strands, nucleating RNP assembly by RNA clamping and displacing RNA-binding proteins from their substrates (14,18–21). Over the years, structural studies and biochemical approaches employed on individual RNA helicases have provided significant insights into the different modes of action used by these enzymes to fulfil their cellular functions [see e.g. (22–26)]. Complementary functional studies have identified RNA helicases acting in many different aspects of gene expression [reviewed in (5,16)].

MULTIFUNCTIONAL RNA HELICASES: DEFINING CHARACTERISTICS AND CONCEPTS

Intriguingly, although many RNA helicases appear to be dedicated to a specific substrate RNA, others have been revealed as multifunctional. The term 'multifunctional' can describe RNA helicases that have alternative molecular functions, for example RNA helicases that function as unwindases and RNA clamps in different contexts. Alternatively, 'multifunctional' can refer to RNA helicases that have only one mode of action but engage a range of different RNA substrates in the context of more than one cellular pathway. In addition, multifunctionality may also encompass both catalytic and noncatalytic functions. The discovery of such multitasking RNA helicases raises key questions. On the mechanistic level, it becomes necessary to understand how enzymes can act on diverse RNAs but also specifically recognize their target RNAs within the complex cellular milieux. Also, the question arises how the functions of RNA helicases with multiple modes of action, e.g. unwinding and ATPase-independent RNA binding, are coordinated and regulated. On the cellular level, the identification of RNA helicases that act in more than one aspect of RNA metabolism raises the possibility that they serve as hubs mediating cross-regulation of different cellular processes.

Prominent and well-characterized examples of RNA helicases acting in multiple cellular pathways are the DEAH/RHA-box proteins *Saccharomyces cerevisiae* (yeast) Prp43 and its human homologue DHX15. Due to their central roles in key cellular processes, these enzymes have served as prototypes for mechanistic studies of this class of enzyme (24,27–31). Through their functions in pre-mRNA splicing, ribosome biogenesis and regulating cap-proximal mRNA methylation as well as a potentially ATPase-independent role in host defence against viral infection, Prp43/DHX15 represent key examples of multifunctional RNA helicases. They therefore serve as an ideal basis from which to conceptually understand the multifunctionality of RNA helicases and how they could link different cellular processes.

Prp43/DHX15 STRUCTURE AND MODE OF ACTION

Members of the DEAH/RHA-box family of RNA helicases, including yeast Prp43 and human DHX15, are characterized by the presence of tandem RecA-like domains connected via a flexible linker and a long C-terminal extension containing structured winged-helix (WH), helical-bundle (HB) and oligosaccharide-binding (OB) domains (Figure 1A) (27,30,31). The first insight into the architecture of DEAH/RHA-box RNA helicases was obtained with a crystal structure of yeast Prp43 (30) and subsequent crystallizations in different functional states revealed the structural basis of how this type of enzyme coordinates NTP hydrolysis with unwinding activity and substrate release (Figure 1B) (24,27,29,31–33). The two RecA-like domains containing conserved sequence motifs involved in RNA binding and NTP hydrolysis form a catalytic cleft, and the C-terminal domains make essential contacts with the RecA-like domains regulating their conformation and promoting formation of an RNA-binding channel (29).

In contrast to their DExD-box counterparts that act locally, it is proposed that DEAH/RHA-box helicases possess processive 3′–5′ unwinding activity (20,34). In an NTP-bound state, the base of the NTP is stacked between conserved arginine and phenylalanine residues (R-/F-motif) in the two RecA domains, maintaining the helicase in an open, autoinhibited state (24,32). In this conformation, an RNA binding groove is formed between a conserved loop (hook turn) in the RecA1 domain and a β-hairpin (hook loop) in RecA2, allowing binding to RNA substrates with 3′ single-stranded (ss) overhangs (Figure 1C). Four nucleotides of the RNA substrate can be accommodated within the groove, and upon RNA loading, the helicase switches to a 'closed' conformation, an essential event that activates the helicase by triggering NTP hydrolysis (Figure 1D) (27). Formation of the closed conformation disrupts interactions of the stacked NTP by extruding the γ-phosphate towards a nucleophilic attack by a proximal water molecule bound to a conserved glutamic acid residue. Release of the γ-phosphate triggers extensive rearrangement of the RecA-like domains resulting in translocation of the more flexible hook loop (RecA2) towards the 5′ end of the RNA enabling an additional nucleotide to be accommodated in the groove. Thus, repeated cycles of NTP hydrolysis and binding are directly coupled to translocation relative to the RNA substrate in one nucleotide step per NTP molecule hydrolysed (Figure 1D). Duplex unwinding can occur by a strand-displacement mechanism as the RNA channel only accommodates ssRNA.

Such a mode of action renders DEAH/RHA-box helicases ideal enzymes for disrupting long stretches of base pairing and displacing RNA-bound proteins. However, beyond this, when anchored within an RNP complex, the 'translocation' of Prp43 (and other DEAH/RHA helicases) relative to the substrate can induce tension in the RNA, thus destabilizing upstream base-paired elements (35,36). In this way, Prp43 can employ its unwinding activity as a winch, facilitating resolution of dense RNP structures at a distance (37).

MECHANISMS OF Prp43/DHX15 REGULATION

The structural studies on Prp43/DHX15 provide insights into how these enzymes interact with their RNA substrates. This is particularly important for understanding how such multifunctional enzymes can target a broad, but
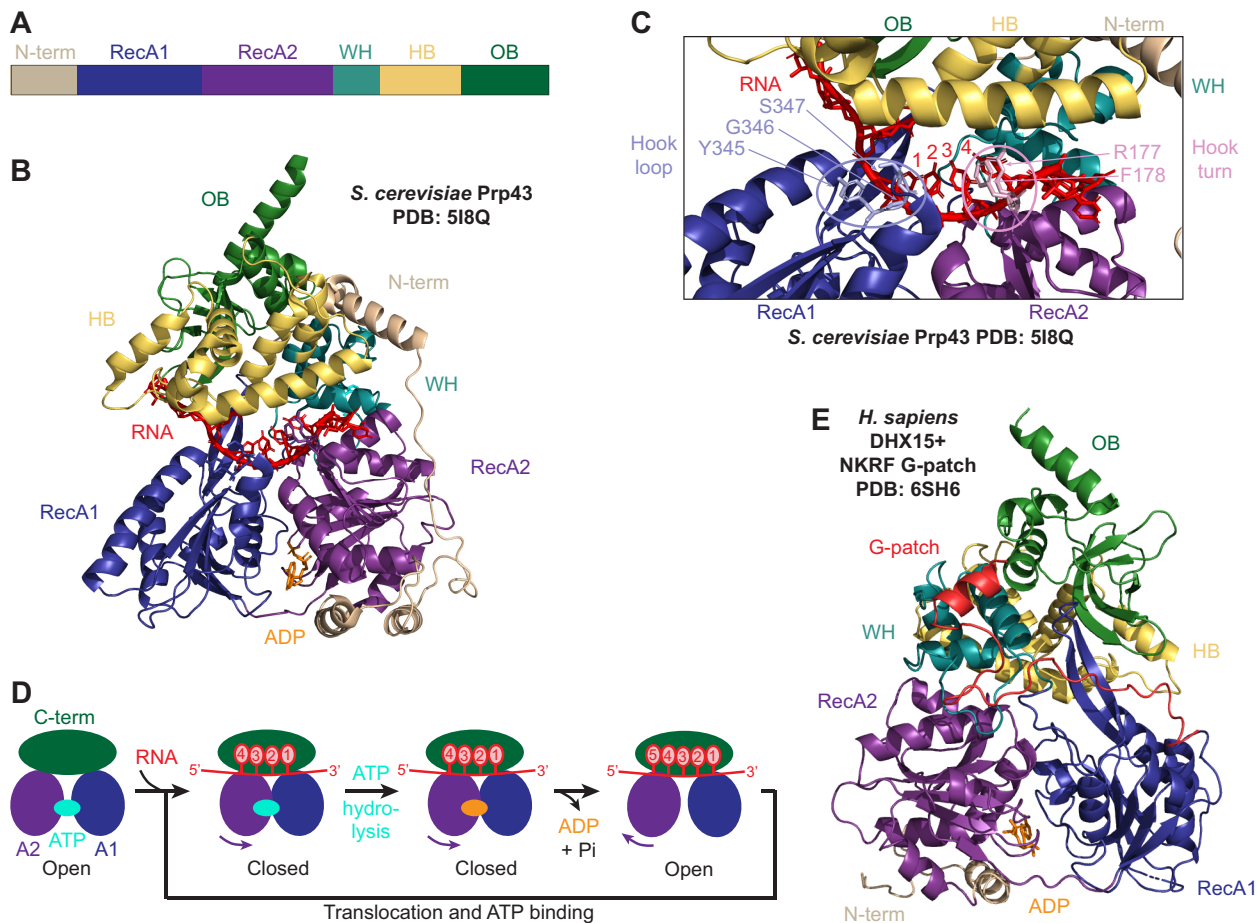
Figure 1. Structure, mechanism and regulation of Prp43/DHX15. (**A**) Schematic view of the domains of Prp43. Domain abbreviations: N-term, N-terminal; RecA, RecA-like; WH, winged helix; HB, helical bundle; OB, oligosaccharide binding. (**B**) Crystal structure of yeast Prp43 [domains coloured as in panel (A)] with ADP (orange) and RNA (red; PDB: 5I8Q). (**C**) Magnified view of the hook-turn and hook-loop motifs of yeast Prp43 (PDB: 5I8Q). (**D**) Schematic model of DEAH/RHA RNA helicase translocation. (**E**) Crystal structure of human DHX15 [domains coloured as in panel (A)] with ADP (orange) and the G-patch domain of NKRF (red; PDB: 6SG6).

nevertheless, specific range of cellular RNAs. Interactions within the catalytic site are predominantly formed with the sugar–phosphate backbone of the RNA, rendering them inherently sequence independent. Nonspecific substrate recognition is in line with the robust catalytic activity of Prp43/DHX15 observed *in vitro* on generic RNA–RNA/RNA–DNA duplexes (24,29,31,32,38–41). The RNA channel of DEAH/RHA-box helicases accommodates only one RNA strand, highlighting the presence of a single-stranded binding platform as an important feature of Prp43/DHX15 helicase substrates. However, this feature is insufficient to endow the necessary substrate specificity within the RNA-dense environments in which the enzymes reside.

The strategy that ensures Prp43/DHX15 target specificity is the coupling of full catalytic activation of the helicase with recruitment to appropriate substrate RNAs by cofactor proteins that can both stimulate catalysis and mediate specific interactions with appropriate RNAs/RNPs (42). This task is accomplished by interactions of Prp43/DHX15 with a family of related cofactor proteins, the G-patch proteins, which share a common glycine-rich G-patch domain (43). The G-patch protein family, which in yeast contains four proteins serving as cofactors of Prp43 and in humans includes ~20 proteins several of which are known to interact with and/or regulate DHX15, has recently been reviewed in detail (42). A crystal structure of DHX15 in complex with a G-patch domain recently revealed that the G-patch domain tethers the two RecA-like domains together; the N-terminal brace helix of the G-patch domain binds the WH domain while the C-terminal brace loop loosely interacts with the more mobile RecA2 domain, thus stabilizing a conformation conducive for increased RNA binding and NTP hydrolysis (Figure 1E) (28).

The combination of a G-patch domain with other structured domains involved in mediating specific protein–protein and protein–RNA interactions that allow recruitment to particular target RNAs/RNPs enables helicase activation to be directly coupled to interactions with a diverse range of specific substrates. The overlapping, and therefore mutually exclusive, interactions between individual G-patch proteins and Prp43/DHX15, alongside the ability of these cofactors to recruit the helicase to

particular substrate RNAs, indicates that they play a role in coordinating the distribution of Prp43/DHX15 between its different cellular functions. This principle is exemplified by the finding that alterations in the levels of specific G-patch proteins in yeast determine the distribution of Prp43 between cellular compartments, affecting its roles in pre-mRNA splicing and ribosome assembly (44). Given that a number of human G-patch proteins have already been identified as DHX15 regulators, it is highly likely that the same *modus operandi* can be extrapolated to human cells. This mode of regulation implies a finely balanced network in which changes in individual G-patch protein levels could broadly regulate RNA metabolism by redirecting available Prp43/DHX15 to different target processes. The potential interconnectedness of different cellular processes involving Prp43/DHX15 therefore always requires consideration.

Prp43 AS AN RNP DISASSEMBLY AND QUALITY CONTROL FACTOR DURING PRE-mRNA SPLICING

Splicing of pre-mRNAs to remove introns is a critical aspect of eukaryotic gene expression. During its functional cycle, the spliceosome, composed of five small nuclear RNPs (snRNPs) and various associated proteins, undergoes extensive structural and compositional rearrangements involving exchange of proteins and remodelling of RNA–RNA interactions largely driven by RNA helicases (37,45).

A conserved function of Prp43/DHX15 in intron lariat spliceosome disassembly

Biochemical analyses and structural studies reveal that, after intron excision and formation of the mature mRNA, Prp43 mediates disassembly of post-splicing complexes (36,46–49). At this stage, the intron lariat is released allowing recycling of the U2, U5 and U6 snRNPs. In this context, the G-patch protein Spp382 (Ntr1) stimulates the catalytic activity of Prp43 to promote spliceosome disassembly (39,40,50–52) (Figures 2A and B and 3). In human intron lariat spliceosomes (ILSs), DHX15 is likewise activated by the Spp382 homologue TFIP11 upon completion of debranching to drive turnover of excised intron lariat complexes (53–55). Recent cryogenic electron microscopy (cryo-EM) structures of yeast and human ILS reveal that Prp43/DHX15 are positioned on the surface of these complexes, proximal to the 3′ end of the U6 snRNA (56,57), which is in line with cross-linking data indicating interactions of Prp43 with U6 (Figure 2A) (36,58). The mechanistic basis of splicing termination and recycling of the U2, U5 and U6 snRNPs is therefore proposed to be Prp43/DHX15 pulling on the 3′ end of the U6 snRNA to promote complex disassembly (Figure 2B).

Quality control of aberrant spliceosomes by Prp43/DHX15

Due to the necessity of high-fidelity splicing to ensure transcriptome integrity, all steps in pre-mRNA splicing are subject to rigorous surveillance. A kinetic proofreading mechanism is described in which slow splicing reactions on suboptimal substrates are overtaken by RNA helicase action leading to active discard of poor substrates from the spliceosome (59–61). Productive splicing is therefore only possible on optimal substrates upon which the catalytic steps of the splicing reactions take place efficiently. In this context, the role of Prp43 in driving post-catalytic spliceosome disassembly is directly coupled with an important function in quality control of aberrant spliceosomes (Figure 3) (62). Most of the RNA helicases involved in pre-mRNA splicing are recruited and released at specific stages of the splicing cycle where they fulfil their productive function as well as act as such internal quality control regulators. In contrast, Prp43/DHX15 have been identified in numerous spliceosomal complexes [see e.g. (63–65)] and Prp43 has been shown to also contribute to the discard of various aberrant intermediates (66). For example, Prp43 acts together with Prp16 to proofread 5′ splice site selection and reject inappropriate substrates (67). Similarly, exon ligation of suboptimal pre-mRNA substrates is slowed by Prp22 whereupon Prp43 serves as a general recycling factor to disassemble the complex (68). By performing essentially the same action at different time points in the splicing cycle, Prp43 can either induce discard of nonspliced suboptimal mRNAs or promote disassembly of intron lariat complexes following successful splicing.

Although the process of spliceosome quality control has not yet been explored in detail in human cells, it is anticipated that the fundamental principle of RNA helicase-mediated discard of suboptimal substrates is evolutionarily conserved and that, via its conserved function in 'winching' the 3′ end of the U6 snRNA, DHX15 will play a central role in this process. In line with this, DHX15 has recently been implicated in quality control of U2 snRNP interactions with pre-mRNA introns (69).

DHX15 in alternative splicing regulation and other splicing-related processes

It is also emerging that DHX15 may function in the regulation of alternative splicing, a prominent process in human cells. For example, it is suggested that DHX15 may function together with two G-patch proteins RBM17 and CHERP to regulate splice site selection via interactions with the U2 snRNP and the 3′ splice site AG (70–72). Notably, beyond the context of spliceosomal disassembly and turnover, a potential role for DHX15 in contributing to a noncanonical U4/U6.U5 tri-snRNP (re-)assembly pathway in Cajal bodies has been suggested (Figure 3) (73). Although DHX15 was shown to be activated by the G-patch protein ZGPAT, also present in these complexes, mechanistic details on the potential role of DHX15 in this process are lacking. Interestingly, the G-patch protein TFIP11, also present in Cajal bodies, was recently shown to regulate U6 snRNA 2′-O-methylation leading to altered U4/U6.U5 tri-snRNP assembly, but this function was shown to be independent of its interaction and activation by DHX15 (74).

FUNCTIONS OF YEAST Prp43 AND HUMAN DHX15 IN RIBOSOME ASSEMBLY

The essential process of translating mRNAs into proteins is carried out by ribosomes, and assembly of these
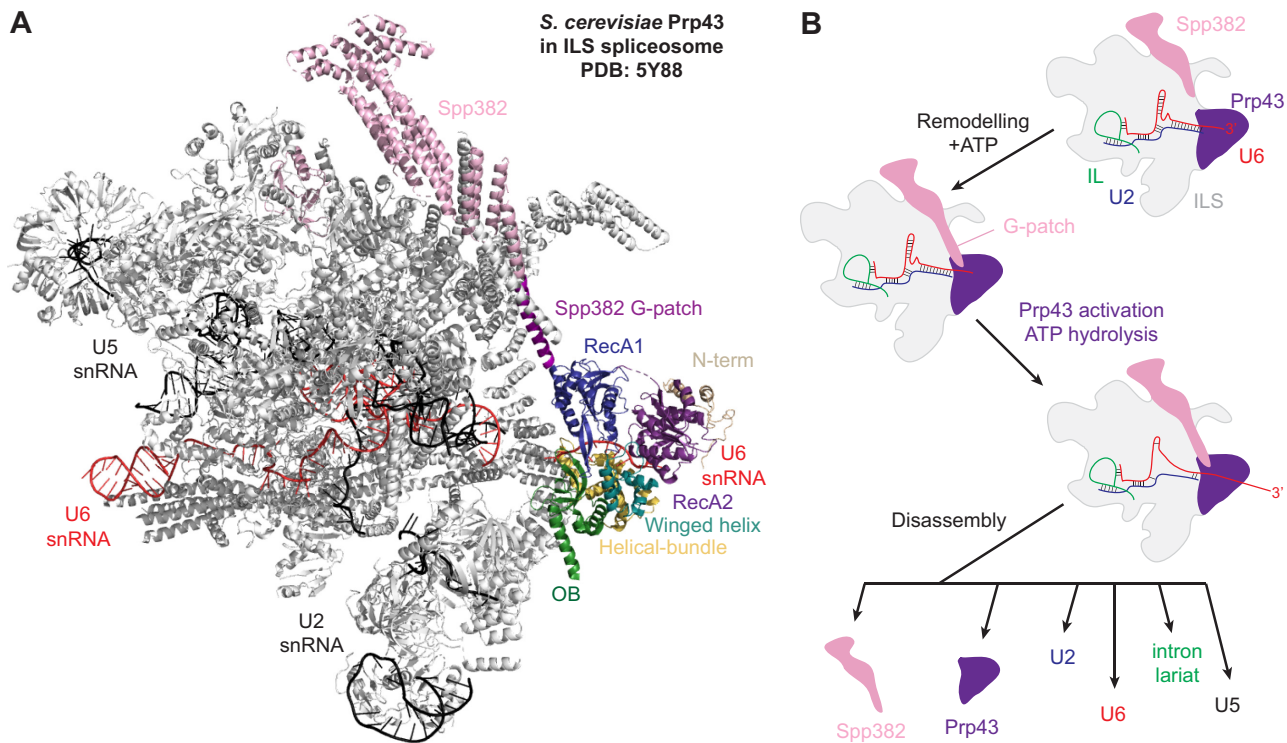
Figure 2. Spliceosome disassembly by Prp43. (**A**) Cryo-EM structure of yeast ILSs with Prp43 (domains coloured as in Figure 1A) and the G-patch protein Spp382 (light pink with G-patch domain in dark pink) highlighted. (**B**) Schematic model of spliceosome disassembly by Prp43. The G-patch protein Spp382 contributes to recruitment of Prp43 to spliceosomes where it binds the 3′ end of the U6 snRNA. Structural rearrangements bring the G-patch domain of Spp382 and Prp43 together stimulating helicase activation. ATP hydrolysis and winching by Prp43 triggers ILS spliceosome disassembly into its constituent components.

macromolecular complexes is therefore a key cellular process. Ribosomal DNA (rDNA) transcription and precursor ribosomal RNA (pre-rRNA) processing, modification and folding are coordinated with assembly of the plethora of ribosomal proteins leading to formation of the small (40S) and large (60S) ribosomal subunits (SSU and LSU, respectively) (75,76).

Prp43 in snoRNA release from pre-LSU particles

Within the context of the yeast ribosome assembly pathway, Prp43 is considered multifunctional as it is one of the few *trans*-acting assembly factors required for both SSU and LSU biogeneses (41,58,77–80). Interactome analyses revealed that Prp43 interacts with RNA polymerase I components, pre-ribosomal particles and small nucleolar RNAs (snoRNAs), and pre-rRNA processing is impaired in cells lacking Prp43 (81). Specific cross-linking sites on the pre-rRNA and certain snoRNAs were identified inspiring functional analyses that showed the requirement of Prp43 and the G-patch protein Pxr1 (also known as Gno1) for release of several snoRNAs from early pre-LSU particles (Figure 3) (32,58). The recovery of chimeric sequencing reads composed of both snoRNA and pre-rRNA sequences in the Prp43 cross-linking data indicates the direct interaction of Prp43 with snoRNA–pre-rRNA duplexes, supporting a direct function of Prp43 in resolving the pre-rRNA base pairing of these snoRNAs (82). This finding makes Prp43 one of the few RNA helicases involved

in ribosome assembly for which substrate RNAs are identified. Lack of Prp43 also affects snoRNP-mediated 2′-*O*-methylation of numerous rRNA nucleotides, but these effects are likely mostly indirect consequences of perturbed pre-LSU biogenesis due to failure to release its substrate snoRNPs (77,82,83).

The efficient, likely processive activity of Prp43 renders it a suitable enzyme for displacing a cluster of snoRNAs base paired in a particular region of the nascent pre-rRNA, but mechanistic questions still remain. In the context of the spliceosome, Prp43 acts as a winch disrupting RNA structures at a distance, but the finding that Prp43 cross-links to snoRNA–pre-rRNA duplexes suggests that this mode of action may not be employed during ribosome assembly. It is tempting to speculate that in this case, instead of the RNA being pulled, Prp43 may physically translocate along the pre-rRNA from a single-stranded region displacing bound snoRNPs in its path.

An undefined role for Prp43 in SSU biogenesis

Beyond promoting snoRNA release from pre-LSU particles, Prp43 has been proposed to function together with the G-patch protein Sqs1 (also known as Pfa1) to structurally remodel cytoplasmic, late pre-SSU particles to facilitate pre-rRNA cleavage at the 3′ end of the SSU rRNA (Figure 3) (41,79). This hypothesis is built on the cross-linking of Prp43 to the 3′ end of the SSU rRNA and the discovery of genetic interactions between Prp43,
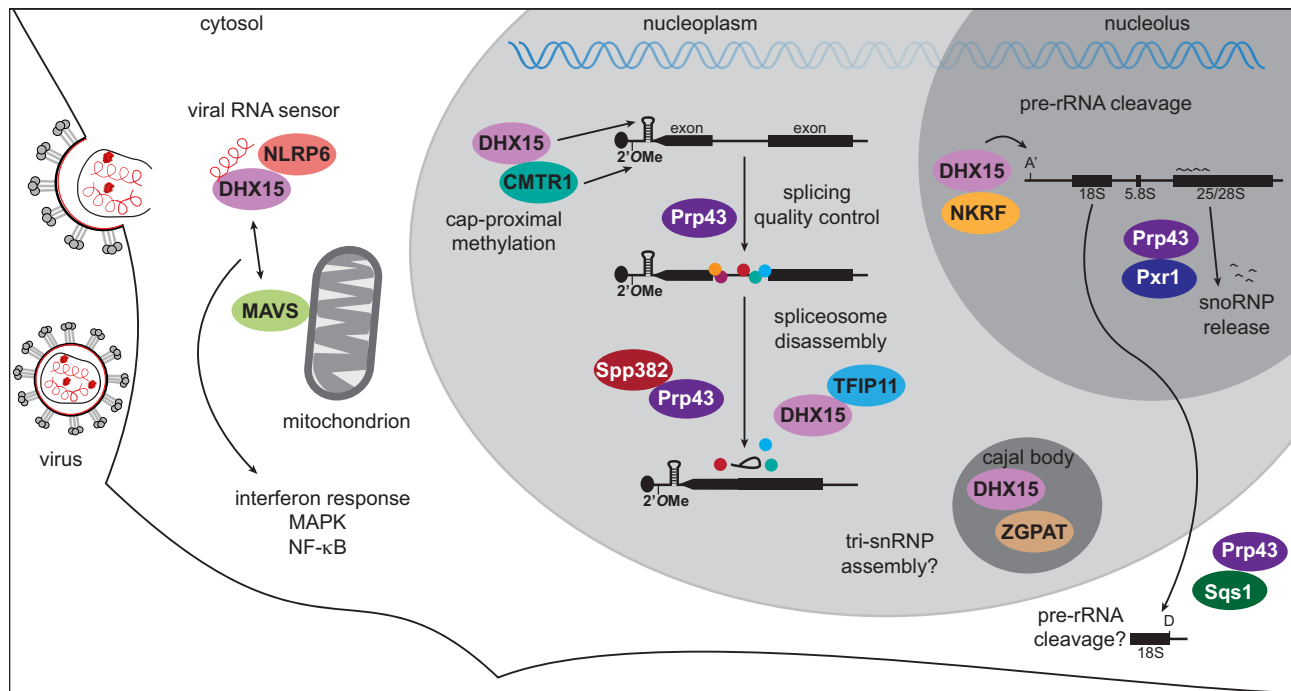
Figure 3. Overview of cellular pathways involving Prp43/DHX15. Cap proximal 2′-*O*-methylation (2′*O*Me) of pre-mRNAs with structured 5′ UTRs requires the concerted action of DHX15 and the G-patch protein/RNA methyltransferase CMTR1. Termination of pre-mRNA splicing is induced by Prp43/DHX15 pulling on the 3′ end of the U6 snRNA. For optimal substrates, this occurs upon successful completion of the splicing reactions, whereas on suboptimal/aberrant substrates where reaction kinetics are slow, Prp43/DHX15 action leads to discard. DHX15 and its G-patch protein cofactor ZGPAT are present in Cajal bodies and a role in regulating tri-snRNP assembly has been speculated. In the context of ribosome assembly, Prp43, together with the G-patch protein Prx1, is involved in release of specific snoRNAs from pre-LSU complexes and potentially plays an indirect role in facilitating cleavage at the 3′ end of the SSU RNA. The catalytic activity of DHX15 and the G-patch protein NKRF are required for a metazoan-specific pre-rRNA cleavage event in the 5′ external transcribed spacer (5′ ETS). Upon viral infection, DHX15 interacts with NPL6 and MAVS in the cytoplasm/on the mitochondrial surface enabling it to fulfil ATPase independent functions as an RNA sensor and/or immune signaller to induce an immune response via the interferon/NF-κB/MAPK signalling pathways.

Sqs1 and Ltv1, a late SSU biogenesis factor, as well as the exacerbated accumulation of the 20S pre-rRNA (the immediate precursor of the SSU rRNA) in cells lacking Prp43 as well as Ltv1 (58,79). However, numerous structural snapshots of late pre-SSU particles from yeast and human cells have recently been captured using affinity purification and cryo-EM [see e.g. (84–87)], but Prp43/DHX15 and Sqs1/PINX1 have not been detected in these particles. Furthermore, a detailed, structure-based model for how 3′ cleavage of the SSU rRNA is regulated has been established without a clear role for Prp43/DHX15. It is possible that Prp43/DHX15 interact only transiently with late pre-40S particles and are lost during affinity purifications. However, their absence raises the question of whether Prp43/DHX15 in fact contact the 3′ end of the SSU rRNA during an earlier stage of pre-SSU maturation and indirectly influence the final pre-rRNA step, for example by structurally remodelling the pre-rRNA or a pre-rRNA–snoRNA duplex to promote recruitment or release of another assembly factor required downstream.

Additional functions of DHX15 in human ribosome assembly

In contrast to the well-defined role of yeast Prp43 in promoting snoRNA release from pre-LSU complexes,

potential functions of DHX15 in human ribosome production remain much less clear. While homologues of several yeast RNA helicases carry out analogous functions in humans, e.g. yeast Dhr1 and human DHX37 are both involved in the release of U3 snoRNA from pre-ribosomal particles (88–91), the functions of other ribosome assembly factors are known to vary between yeast and humans (76). It remains unexplored whether DHX15 mediates release of snoRNAs from human pre-LSU particles, and thus far, DHX15 has not been linked to late pre-SSU maturation (86,87,92). In contrast to depletion of yeast Prp43, efficient, RNAi-mediated depletion of human DHX15 does not strongly affect pre-rRNA processing. This could simply reflect functionality of residual protein, but intriguingly lack of DHX15, its catalytic activity or its regulatory G-patch protein NKRF leads to inefficient processing at a metazoan-specific cleavage site in the 5′ ETS of the nascent pre-rRNA transcript (Figure 3) (38). Exploring more fully the role of DHX15 in human ribosome assembly will be necessary to understand whether the functions of yeast Prp43 are conserved to humans or if Prp43 functions are outsourced to other helicases in human cells. Also, it will be interesting to discover if DHX15 has further additional metazoan-specific functions in ribosome production beyond facilitating early pre-rRNA cleavage.

A role for Prp43/DHX15 in pre-ribosome quality control?

The role of Prp43 in driving snoRNP release from pre-ribosomes draws parallels to the spliceosome disassembly function of the helicase in which snRNPs are displaced from the pre-mRNA substrate. It remains unknown, however, whether this analogy extends to a role of Prp43 in promoting disassembly of aberrant pre-ribosomal particles by prematurely exerting its disassembly function. The metazoan-specific pre-rRNA processing step for which DHX15 is required is linked to early pre-ribosome quality control (93), thus adding to the conjecture that DHX15 may act as a surveillance factor for human pre-ribosomes. Intriguingly, Sqs1, which associates with Prp43 in the context of ribosome biogenesis, contains not only a G-patch domain but also an arch-interacting motif (AIM) via which it contacts another helicase Mtr4 (see below) (94). As Mtr4 is responsible for resolving secondary structures to allow RNA degradation by the exosome, this raises the possibility that Prp43–Sqs1 couple disassembly of aberrant pre-ribosomes with their turnover. However, further research is required to determine whether Prp43/DHX15 function as general RNP disassembly machineries.

DHX15-MEDIATED REGULATION OF mRNA CAP-PROXIMAL 2′-*O*-METHYLATION

So far, yeast Prp43 has only been implicated in the different aspects of pre-mRNA splicing and ribosome biogenesis discussed above; however, the multifunctionality of human DHX15 extends beyond these processes. Recently, a role for DHX15 in facilitating cap-proximal 2′-*O*-methylation of mRNAs was described (Figure 3) (95,96). In this process, stimulation of the unwinding activity of DHX15 is directly coupled to methylation activity via interactions with CMTR1, which possesses both a G-patch domain and a methyltransferase domain. DHX15 is specifically implicated in structural remodelling of mRNAs with highly structured 5′ ends (95), and the typically shorter and less structured 5′UTRs of yeast mRNAs (97) may rationalize the lack of an analogous role for yeast Prp43. This finding implies that some pre-mRNAs likely encounter DHX15 in the context of both pre-mRNA splicing and capping, and as these processes have been suggested to take place concurrently, it is possible that different DHX15 molecules may simultaneously undertake different aspects of maturation on a single mRNA.

DHX15 IN INNATE IMMUNITY AGAINST VIRAL INFECTIONS

Some cellular RNA helicases are hijacked by viruses to promote viral replication, whereas others play important roles in the innate immune response to viral infection by acting as sensors of exogenous RNA (98). Viral infections are typically detected by membrane-bound receptors and cytoplasmic sensors [e.g. retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5)] that relay signals to induce expression of interferons (IFNs), interferon-stimulated genes (ISGs) and inflammatory cytokines. Several studies implicate DHX15 in different aspects of the immune responses to viral infection (Figure 3). DHX15 is a component of the RIG-I and MDA5 interactomes, and depletion of DHX15 increases susceptibility to infection by the ssRNA Paramyxoviridae, Rhabdoviridae and Picornaviridae viruses (99). Furthermore, DHX15, which upon viral infection directly interacts with the mitochondrial antiviral signalling protein MAVS via its helicase core, acts in an ATP hydrolysis-independent manner downstream of MAVS to activate the NF-κB and MAPK pathways in response to poly(I:C) treatment and infection with the encephalomyocarditis and Sendai RNA viruses, thus functioning as an immune signalling adaptor (100). DHX15 has also been proposed to act as a viral RNA sensor in response to infection with enteric viruses, such as encephalomyocarditis virus and norovirus (101,102). Here, DHX15 directly interacts with the nucleotide oligomerization domain-like receptor protein 6 (NLRP6), which has key roles in antibacterial and antiviral immunity through activation of an inflammasome complex (101,103–106). Both NLRP6 and DHX15 associate with ss viral RNAs, but greater RNA binding by DHX15 and strong reduction of NLRP6 RNA interactions in the absence of DHX15 suggest that DHX15 serves as the main RNA sensor (101). It has recently emerged that activation of the NLRP6 inflammasome is driven by liquid–liquid phase separation and that DHX15 forms condensates together with NLRP6 and RNA (103). NLRP6 enhances the interaction between DHX15 and MAVS, and the trimeric complex is required for activation of type I (IFN-I, IFN-β) and type III (IFN-λ3) ISGs and cytokine IL-18 production in intestinal epithelial cells (101,102,107).

As structural and biochemical evidence support binding of DHX15 to ssRNAs, a potential function as an ssRNA sensor is plausible. However, whether DHX15 binds ss viral RNAs in the same manner as endogenous substrates, how ATPase activity is impaired so that these RNAs remain bound for sufficient time to trigger a downstream immune response remains unclear. Intriguingly, DHX15 was also suggested to sense double-stranded (ds) viral RNAs in myeloid dendritic cells and weak binding to synthetic poly(I:C) has been observed *in vitro* (99,108). Rationalizing these results predicates the existence of an alternative RNA binding mode, and as DHX15 appears to play noncatalytic roles in the context of viral infection and interacts with a specific set of protein interaction partners, this is possible. Indeed, it has been suggested the ds RNA binding is mediated by the DHX15 C-terminal region. While a direct role of DHX15 in the cellular response to viral infection is supported by evidence of physical interactions between DHX15 and components of the innate immune response, e.g. NLRP6, it is important to note that some of the effects observed in cells lacking DHX15 could be indirect.

COMPARISON OF Prp43/DHX15 TO OTHER MULTIFUNCTIONAL RNA HELICASES

Although perhaps the most broad-spectrum and best characterized, DHX15 is not unique as a multitasking RNA helicase. For example, DHX9 has been implicated in diverse cellular processes, including genome maintenance, regulation of transcription, mRNA export, miRNA

processing, circular RNA biogenesis, translation and innate immunity (109). Various roles for UPF1 in DNA/RNA metabolism, such as DNA repair, telomere maintenance, nonsense-mediated decay, histone mRNA turnover, Staufen-mediated mRNA decay and release of nascent transcripts from gene loci, have been described (110–113). Likewise, the DEAD-box RNA helicase eIF4A-III functions in ribosome assembly and translation as well as acts as an RNA clamp within exon junction complexes (19,114,115). The RNA exosome-associated Ski2-like helicase MTR4 is also multifunctional through its contributions to the processing and decay of a wide variety of RNA species. It is striking that, like Prp43/DHX15, UPF1, eIF4A-III and MTR4 associate with cofactor proteins. In the cases of eIF4A-III, these are proteins of the MIF4G domain family (CTIF, NOM1 and CWC22), which have overlapping binding sites and influence recruitment of the helicase to appropriate substrates as well as regulate its catalytic activity (15,114,116–118). Mtr4/MTR4 is likewise recruited to different target RNAs via proteins containing AIMs, such as the ribosome assembly factors Nop53, Utp18 and NVL, as well as the nuclear exosome targeting complex component ZCCHC8 and its negative regulator NRDE2 (94,119,120). These observations suggest that a shared feature of multifunctional RNA helicases, irrespective of their familial origin, is their recruitment to specific substrates by cofactor proteins possessing a common domain/motif via which they bind a specific region of the helicase.

WHY DO WE NEED BOTH DEDICATED AND MULTIFUNCTIONAL RNA HELICASES?

It is clear that helicases with particular molecular functions, e.g. local strand unwinders, RNA clampers and translocating helicases, are needed in different contexts, depending on the nature of the RNA/RNP rearrangement. However, the existence of dedicated and multifunctional RNA helicases raises the questions of why it is beneficial to have both and what determines whether an RNA/RNP remodelling event is accomplished by a dedicated or a multifunctional enzyme. At first glance, the answer to this question is not intuitive as apparently analogous functions to those performed by Prp43/DHX15 in pre-mRNA splicing and ribosome biogenesis are fulfilled by other, non-multifunctional RNA helicases. For example, like Prp43, the dedicated helicase Dhr1 mediates snoRNA release from pre-ribosomes and similar to the role of Prp43 in recycling of the snRNPs from the intron lariat, Prp22, for which no additional functions are described, initiates spliceosome disassembly by destabilizing interactions between the U5 snRNP and mRNA. Most RNA helicases act within RNP complexes, so it is possible that whether only catalytic activity or also the formation of specific protein–protein interactions is required may influence whether a dedicated or multifunctional RNA helicase is utilized.

The increased multifunctionality of human DHX15 compared to yeast Prp43 could suggest that DHX15 and other multifunctional RNA helicases represent optimized, flexible machines conscripted to increasingly diverse cellular functions through the evolution of additional cofactor proteins. This is in line with the >4-fold increase in G-patch proteins between yeast and humans and could suggest that rather than re-inventing new helicases to meet the demands for additional RNA/RNP remodelling activities in more complex systems, it is preferable to repurpose the robust machineries already available through specialized adaptor proteins.

FUTURE PERSPECTIVE: MULTIFUNCTIONAL RNA HELICASES IN THE CROSS-REGULATION OF GENE EXPRESSION PROCESSES

Importantly, RNA helicase multifunctionality offers the opportunity for co-regulation of different gene expression processes. Co-regulation of key mRNA maturation events such as capping and splicing with highly energy consuming pathways like ribosome assembly would allow efficient and coordinated adaptation of gene expression in different conditions. Indeed, other examples of crosstalk between these processes exist, such as ribosomal proteins moonlighting as alternative splicing regulators (121). While the concept of cross-regulation of cellular processes by redistribution of Prp43 has been demonstrated by overexpression of individual G-patch cofactors, physiological relevance of this interconnected helicase–cofactor network has yet to be discovered. Changes in G-patch protein levels are observed upon exposure to cellular stress and in cancer [e.g. GPATCH2, PINX1 and RBM5 (122–124)], so it is possible that redistribution of the multifunctional helicase between different RNA substrates contributes to the adaptive response and/or cellular transformation, but this is yet to be proven.

Further work is required to understand how other multifunctional RNA helicases, such as DHX9, are regulated to enable parallels and differences with the mechanisms of DHX15 regulation to be uncovered. In addition, better understanding of the functional diversification of DHX15 will likely shed new light on why it is preferable to have specifically this helicase operating as a multifunctional enzyme.

REFERENCES

1. Vicens,Q. and Kieft,J.S. (2022) Thoughts on how to think (and talk) about RNA structure. *Proc. Natl Acad. Sci. U.S.A.*, **119**, e2112677119.
2. Herschlag,D. (1995) RNA chaperones and the RNA folding problem. *J. Biol. Chem.*, **270**, 20871–20874.

3. Herschlag,D., Bonilla,S. and Bisaria,N. (2018) The story of RNA folding, as told in epochs. *Cold Spring Harb. Perspect. Biol.*, **10**, a032433.

4. Schroeder,R., Barta,A. and Semrad,K. (2004) Strategies for RNA folding and assembly. *Nat. Rev. Mol. Cell Biol.*, **5**, 908–919.

5. Jarmoskaite,I. and Russell,R. (2014) RNA helicase proteins as chaperones and remodelers. *Annu. Rev. Biochem.*, **83**, 697–725.

6. Duss,O., Stepanyuk,G.A., Puglisi,J.D. and Williamson,J.R. (2019) Transient protein–RNA interactions guide nascent ribosomal RNA folding. *Cell*, **179**, 1357.e16–1369.

7. Bohnsack,K.E. and Bohnsack,M.T. (2019) RNA-binding proteins chaperone ribonucleoprotein complex assembly to solve the RNA-folding problem. *Cell*, **179**, 1248–1250.

8. Huang,H. and Karbstein,K. (2021) Assembly factors chaperone ribosomal RNA folding by isolating helical junctions that are prone to misfolding. *Proc. Natl Acad. Sci. U.S.A.*, **118**, e2101164118.

9. Rodgers,M.L. and Woodson,S.A. (2019) Transcription increases the cooperativity of ribonucleoprotein assembly. *Cell*, **179**, 1370–1381.

10. Rodgers,M.L. and Woodson,S.A. (2021) A roadmap for rRNA folding and assembly during transcription. *Trends Biochem. Sci.*, **46**, 889–901.

11. Hohmann,K.F., Blümler,A., Heckel,A. and Fürtig,B. (2021) The RNA chaperone StpA enables fast RNA refolding by destabilization of mutually exclusive base pairs within competing secondary structure elements. *Nucleic Acids Res.*, **49**, 11337–11349.

12. Hua,B., Panja,S., Wang,Y., Woodson,S.A. and Ha,T. (2018) Mimicking co-transcriptional RNA folding using a superhelicase. *J. Am. Chem. Soc.*, **140**, 10067–10070.

13. Matera,A.G. and Wang,Z. (2014) A day in the life of the spliceosome. *Nat. Rev. Mol. Cell Biol.*, **15**, 108–121.

14. Linder,P. and Jankowsky,E. (2011) From unwinding to clamping—the DEAD box RNA helicase family. *Nat. Rev. Mol. Cell Biol.*, **12**, 505–516.

15. Sloan,K.E. and Bohnsack,M.T. (2018) Unravelling the mechanisms of RNA helicase regulation. *Trends Biochem. Sci.*, **43**, 237–250.

16. Bourgeois,C.F., Mortreux,F. and Auboeuf,D. (2016) The multiple functions of RNA helicases as drivers and regulators of gene expression. *Nat. Rev. Mol. Cell Biol.*, **17**, 426–438.

17. Fairman-Williams,M.E., Guenther,U.-P. and Jankowsky,E. (2010) SF1 and SF2 helicases: family matters. *Curr. Opin. Struct. Biol.*, **20**, 313–324.

18. Fairman,M.E., Maroney,P.A., Wang,W., Bowers,H.A., Gollnick,P., Nilsen,T.W. and Jankowsky,E. (2004) Protein displacement by DExH/D 'RNA helicases' without duplex unwinding. *Science*, **304**, 730–734.

19. Ballut,L., Marchadier,B., Baguet,A., Tomasetto,C., Seraphin,B. and Le Hir,H. (2005) The exon junction core complex is locked onto RNA by inhibition of eIF4AIII ATPase activity. *Nat. Struct. Mol. Biol.*, **12**, 861–869.

20. Pyle,A.M. (2008) Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu. Rev. Biophys.*, **37**, 317–336.

21. Jankowsky,E., Gross,C.H., Shuman,S. and Pyle,A.M. (2001) Active disruption of an RNA–protein interaction by a DExH/D RNA helicase. *Science*, **291**, 121–125.

22. Mallam,A.L., Del Campo,M., Gilman,B., Sidote,D.J. and Lambowitz,A.M. (2012) Structural basis for RNA-duplex recognition and unwinding by the DEAD-box helicase Mss116p. *Nature*, **490**, 121–125.

23. Oberer,M., Marintchev,A. and Wagner,G. (2005) Structural basis for the enhancement of eIF4A helicase activity by eIF4G. *Genes Dev.*, **19**, 2212–2223.

24. Tauchert,M.J., Fourmann,J.-B., Luhrmann,R. and Ficner,R. (2017) Structural insights into the mechanism of the DEAH-box RNA helicase Prp43. *eLife*, **6**, e21510.

25. Jackson,R.N., Klauer,A.A., Hintze,B.J., Robinson,H., van Hoof,A. and Johnson,S.J. (2010) The crystal structure of Mtr4 reveals a novel arch domain required for rRNA processing. *EMBO J.*, **29**, 2205–2216.

26. Linder,P. and Jankowsky,E. (2011) From unwinding to clamping—the DEAD box RNA helicase family. *Nat. Rev. Mol. Cell Biol.*, **12**, 505–516.

27. Hamann,F., Enders,M. and Ficner,R. (2019) Structural basis for RNA translocation by DEAH-box ATPases. *Nucleic Acids Res.*, **47**, 4349–4362.

28. Studer,M.K., Ivanović,L., Weber,M.E., Marti,S. and Jonas,S. (2020) Structural basis for DEAH-helicase activation by G-patch proteins. *Proc. Natl Acad. Sci. U.S.A.*, **117**, 7159–7170.

29. He,Y., Staley,J.P., Andersen,G.R. and Nielsen,K.H. (2017) Structure of the DEAH/RHA ATPase Prp43p bound to RNA implicates a pair of hairpins and motif Va in translocation along RNA. *RNA*, **23**, 1110–1124.

30. Walbott,H., Mouffok,S., Capeyrou,R., Lebaron,S., Humbert,O., van Tilbeurgh,H., Henry,Y. and Leulliot,N. (2010) Prp43p contains a processive helicase structural architecture with a specific regulatory domain. *EMBO J.*, **29**, 2194–2204.

31. Murakami,K., Nakano,K., Shimizu,T. and Ohto,U. (2017) The crystal structure of human DEAH-box RNA helicase 15 reveals a domain organization of the mammalian DEAH/RHA family. *Acta Crystallogr. F Struct. Biol. Commun.*, **73**, 347–355.

32. Robert-Paganin,J., Halladjian,M., Blaud,M., Lebaron,S., Delbos,L., Chardon,F., Capeyrou,R., Humbert,O., Henry,Y., Henras,A.K. *et al.* (2017) Functional link between DEAH/RHA helicase Prp43 activation and ATP base binding. *Nucleic Acids Res.*, **45**, 1539–1552.

33. He,Y., Andersen,G.R. and Nielsen,K.H. (2010) Structural basis for the function of DEAH helicases. *EMBO Rep.*, **11**, 180–186.

34. Jankowsky,E. (2011) RNA helicases at work: binding and rearranging. *Trends Biochem. Sci.*, **36**, 19–29.

35. Semlow,D.R., Blanco,M.R., Walter,N.G. and Staley,J.P. (2016) Spliceosomal DEAH-Box ATPases remodel pre-mRNA to activate alternative splice sites. *Cell*, **164**, 985–998.

36. Toroney,R., Nielsen,K.H. and Staley,J.P. (2019) Termination of pre-mRNA splicing requires that the ATPase and RNA unwindase Prp43p acts on the catalytic snRNA U6. *Genes Dev.*, **33**, 1555–1574.

37. De Bortoli,F., Espinosa,S. and Zhao,R. (2021) DEAH-Box RNA helicases in pre-mRNA splicing. *Trends Biochem. Sci.*, **46**, 225–238.

38. Memet,I., Doebele,C., Sloan,K.E. and Bohnsack,M.T. (2017) The G-patch protein NF-kappaB-repressing factor mediates the recruitment of the exonuclease XRN2 and activation of the RNA helicase DHX15 in human ribosome biogenesis. *Nucleic Acids Res.*, **45**, 5359–5374.

39. Tanaka,N. and Schwer,B. (2006) Mutations in PRP43 that uncouple RNA-dependent NTPase activity and pre-mRNA splicing function. *Biochemistry*, **45**, 6510–6521.

40. Tanaka,N., Aronova,A. and Schwer,B. (2007) Ntr1 activates the Prp43 helicase to trigger release of lariat-intron from the spliceosome. *Genes Dev.*, **21**, 2312–2325.

41. Lebaron,S., Papin,C., Capeyrou,R., Chen,Y.-L., Froment,C., Monsarrat,B., Caizergues-Ferrer,M., Grigoriev,M. and Henry,Y. (2009) The ATPase and helicase activities of Prp43p are stimulated by the G-patch protein Pfa1p during yeast ribosome biogenesis. *EMBO J.*, **28**, 3808–3819.

42. Bohnsack,K.E., Ficner,R., Jonas,S. and Bohnsack,M.T. (2021) Regulation of DEAH-box RNA helicases by G-patch proteins. *Biol. Chem.*, **402**, 561–579.

43. Aravind,L. and Koonin,E.V (1999) G-patch: a new conserved domain in eukaryotic RNA-processing proteins and type D retroviral polyproteins. *Trends Biochem. Sci.*, **24**, 342–344.

44. Heininger,A.U., Hackert,P., Andreou,A.Z., Boon,K.-L., Memet,I., Prior,M., Clancy,A., Schmidt,B., Urlaub,H., Schleiff,E. *et al.* (2016) Protein cofactor competition regulates the action of a multifunctional RNA helicase in different pathways. *RNA Biol.*, **13**, 320–330.

45. De,I., Schmitzová,J. and Pena,V. (2016) The organization and contribution of helicases to RNA splicing. *Wiley Interdiscip. Rev. RNA*, **7**, 259–274.

46. Arenas,J.E. and Abelson,J.N. (1997) Prp43: an RNA helicase-like factor involved in spliceosome disassembly. *Proc. Natl Acad. Sci. U.S.A.*, **94**, 11798–11802.

47. Martin,A., Schneider,S. and Schwer,B. (2002) Prp43 is an essential RNA-dependent ATPase required for release of lariat-intron from the spliceosome. *J. Biol. Chem.*, **277**, 17743–17750.

48. Tsai,R.-T., Fu,R.-H., Yeh,F.-L., Tseng,C.-K., Lin,Y.-C., Huang,Y.-H. and Cheng,S.-C. (2005) Spliceosome disassembly catalyzed by Prp43 and its associated components Ntr1 and Ntr2. *Genes Dev.*, **19**, 2991–3003.

49. Burke,J.E., Longhurst,A.D., Merkurjev,D., Sales-Lee,J., Rao,B., Moresco,J.J., Yates,J.R. 3rd, Li,J.J. and Madhani,H.D. (2018)

Spliceosome profiling visualizes operations of a dynamic RNP at nucleotide resolution. *Cell*, **173**, 1014–1030.

50. Tsai,R.-T., Tseng,C.-K., Lee,P.-J., Chen,H.-C., Fu,R.-H., Chang,K., Yeh,F.-L. and Cheng,S.-C. (2007) Dynamic interactions of Ntr1–Ntr2 with Prp43 and with U5 govern the recruitment of Prp43 to mediate spliceosome disassembly. *Mol. Cell. Biol.*, **27**, 8027–8037.

51. Fourmann,J.-B., Schmitzová,J., Christian,H., Urlaub,H., Ficner,R., Boon,K.-L., Fabrizio,P. and Lührmann,R. (2013) Dissection of the factor requirements for spliceosome disassembly and the elucidation of its dissociation products using a purified splicing system. *Genes Dev.*, **27**, 413–428.

52. Boon,K.-L., Auchynnikava,T., Edwalds-Gilbert,G., Barrass,J.D., Droop,A.P., Dez,C. and Beggs,J.D. (2006) Yeast Ntr1/Spp382 mediates Prp43 function in postspliceosomes. *Mol. Cell. Biol.*, **26**, 6016–6023.

53. Yoshimoto,R., Kataoka,N., Okawa,K. and Ohno,M. (2009) Isolation and characterization of post-splicing lariat-intron complexes. *Nucleic Acids Res.*, **37**, 891–902.

54. Wen,X., Tannukit,S. and Paine,M.L. (2008) TFIP11 interacts with mDEAH9, an RNA helicase involved in spliceosome disassembly. *Int. J. Mol. Sci.*, **9**, 2105–2113.

55. Tannukit,S., Crabb,T.L., Hertel,K.J., Wen,X., Jans,D.A. and Paine,M.L. (2009) Identification of a novel nuclear localization signal and speckle-targeting sequence of tuftelin-interacting protein 11, a splicing factor involved in spliceosome disassembly. *Biochem. Biophys. Res. Commun.*, **390**, 1044–1050.

56. Wan,R., Yan,C., Bai,R., Lei,J. and Shi,Y. (2017) Structure of an intron lariat spliceosome from *Saccharomyces cerevisiae*. *Cell*, **171**, 120–132.

57. Zhang,X., Zhan,X., Yan,C., Zhang,W., Liu,D., Lei,J. and Shi,Y. (2019) Structures of the human spliceosomes before and after release of the ligated exon. *Cell Res.*, **29**, 274–285.

58. Bohnsack,M.T., Martin,R., Granneman,S., Ruprecht,M., Schleiff,E. and Tollervey,D. (2009) Prp43 bound at different sites on the pre-rRNA performs distinct functions in ribosome synthesis. *Mol. Cell*, **36**, 583–592.

59. Hopfield,J.J. (1974) Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. *Proc. Natl Acad. Sci. U.S.A.*, **71**, 4135–4139.

60. Ninio,J. (1975) Kinetic amplification of enzyme discrimination. *Biochimie*, **57**, 587–595.

61. Semlow,D.R. and Staley,J.P. (2012) Staying on message: ensuring fidelity in pre-mRNA splicing. *Trends Biochem. Sci.*, **37**, 263–273.

62. Pandit,S., Lynn,B. and Rymond,B.C. (2006) Inhibition of a spliceosome turnover pathway suppresses splicing defects. *Proc. Natl Acad. Sci. U.S.A.*, **103**, 13700–13705.

63. Bessonov,S., Anokhina,M., Will,C.L., Urlaub,H. and Lührmann,R. (2008) Isolation of an active step I spliceosome and composition of its RNP core. *Nature*, **452**, 846–850.

64. Bessonov,S., Anokhina,M., Krasauskas,A., Golas,M.M., Sander,B., Will,C.L., Urlaub,H., Stark,H. and Lührmann,R. (2010) Characterization of purified human Bact spliceosomal complexes reveals compositional and morphological changes during spliceosome activation and first step catalysis. *RNA*, **16**, 2384–2403.

65. Agafonov,D.E., Deckert,J., Wolf,E., Odenwälder,P., Bessonov,S., Will,C.L., Urlaub,H. and Lührmann,R. (2011) Semiquantitative proteomic analysis of the human spliceosome via a novel two-dimensional gel electrophoresis method. *Mol. Cell. Biol.*, **31**, 2667–2682.

66. Pandit,S., Paul,S., Li,Z., Min,C., Durbin,N., Harrison,S.M.W. and Rymond,B.C. (2009) Spp382p interacts with multiple yeast splicing factors, including possible regulators of Prp43 DExD/H-box protein function. *Genetics*, **183**, 195–206.

67. Koodathingal,P., Novak,T., Piccirilli,J.A. and Staley,J.P. (2010) The DEAH box ATPases Prp16 and Prp43 cooperate to proofread 5′ splice site cleavage during pre-mRNA splicing. *Mol. Cell*, **39**, 385–395.

68. Mayas,R.M., Maita,H., Semlow,D.R. and Staley,J.P. (2010) Spliceosome discards intermediates via the DEAH box ATPase Prp43p. *Proc. Natl Acad. Sci. U.S.A.*, **107**, 10020–10025.

69. Maul-Newby,H.M., Amorello,A.N., Sharma,T., Kim,J.H., Modena,M.S., Prichard,B.E. and Jurica,M.S. (2022) A model for DHX15 mediated disassembly of A-complex spliceosomes. *RNA*, **28**, 583–595.

70. Han,H., Braunschweig,U., Gonatopoulos-Pournatzis,T., Weatheritt,R.J., Hirsch,C.L., Ha,K.C.H., Radovani,E., Nabeel-Shah,S., Sterne-Weiler,T., Wang,J. *et al.* (2017) Multilayered control of alternative splicing regulatory networks by transcription factors. *Mol. Cell*, **65**, 539–553.

71. Lallena,M.J., Chalmers,K.J., Llamazares,S., Lamond,A.I. and Valcárcel,J. (2002) Splicing regulation at the second catalytic step by Sex-lethal involves 3′ splice site recognition by SPF45. *Cell*, **109**, 285–296.

72. Hegele,A., Kamburov,A., Grossmann,A., Sourlis,C., Wowro,S., Weimann,M., Will,C.L., Pena,V., Lührmann,R. and Stelzl,U. (2012) Dynamic protein–protein interaction wiring of the human spliceosome. *Mol. Cell*, **45**, 567–580.

73. Chen,Z., Gui,B., Zhang,Y., Xie,G., Li,W., Liu,S., Xu,B., Wu,C., He,L., Yang,J. *et al.* (2017) Identification of a 35S U4/U6.U5 tri-small nuclear ribonucleoprotein (tri-snRNP) complex intermediate in spliceosome assembly. *J. Biol. Chem.*, **292**, 18113–18128.

74. Duchemin,A., O'Grady,T., Hanache,S., Mereau,A., Thiry,M., Wacheul,L., Michaux,C., Perpète,E., Hervouet,E., Peixoto,P. *et al.* (2021) DHX15-independent roles for TFIP11 in U6 snRNA modification, U4/U6.U5 tri-snRNP assembly and pre-mRNA splicing fidelity. *Nat. Commun.*, **12**, 6648.

75. Klinge,S. and Woolford,J.L.J. (2019) Ribosome assembly coming into focus. *Nat. Rev. Mol. Cell Biol.*, **20**, 116–131.

76. Bohnsack,K.E. and Bohnsack,M.T. (2019) Uncovering the assembly pathway of human ribosomes and its emerging links to disease. *EMBO J.*, **38**, e100278.

77. Leeds,N.B., Small,E.C., Hiley,S.L., Hughes,T.R. and Staley,J.P. (2006) The splicing factor Prp43p, a DEAH box ATPase, functions in ribosome biogenesis. *Mol. Cell. Biol.*, **26**, 513–522.

78. Combs,D.J., Nagel,R.J., Ares,M.J. and Stevens,S.W. (2006) Prp43p is a DEAH-box spliceosome disassembly factor essential for ribosome biogenesis. *Mol. Cell. Biol.*, **26**, 523–534.

79. Pertschy,B., Schneider,C., Gnadig,M., Schafer,T., Tollervey,D. and Hurt,E. (2009) RNA helicase Prp43 and its co-factor Pfa1 promote 20 to 18 S rRNA processing catalyzed by the endonuclease Nob1. *J. Biol. Chem.*, **284**, 35079–35091.

80. Chen,Y.-L., Capeyrou,R., Humbert,O., Mouffok,S., Kadri,Y.Al, Lebaron,S., Henras,A.K. and Henry,Y. (2014) The telomerase inhibitor Gno1p/PINX1 activates the helicase Prp43p during ribosome biogenesis. *Nucleic Acids Res.*, **42**, 7330–7345.

81. Lebaron,S., Froment,C., Fromont-Racine,M., Rain,J.-C., Monsarrat,B., Caizergues-Ferrer,M. and Henry,Y. (2005) The splicing ATPase Prp43p is a component of multiple preribosomal particles. *Mol. Cell. Biol.*, **25**, 9269–9282.

82. Aquino,G.R.R., Krogh,N., Hackert,P., Martin,R., Gallesio,J.D., van Nues,R.W., Schneider,C., Watkins,N.J., Nielsen,H., Bohnsack,K.E. *et al.* (2021) RNA helicase-mediated regulation of snoRNP dynamics on pre-ribosomes and rRNA 2′-*O*-methylation. *Nucleic Acids Res.*, **49**, 4066–4084.

83. Bailey,A.D., Talkish,J., Ding,H., Igel,H., Duran,A., Mantripragada,S., Paten,B. and Ares,M. (2022) Concerted modification of nucleotides at functional centers of the ribosome revealed by single-molecule RNA modification profiling. *eLife*, **11**, e76562.

84. Heuer,A., Thomson,E., Schmidt,C., Berninghausen,O., Becker,T., Hurt,E. and Beckmann,R. (2017) Cryo-EM structure of a late pre-40S ribosomal subunit from *Saccharomyces cerevisiae*. *eLife*, **6**, e30189.

85. Scaiola,A., Peña,C., Weisser,M., Böhringer,D., Leibundgut,M., Klingauf-Nerurkar,P., Gerhardy,S., Panse,V.G. and Ban,N. (2018) Structure of a eukaryotic cytoplasmic pre-40S ribosomal subunit. *EMBO J.*, **37**, e98499.

86. Ameismeier,M., Cheng,J., Berninghausen,O. and Beckmann,R. (2018) Visualizing late states of human 40S ribosomal subunit maturation. *Nature*, **558**, 249–253.

87. Ameismeier,M., Zemp,I., van den Heuvel,J., Thoms,M., Berninghausen,O., Kutay,U. and Beckmann,R. (2020) Structural basis for the final steps of human 40S ribosome maturation. *Nature*, **587**, 683–687.

88. Sardana,R., Liu,X., Granneman,S., Zhu,J., Gill,M., Papoulas,O., Marcotte,E.M., Tollervey,D., Correll,C.C. and Johnson,A.W. (2015) The DEAH-box helicase Dhr1 dissociates U3 from the pre-rRNA to

promote formation of the central pseudoknot. *PLoS Biol.*, **13**, e1002083.

89. Choudhury,P., Hackert,P., Memet,I., Sloan,K.E. and Bohnsack,M.T. (2019) The human RNA helicase DHX37 is required for release of the U3 snoRNP from pre-ribosomal particles. *RNA Biol.*, **16**, 54–68.

90. Du,Y., An,W., Zhu,X., Sun,Q., Qi,J. and Ye,K. (2020) Cryo-EM structure of 90S small ribosomal subunit precursors in transition states. *Science*, **369**, 1477–1481.

91. Cheng,J., Lau,B., La Venuta,G., Ameismeier,M., Berninghausen,O., Hurt,E. and Beckmann,R. (2020) 90S pre-ribosome transformation into the primordial 40S subunit. *Science*, **369**, 1470–1476.

92. Sloan,K.E., Knox,A.A., Wells,G.R., Schneider,C. and Watkins,N.J. (2019) Interactions and activities of factors involved in the late stages of human 18S rRNA maturation. *RNA Biol.*, **16**, 196–210.

93. Wang,M. and Pestov,D.G. (2011) 5′-end surveillance by Xrn2 acts as a shared mechanism for mammalian pre-rRNA maturation and decay. *Nucleic Acids Res.*, **39**, 1811–1822.

94. Thoms,M., Thomson,E., Baßler,J., Gnädig,M., Griesel,S. and Hurt,E. (2015) The exosome is recruited to RNA substrates through specific adaptor proteins. *Cell*, **162**, 1029–1038.

95. Toczydlowska-Socha,D., Zielinska,M.M., Kurkowska,M., Astha,Almeida, Stefaniak,C.F., F.,Purta,E. and Bujnicki,J.M. (2018) Human RNA cap1 methyltransferase CMTr1 cooperates with RNA helicase DHX15 to modify RNAs with highly structured 5′ termini. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **373**, 20180161.

96. Inesta-Vaquera,F., Chaugule,V.K., Galloway,A., Chandler,L., Rojas-Fernandez,A., Weidlich,S., Peggie,M. and Cowling,V.H. (2018) DHX15 regulates CMTR1-dependent gene expression and cell proliferation. *Life Sci. Alliance*, **1**, e201800092.

97. Leppek,K., Das,R. and Barna,M. (2018) Functional 5′ UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nat. Rev. Mol. Cell Biol.*, **19**, 158–174.

98. Taschuk,F. and Cherry,S. (2020) DEAD-box helicases: sensors, regulators, and effectors for antiviral defense. *Viruses*, **12**, 181.

99. Pattabhi,S., Knoll,M.L., Gale,M. Jr and Loo,Y.-M. (2019) DHX15 is a coreceptor for RLR signaling that promotes antiviral defense against RNA virus infection. *J. Interferon Cytokine Res.*, **39**, 331–346.

100. Mosallanejad,K., Sekine,Y., Ishikura-Kinoshita,S., Kumagai,K., Nagano,T., Matsuzawa,A., Takeda,K., Naguro,I. and Ichijo,H. (2014) The DEAH-box RNA helicase DHX15 activates NF-κB and MAPK signaling downstream of MAVS during antiviral responses. *Sci. Signal.*, **7**, ra40.

101. Wang,P., Zhu,S., Yang,L., Cui,S., Pan,W., Jackson,R., Zheng,Y., Rongvaux,A., Sun,Q., Yang,G. *et al.* (2015) Nlrp6 regulates intestinal antiviral innate immunity. *Science*, **350**, 826–830.

102. Xing,J., Zhou,X., Fang,M., Zhang,E., Minze,L.J. and Zhang,Z. (2021) DHX15 is required to control RNA virus-induced intestinal inflammation. *Cell Rep.*, **35**, 109205.

103. Shen,C., Li,R., Negro,R., Cheng,J., Vora,S.M., Fu,T.-M., Wang,A., He,K., Andreeva,L., Gao,P. *et al.* (2021) Phase separation drives RNA virus-induced activation of the NLRP6 inflammasome. *Cell*, **184**, 5759–5774.

104. Wlodarska,M., Thaiss,C.A., Nowarski,R., Henao-Mejia,J., Zhang,J.-P., Brown,E.M., Frankel,G., Levy,M., Katz,M.N., Philbrick,W.M. *et al.* (2014) NLRP6 inflammasome orchestrates the colonic host–microbial interface by regulating goblet cell mucus secretion. *Cell*, **156**, 1045–1059.

105. Elinav,E., Strowig,T., Kau,A.L., Henao-Mejia,J., Thaiss,C.A., Booth,C.J., Peaper,D.R., Bertin,J., Eisenbarth,S.C., Gordon,J.I. *et al.* (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*, **145**, 745–757.

106. Levy,M., Shapiro,H., Thaiss,C.A. and Elinav,E. (2017) NLRP6: a multifaceted innate immune sensor. *Trends Immunol.*, **38**, 248–260.

107. Mosallanejad,K., Sekine,Y., Ishikura-Kinoshita,S., Kumagai,K., Nagano,T., Matsuzawa,A., Takeda,K., Naguro,I. and Ichijo,H.

108. Lu,H., Lu,N., Weng,L., Yuan,B., Liu,Y.-J. and Zhang,Z. (2014) DHX15 senses double-stranded RNA in myeloid dendritic cells. *J. Immunol.*, **193**, 1364–1372.

109. Pan,Y.-Q. and Xing,L. (2021) The current view on the helicase activity of RNA helicase A and its role in gene expression. *Curr. Protein Pept. Sci.*, **22**, 29–40.

110. Azzalin,C.M. and Lingner,J. (2006) The human RNA surveillance factor UPF1 is required for S phase progression and genome stability. *Curr. Biol.*, **16**, 433–439.

111. Chamieh,H., Ballut,L., Bonneau,F. and Le Hir,H. (2008) NMD factors UPF2 and UPF3 bridge UPF1 to the exon junction complex and stimulate its RNA helicase activity. *Nat. Struct. Mol. Biol.*, **15**, 85–93.

112. Chawla,R., Redon,S., Raftopoulou,C., Wischnewski,H., Gagos,S. and Azzalin,C.M. (2011) Human UPF1 interacts with TPP1 and telomerase and sustains telomere leading-strand replication. *EMBO J.*, **30**, 4047–4058.

113. Kim,Y.K., Furic,L., Desgroseillers,L. and Maquat,L.E. (2005) Mammalian Staufen1 recruits Upf1 to specific mRNA 3′UTRs so as to elicit mRNA decay. *Cell*, **120**, 195–208.

114. Choe,J., Ryu,I., Park,O.H., Park,J., Cho,H., Yoo,J.S., Chi,S.W., Kim,M.K., Song,H.K. and Kim,Y.K. (2014) eIF4AIII enhances translation of nuclear cap-binding complex-bound mRNAs by promoting disruption of secondary structures in 5′UTR. *Proc. Natl Acad. Sci. U.S.A.*, **111**, E4577–E4586.

115. Alexandrov,A., Colognori,D. and Steitz,J.A. (2011) Human eIF4AIII interacts with an eiF4G-like partner, NOM1, revealing an evolutionarily conserved function outside the exon junction complex. *Genes Dev.*, **25**, 1078–1090.

116. Barbosa,I., Haque,N., Fiorini,F., Barrandon,C., Tomasetto,C., Blanchette,M. and Le Hir,H. (2012) Human CWC22 escorts the helicase eIF4AIII to spliceosomes and promotes exon junction complex assembly. *Nat. Struct. Mol. Biol.*, **19**, 983–990.

117. Buchwald,G., Schussler,S., Basquin,C., Le Hir,H. and Conti,E. (2013) Crystal structure of the human eIF4AIII–CWC22 complex shows how a DEAD-box protein is inhibited by a MIF4G domain. *Proc. Natl Acad. Sci. U.S.A.*, **110**, E4611–E4618.

118. Alexandrov,A., Colognori,D. and Steitz,J.A. (2011) Human eIF4AIII interacts with an eIF4G-like partner, NOM1, revealing an evolutionarily conserved function outside the exon junction complex. *Genes Dev.*, **25**, 1078–1090.

119. Lingaraju,M., Johnsen,D., Schlundt,A., Langer,L.M., Basquin,J., Sattler,M., Heick Jensen,T., Falk,S. and Conti,E. (2019) The MTR4 helicase recruits nuclear adaptors of the human RNA exosome using distinct arch-interacting motifs. *Nat. Commun.*, **10**, 3393.

120. Wang,J., Chen,J., Wu,G., Zhang,H., Du,X., Chen,S., Zhang,L., Wang,K., Fan,J., Gao,S. *et al.* (2019) NRDE2 negatively regulates exosome functions by inhibiting MTR4 recruitment and exosome interaction. *Genes Dev.*, **33**, 536–549.

121. Zhang,Y., O'Leary,M.N., Peri,S., Wang,M., Zha,J., Melov,S., Kappes,D.J., Feng,Q., Rhodes,J., Amieux,P.S. *et al.* (2017) Ribosomal proteins Rpl22 and Rpl22l1 control morphogenesis by regulating pre-mRNA splicing. *Cell Rep.*, **18**, 545–556.

122. Lin,M.-L., Fukukawa,C., Park,J.-H., Naito,K., Kijima,K., Shimo,A., Ajiro,M., Nishidate,T., Nakamura,Y. and Katagiri,T. (2009) Involvement of G-patch domain containing 2 overexpression in breast carcinogenesis. *Cancer Sci.*, **100**, 1443–1450.

123. Niu,Z., Jin,W., Zhang,L. and Li,X. (2012) Tumor suppressor RBM5 directly interacts with the DExD/H-box protein DHX15 and stimulates its helicase activity. *FEBS Lett.*, **586**, 977–983.

124. Zhou,X.Z. and Lu,K.P. (2001) The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. *Cell*, **107**, 347–359.