ANNUAL REVIEWS

Annual Review of Biochemistry

The Role of DEAD-Box ATPases in Gene Expression and the Regulation of RNA–Protein Condensates

Karsten Weis[1] and Maria Hondele[2]

[1]Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich, Switzerland; email: karsten.weis@bc.biol.ethz.ch

[2]Biozentrum, University of Basel, Basel, Switzerland; email: maria.hondele@unibas.ch

Annu. Rev. Biochem. 2022. 91:197–219

First published as a Review in Advance on March 18, 2022

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

https://doi.org/10.1146/annurev-biochem-032620-105429

Keywords

DEAD-box ATPases, gene expression, ribonucleoprotein complexes, RNA unwinding, biomolecular condensates, membraneless organelles

Abstract

DEAD-box ATPases constitute a very large protein family present in all cells, often in great abundance. From bacteria to humans, they play critical roles in many aspects of RNA metabolism, and due to their widespread importance in RNA biology, they have been characterized in great detail at both the structural and biochemical levels. DEAD-box proteins function as RNA-dependent ATPases that can unwind short duplexes of RNA, remodel ribonucleoprotein (RNP) complexes, or act as clamps to promote RNP assembly. Yet, it often remains enigmatic how individual DEAD-box proteins mechanistically contribute to specific RNA-processing steps. Here, we review the role of DEAD-box ATPases in the regulation of gene expression and propose that one common function of these enzymes is in the regulation of liquid–liquid phase separation of RNP condensates.

Contents

GENERAL OVERVIEW OF THE DEAD-BOX AND DExH BOX SF2 HELICASES

RNA has likely existed since the origin of life. Being single-stranded and not confined to one cellular location, RNA is more reactive and dynamic than its relative, DNA. For example, messenger RNAs (mRNAs) are produced and processed in the nucleus, but they subsequently migrate to the cytoplasm, where they recruit ribosomes and matching transfer RNAs (tRNAs) to undergo translation. RNAs can also fold into well-defined structures that influence their function. In the case of mRNAs, this is exemplified by the effects of structured untranslated regions (UTRs) on ribosome recruitment and translation efficiency (1, 2).

However, due to the four-base composition of RNA, there is also a chance of unintended, random base pairing, either with itself or with other RNAs (3). Furthermore, the phosphate backbone makes the RNA molecule highly negatively charged overall. As a consequence, RNA polymers are intrinsically prone to aggregate and form thermodynamically stable but not always useful, and potentially even harmful, side products. As one measure of protection, RNAs interact at any stage of their life with RNA-binding proteins and form ribonucleoprotein (RNP) complexes (4–7). Yet, the proteins that coat the RNA also create challenges for cells, since many RNA-binding proteins have affinities in a range that prevents easy or rapid dissociation, and therefore, the RNP coat cannot be readily exchanged all by itself. However, a dynamic RNP composition is critical for correct RNA processing, transport, and function (5, 8–14).

To keep RNP complexes dynamic and to safeguard RNA against misfolding or aggregation, cells contain enzymes that can unfold thermodynamically stable RNA structures and remodel RNA–protein complexes. These enzymes are expressed by all cells from bacteria to humans, often in great abundance. They are highly conserved across evolution and can even be found in many viruses. These enzymes consist of a multitude of RNA helicases or RNA chaperones, and their basic mechanistic principle is simple: They utilize ATP to bind and remodel RNA and RNA–protein complexes (15–17).

Most cellular RNA helicases (Enzyme Commission number 3.6.4.13) belong to the superfamily 2 (SF2) of nucleic acid helicases (18, 19). SF2 helicases encompass at least nine families plus one

group that all share a highly similar core structure characterized by two so-called RecA domains, D1 and D2, which are connected by a flexible linker (16, 17). This RecA core, named after its homology to the bacterial recombination factor RecA (20), contains 12 conserved sequence motifs for RNA binding, ATP binding, and interaction between the RecA domains (16, 17, 21).

The two most abundant families of SF2 helicases are DEAD-box proteins and the subfamilies of DExH-box proteins. They both received their names from the amino acid motifs that are found in the catalytic core responsible for ATP hydrolysis. The more complex organisms are, the greater their number of DEAD-box or DExH-box ATPases; e.g., bacteria express approximately 5; *Saccharomyces cerevisiae*, 26; and human cells, 37 DEAD-box proteins (16). Despite the very similar core structures of DEAD- and DExH-box ATPases, several divergences result in major functional differences between these groups of enzymes. First and most importantly, DEAD-box ATPases harbor only one surface on the RecA core that interacts with single-stranded RNA or RNA duplexes to trigger unwinding or protein displacement. On their own, DEAD-box ATPases are therefore nonprocessive and act in a single turnover reaction on short RNA duplexes. In contrast, DExH-box ATPases have additional C-terminal globular domains that allow for stable RNA binding and translocation along one of the RNA strands in the 3′–5′ direction. This enables a processive movement of DExH-box ATPases along RNA duplexes, during which they displace one RNA strand and any bound protein molecules (17, 21, 22).

DEAD-BOX ATPASES: GENERAL STRUCTURE AND BIOCHEMICAL FUNCTION

The family of DEAD-box ATPases was first discovered more than 30 years ago (23), and since then, it has become clear that these proteins function in all facets of RNA biology. They chaperone mRNAs from transcription to decay, are heavily involved in nucleolar ribosomal RNA (rRNA) maturation and ribosome biogenesis, and function in many other RNA-processing reactions as well (16, 21, 24). In the unbound (apo) state, the two RecA domains of most DEAD-box proteins have a flexible orientation and an open conformation (**Figure 1**) (17, 22, 25–27). ATP and RNA binding are usually coupled. ATP primarily binds the D1 RecA domain, and single- or double-stranded RNA interacts with a basic surface on the D2 RecA. RNA binding is generally not sequence specific, since the interaction with the RecA domain involves only the phosphate groups of one strand and the hydroxyl group of the other but not the bases (17, 22, 28). ATP binding enables additional interactions between the two RecA domains, involving some of the 12 conserved sequence motifs increasing the affinity between D1 and D2. This leads to a conformational rearrangement, which brings the two domains to a relatively fixed and closed D1–D2 orientation that buries ATP (**Figure 1**) (17, 22).

Domain closure has two critical consequences. First, it creates an enlarged RNA-binding surface that spans both RecA domains, resulting in a more stable interaction with the phosphate backbone of one RNA strand. Importantly, RNA binding occurs in a kinked or arch-like shape, which is incompatible with RNA duplex formation and thus causes the eviction of the other RNA strand (28–33). Second, domain closure promotes ATP hydrolysis, which in turn lowers the D1–D2 affinity, resulting in domain opening, decreased RNA affinity, and release of all substrates, thus completing the cycle (**Figure 1**) (17, 22, 34). The efficiency of unwinding decreases with RNA duplex length: DEAD-box proteins are nonprocessive, and the RNA–RNA interactions have to be outcompeted by the RNA–RecA affinity, which becomes very inefficient for duplexes longer than ~15 base pairs (16, 35).

Although RNA duplex unwinding by DEAD-box ATPases has been very well characterized, it is not the only remodeling or chaperoning activity of these enzymes. They were also shown to

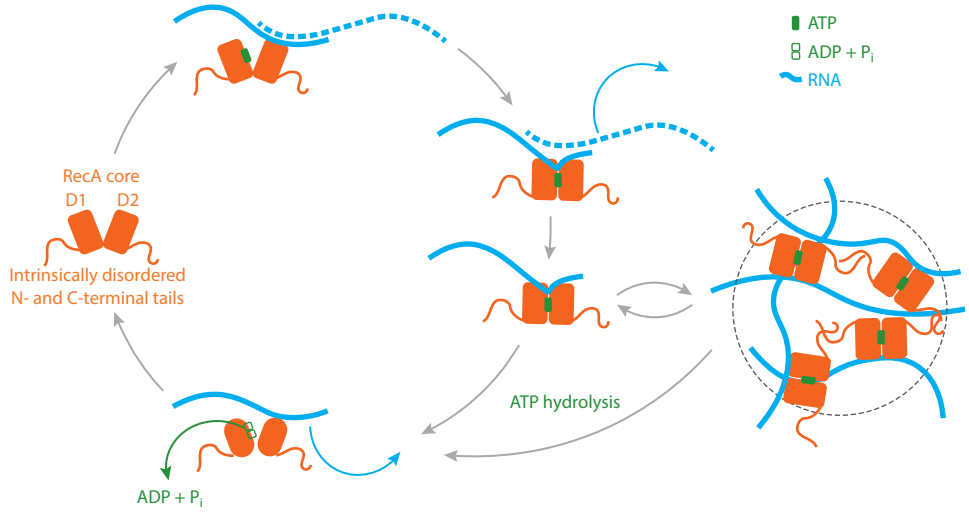

Figure 1

Model of the DEAD-box ATPase cycle. ATP binding brings the two RecA domains (D1 and D2) of the DEAD-box protein into a closed conformation, creating an extended RNA-binding surface that enables interactions with the RNA phosphate backbone of one strand. Binding causes bending of the RNA strand into an arch, which is incompatible with its interaction with another RNA strand as a duplex and can also displace associated proteins. ATP hydrolysis breaks the interdomain contacts between the D1 and D2 RecA domains and triggers release of RNA and ADP + P_i, and the cycle can restart. In the ATP-bound state, DEAD-box proteins can also form stable clamps on RNA, which can lead through multivalency to the formation of higher-order RNP complexes or RNP condensates. Abbreviation: RNP, ribonucleoprotein.

promote RNA-strand annealing or exchange (36), to remodel G-quadruplex structures in both DNA and RNA, and to unwind RNA–DNA hybrids (R-loops) that have important physiological functions but can also trigger DNA damage and genome instability (16, 19, 37).

Another fundamental but less well-characterized function of DEAD-box ATPases is their ability to dissociate or remodel RNA–protein complexes or to function as RNPases. DEAD-box proteins can displace proteins without even unwinding an RNA duplex (38, 39), and this process seems to be somewhat selective toward RNA interactions on the surface of RNPs (16, 17, 33).

The ATPase activity and the frequency of the DEAD-box ATPase cycle are crucial to regulating RNA binding and chaperoning in many RNA-processing reactions. It is therefore not surprising that DEAD-box ATPases are tightly controlled. In fact, many DEAD-box ATPases are inefficient enzymes by themselves and require interactions with activators to overcome kinetic bottlenecks. Notably, in many instances, it is not the ATP hydrolysis step but rather the release of substrate (RNA, ADP + P_i) that is rate limiting and critical for resetting the ATPase cycle (15, 22, 40).

The family of MIF4G-domain-containing proteins [named after the middle domain of the eukaryotic initiation factor 4G (eIF4G)] is the most prominent among DEAD-box ATPase regulators. Interestingly, MIF4G proteins can both activate and inhibit enzymatic activity. The MIF4G fold consists of α-helical HEAT repeats that, through interactions with both RecA domains, hold the domains in a specific orientation. Activating MIF4G proteins like Gle1 for DDX19/Dbp5 or Not1 for DDX6/Dhh1 stabilize a transition state that facilitates ADP/P_i and RNA release (41–44). By contrast, binding of inhibiting MIF4G domains induces different RecA core conformations; for example, the MIF4G domain of CWC22 latches onto both RecA folds of eIF4AIII and spatially

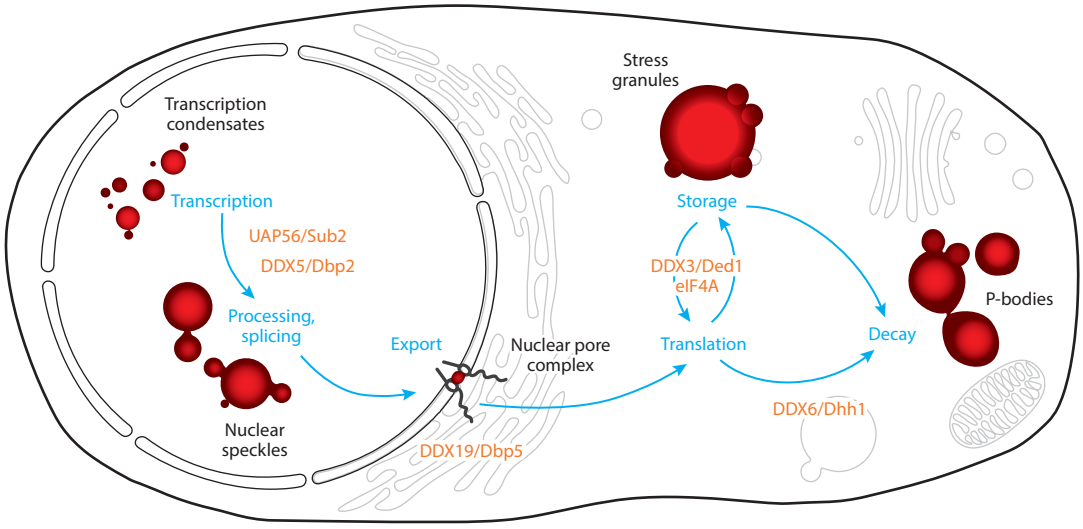

Figure 2

Different DEAD-box ATPases function in multiple steps of gene expression that are closely associated with distinct biomolecular condensates and membraneless organelles. This includes the processes of transcription, pre-mRNA splicing, transport though the nuclear pore complex, translation, and mRNA degradation. Abbreviation: P-bodies, processing bodies

separates the RNA- and ATP-binding residues on D1 from their counterparts on D2 to prevent substrate binding (22, 45).

In the absence of ATP hydrolysis or substrate release, e.g., due to the action of inhibitory proteins, or by the introduction of hydrolysis-deficient, catalytically dead mutants (such as the DQAD variant of the DEAD motif), DEAD-box proteins remain stably bound to the RNA and can also serve as an RNA clamp or scaffold that aids with the recruitment of additional proteins to the RNA (16, 46). Such a stably bound enzyme complex can further trigger the formation of multivalent, higher-order RNP granules that can assemble into large biomolecular condensates and manifest as constitutive membraneless organelles in cells (**Figures 1** and **2**) (reviewed in 47–52). Similarly, nonhydrolyzable ATP analogs, especially those mimicking the ground state, can induce the formation of long-lived complexes with RNA (34). In contrast, mutants that prevent ATP binding (e.g., the Q-motif) (53, 54) and mutants that prevent the switch from the open to the closed D1–D2 state (e.g., motif VI, HRxGRxxR) (55) decrease RNA binding and, in consequence, overall ATP consumption (reviewed in 16, 18).

DEAD-BOX ATPASES INVOLVED IN THE REGULATION OF GENE EXPRESSION

With such a similar core mechanism that is shared by all DEAD-box proteins, several central questions regarding their physiological function have remained unanswered: Why are there so many different DEAD-box ATPases, almost 40 in human cells? How can they function in specific RNA-processing steps, given their apparent lack of sequence specificity? Why are these enzymes so heavily expressed and among the most abundant cellular proteins, with close to the same expression levels as ribosomal proteins? Recent work has begun to provide the first insights into these questions. Whereas their RecA core fold may have a very similar structure and general function, DEAD-box proteins differ in their cellular localization, catalytic activity, and regulation by cofactors. In addition, they also contain a striking array of intrinsically disordered N- and

C-terminal extensions or tails that critically contribute to the diverse cellular roles of DEAD-box ATPases. These tails can autoinhibit or regulate the ATPase activity (for example, 41, 56–59) and orchestrate interactions with other protein complexes or RNA targets (for example, 33, 60–65). In addition, in many cases these tails allow for the condensation of these abundant proteins in membraneless organelles (**Figures 1** and **2**) (49, 50, 66–72). Condensation can enrich these enzymes together with their RNA substrates in specific subcellular locations, which might facilitate remodeling by mass action. In the following sections, we summarize our current knowledge of the role of DEAD-box ATPases in the regulation of RNP remodeling and condensation, focusing on the subgroup of DEAD-box proteins that function directly in the regulation of gene expression.

UAP56/Sub2

UAP56/Sub2 (also known as DDX39B) is a highly conserved DEAD-box ATPase that is found in all eukaryotes. It stands out because it contains a DECD motif instead of the eponymous DEAD motif, and this alanine-to-cysteine exchange is conserved in all characterized UAP56/Sub2 orthologs. This suggests that the cysteine in the DEAD-box of UAP56/Sub2 is functionally important, and at least in budding yeast, a strain expressing the DEAD variant of Sub2/yUAP56 is not viable (73). However, as expected, the cysteine is not critical for the ATPase activity of UAP56 (74), and the role of this uniquely conserved alanine substitution remains enigmatic.

UAP56 was originally identified as an interaction partner of the splicing factor $U2AF^{65}$ (73), and UAP56 and its yeast ortholog Sub2 were found to be required for spliceosome assembly in vivo and in vitro (73, 75, 76). However, UAP56/Sub2 was soon discovered to also function in global mRNA export (77–79), even for mRNAs that do not contain introns (77, 78). It has now been firmly established that UAP56/Sub2 is an essential component of the transcription and export (TREX) complex that is generally required for early steps in nuclear mRNP biogenesis (80).

TREX is an evolutionarily conserved multisubunit assembly that contains, in addition to UAP56/Sub2, the mRNA export adaptor ALYREF/Yra1 and the THO (transcription-dependent hyperrecombination) complex. In a mechanistically poorly understood series of events, UAP56/Sub2 is required to bring ALYREF/Yra1 to the THO complex, which in turn triggers loading of the essential export factor NXF1/Mex67 onto mRNA, a critical event during the nuclear export of mRNAs (77, 80–84).

TREX is recruited to mRNAs during transcription and subsequently influences elongation, 3′ end processing, and transcript release (50, 78, 80, 85–90). Timely mRNP processing by TREX is also critical to prevent the formation of R-loops that can form when the nascent transcript anneals with the single-stranded DNA template that is formed behind the elongating RNA polymerase II (37, 91). Formation of unintended R-loops is dangerous because they can trigger replication stress and hyperrecombination and thus affect genome integrity. The helicase activity of UAP56 might be critical to unwind harmful RNA–DNA hybrids and to protect the stability of the genome (91).

Recently, the structural organizations of the yeast and human TREX were characterized by cryo–electron microscopy (92–94). Intriguingly, TREX multimerizes to form a tetramer in humans and a dimer in yeast. Thus, TREX is capable of making multivalent, simultaneous interactions with different mRNA regions in *cis* or distinct mRNA molecules in *trans*. The structures also provide new insight into how THO can stimulate the ATPase activity of UAP56/Sub2 (92–95): Interactions with MIF4G modules in Tho2/THOC2 hold UAP56/Sub2 in a semiopen, activated conformation comparable to that observed for other MIF4G DEAD-box helicase systems (22, 52). The position of UAP56/Sub2 in the complex, together with the conformational changes expected to occur during the ATPase cycle of UAP56/Sub2, further suggests a seesaw-like movement of the complex that could explain how TREX remodels mRNPs or R-loops (92).

In mammalian cells, UAP56 localizes to nuclear speckles, membraneless organelles that contain high concentrations of RNA-processing factors (84, 96). Inhibition of the catalytic activity of UAP56 increases the size of nuclear speckles and blocks mRNA release from this compartment (50). Similarly, mutants in yeast Sub2 inhibit mRNA release from the site of transcription and lead to the formation of RNP granules that show hallmarks of liquid–liquid phase separation (50, 78). Intriguingly, TREX itself contains several domains of low complexity, and such domains often promote phase separation. It will be interesting to examine how the multivalent interactions between TREX and mRNPs, together with the enzymatic activity of UAP56/Sub2, regulate condensation reactions that might occur during transcription, R-loop formation, or pre-mRNA splicing and, in turn, how condensation affects these molecular processes.

DDX5/Dbp2

Human DDX5, initially called p68, is one of the founding members of the DEAD-box ATPase family. When its complementary DNA (cDNA) was sequenced, it became clear that it has extensive sequence homology with the translation initiation factor eIF4A (97). Together with the identification of additional homologous proteins in organisms such as *Escherichia coli*, budding yeast, and *Drosophila*, this led to the definition and birth of the DEAD-box protein family (23). Human DDX5/p68 has a paralog, DDX17/p72. Both show high sequence conservation with the yeast protein Dbp2, and DDX5 expression in yeast complements a *dbp2Δ* strain, demonstrating functional conservation from yeast to humans (98, 99).

Despite its seniority in the DEAD-box family, the detailed cellular function(s) of DDX5/Dbp2 has remained unclear. DDX5/Dbp2 has been implicated in a host of ostensibly unconnected cellular pathways, including transcriptional regulation, pre-mRNA splicing, mRNA export, mRNA quality control, ribosome biogenesis, cellular signaling, and recently, the control of G-quadruplex destabilization (99–101). Such an array of diverse cellular functions might be reflected in the variability of its localization: Although DDX5/Dbp2 is predominantly localized in the nucleus, it can also be enriched in the nucleolus or localized to the cytoplasm at different stages of the cell cycle and in different cell types (99). DDX5/Dbp2 has robust ATP-dependent RNA-binding and helicase activity but does not seem to display a clear sequence specificity; hence, it is conceivable that it functions promiscuously on a wide range of RNP targets. However, it is likely that at least some of the ascribed functions of DDX5/Dbp2, e.g., in cellular signaling, are indirect consequences of its role in the regulation of gene expression and that selectivity for specific RNA targets is achieved through cofactors that regulate its localization or ATPase activity but have yet to be identified.

In budding yeast, the RNA-binding sites of Dbp2 have been mapped by UV cross-linking, and Dbp2p was found to interact with mRNAs and ribosomal RNAs but also with other noncoding RNAs, including small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), and tRNAs (102). Of note, the cross-linking sites in noncoding RNAs often overlap with regions where R-loops form, e.g., in the 3′ end of snoRNAs. These regions also coincide with binding sites for Sen1, a SF1B 5′–3′ RNA/DNA helicase and a member of the Nrd1 complex, which plays an essential role in the transcriptional termination of snRNAs, snoRNAs, cryptic unstable transcripts (CUTs), and some mRNAs. In addition, a physical interaction between Sen1 and Dbp2 was detected by coimmunoprecipitation, which led to the suggestion that the regulation of R-loop formation is a unifying physiological function of Dbp2 (102). Other DEAD-box ATPases, including Sub2/UAP56 (37) (see the section titled UAP56/Sub2) and Dbp5/DDX19 (103), were implicated in the control of R-loop formation, and therefore, the unwinding of harmful RNA–DNA hybrids could be a critical task of DEAD-box ATPases in the nucleus.

A role in transcriptional termination and R-loop prevention could also be connected to a function of Dbp2 in nuclear mRNP assembly that is required for proper mRNA export. On one hand, DBP2 displays genetic interaction with the mRNA export adaptor YRA1, and Yra1 inhibits the helicase activity of Dbp2 in vitro (104). On the other hand, Dbp2 enhances the loading of Yra1, Nab2, and Mex67 onto poly(A)+ RNA (104). However, it has not been further examined how a reduction of the enzymatic activity of Dbp2 promotes the formation of an export-competent mRNP.

Like many DEAD-box ATPases, DDX5/Dbp2 contains N- and C-terminal extensions flanking its two RecA domains. In DDX5/Dbp2, these tails are predicted to be intrinsically disordered and are particularly enriched in arginine-glycine-glycine repeats. Accordingly, Dbp2 readily phase separates in the presence of ATP and RNA in vitro (50), but whether DDX5/Dbp2 also forms condensates in vivo and whether condensation modulates or contributes to the physiological function of DDX5/Dbp2 await further exploration.

DDX19/Dbp5

DDX19/Dbp5 was initially characterized in budding yeast (105, 106). Mutations in *DBP5* block nuclear mRNA export and, given the localization of DDX19/Dbp5 both in the cytoplasm and at the nuclear pore complex (NPC), it was immediately proposed that its ATPase activity is harnessed to promote the directional export of mRNAs through the NPC (105, 106). This initial hypothesis has been corroborated in the last 20 years. Multiple lines of evidence support a model in which Dbp5 functions at the NPC to remodel exporting RNPs and DDX19/Dbp5-mediated removal of essential export factors such as Nab2 (107) or Mex67/NXF1 (108) confers directionality to the mRNA export process (109).

On its own, DDX19/Dbp5 exists in an autoinhibited conformation (41, 57), and its enzymatic activity is greatly stimulated by the NPC-associated cofactor Gle1 (44, 110, 111). Like other DEAD-box ATPase activators, Gle1 contains a MIF4G domain, which interacts with both RecA domains of Dbp5 to overcome autoinhibition and speed up the ATPase cycle (41). Gle1, together with the nucleoporin Nup159/NUP214, also tethers Dbp5 to the NPC (110, 112–117), which ensures localized activation of Dbp5/DDX19 specifically at the cytoplasmic side of the NPC, which is presumably essential for the function of Dbp5 in mRNA export. A peculiarity of the NPC-associated activation of Dbp5 is the fact that the interaction between Dbp5 and Gle1, at least in fungi, is stabilized by the small molecule inositol hexakisphosphate ($InsP_6$), which potentially adds another layer of regulation to the Dbp5 ATPase cycle and mRNA export (41, 109, 118). In this context, it is of interest that Dbp5 leaves the NPC and relocalizes to the nuclear interior following ethanol stress and UV irradiation (103, 119, 120).

In addition to its canonical function in mRNA export, Dbp5 has also been implicated in a variety of other cellular processes. These include the regulation of transcription in the nucleus and termination of translation in the cytoplasm. Interestingly, when Dbp5 is permanently tethered to the NPC by artificially fusing it to the nucleoporin Nup159, yeast cells are viable, suggesting that these nuclear and cytoplasmic functions of Dbp5 are not essential during normal growth conditions (121).

Dbp5 was also shown to function in the export of tRNAs, ribosomal precursors, and telomerase RNA (122–124). Given its apparent lack of RNA-sequence specificity, it is not obvious how Dpb5 would restrict its activity at the NPC to remodel exclusively mRNAs, and therefore, a more general RNPase role for Dbp5 in the export of different RNA substrates could be plausible. However, at least in the export of rRNA, the ATPase activity of Dbp5 appears to be dispensable, indicating that Dbp5 relies on different modes of action to promote the export of distinct RNA classes (123).

Clearly, additional work is needed to better understand the mechanisms of RNA export and to better define how DDX19/Dbp5 functions in the directional transport of mRNA and potentially other RNA substrates across the NPC. Interestingly, trypanosomes, and potentially also other organisms outside the opisthokonta clade (previously called the Fungi/Metazoa group), seem to lack parts of or even the entire mRNA export platform consisting of DDX19/Dbp5, Gle1, and NUP214/Nup159 (125, 126). Furthermore, in *Drosophila* tissue culture cells, it was observed that mRNA export does not require Dbp5 function (127). Since the directional export of mRNA is likely to be essential in all eukaryotic cells, additional pathways seem to exist that translocate mRNAs across the NPC.

In addition, one needs to consider other functions of DEAD-box ATPases such as Dbp5/DDX19 at the NPC. Given the predicted high RNP concentrations within the crowded, potentially phase-separated NPC transport channel (128), this could include a role in the disentanglement of short RNA–RNA duplexes or the inhibition of aggregation of condensation-prone RNPs that could otherwise clog the NPC and block RNA transport.

eIF4A

The eukaryotic translation initiation factor 4A (eIF4A/DDX2) was the first DEAD-box protein whose sequence was identified (129). eIF4A is among the most abundant DEAD-box proteins and plays an essential role in early steps of protein translation. It has also been implicated in cancer cell growth and is critical for the replication of many viruses (130–132). It is therefore not surprising that eIF4A has been extensively characterized and that small-molecule inhibitors of its activity have been identified that show promise as anticancer therapeutics (1, 131, 132). Several landmark studies that established central principles for the structure and function of DEAD-box proteins were performed with eIF4A (132). This includes the detailed biochemical characterization of its activities as an RNA-dependent ATPase and ATP-dependent helicase (35, 133–137) and the first structural characterization of a DEAD-box protein (25, 138, 139). While eIF4A has served as a paradigm for DEAD-box protein function, its overall domain organization is an exception in the family, as it is made up exclusively of the central RecA core and lacks the N- and C-terminal extensions that are present in most other family members.

A crucial function of eIF4A is in translation initiation. As part of the heterotrimeric eIF4F complex, eIF4A facilitates the recruitment of the 40S ribosomal subunit to capped mRNAs (131, 132). In addition to eIF4A, eIF4F also contains eIF4G, which is a large scaffold protein, and eIF4E, which directly binds to the m7G cap structure. eIF4A and its interaction partners function with a large network of translation initiation factors and, together with the 40S subunit, form the 43S preinitiation complex that is thought to scan the 5′ UTR in a 5′–3′ direction until it encounters the initiation codon. Given its ability to unwind RNA duplexes, the prevailing model is that eIF4A functions in the scanning mechanism by unwinding any secondary structures present in the 5′ UTR of mRNAs. This model is supported by the observation that the translation of mRNAs with strong secondary structures in their 5′ UTR is more affected by eIF4A mutants than is the translation of mRNAs with little structure (140). eIF4A alone is a weak, nonprocessive helicase, and the scanning process is likely aided by other cofactors and additional unwinding enzymes such as DDX3/Ded1 (see the section titled DDX3/Ded1) (141–146). However, eIF4A has also been shown to enhance preinitiation complex recruitment to mRNAs regardless of the amount of secondary structure (147) and independent of its helicase function (148). These results reveal that eIF4A has a critical function in translation initiation beyond the unwinding of 5′ UTR structures.

Interestingly, eIF4A was also recently shown to regulate the formation of stress granules (51), membraneless organelles that form through the condensation of untranslated mRNPs (149–151).

Intermolecular RNA–RNA interactions contribute to stress granule formation and RNP condensation, likely by enhancing the interaction valency (152–156). The ATP-dependent RNA-binding activity of eIF4A can counteract RNA–RNA multimerization and thus inhibit RNA condensation in vitro and stress granule formation in vivo (51). This adds important additional evidence that DEAD-box ATPases regulate RNP condensation in cells through their ability to function as ATP-dependent RNA-binding proteins that can unwind short RNA duplexes.

Mammalian and plant cells express three eIF4A proteins. eIF4A-I (DDX2A) and eIF4A-II (DDX2B) share more than 90% sequence identity and are thought to have largely overlapping cellular functions. However, they are differentially expressed and might not functionally complement each other (132). The third eIF4A protein is eIF4A-III (DDX48). It is found predominantly in the nucleus and plays a critical role in the formation of the exon-junction complex (EJC), a protein mark that is deposited during pre-mRNA splicing, influencing downstream processing reactions such as mRNA export, translation, and nonsense-mediated decay (157, 158). Reconstitution and structural studies have demonstrated that eIF4A-III is at the core of the EJC. Through interactions with other EJC members, mainly MAGOH-Y14, eIF4A-III is kept in a closed, RNA-bound state, even when ATP hydrolysis has occurred (26, 27, 46, 159). This stably locks eIF4A-III and the entire EJC onto the RNA substrate, providing a paradigm of how DEAD-box ATPases can function as clamps to promote stable RNP complex formation.

DDX6/Dhh1

DDX6/Dhh1, also termed RCKp54 in mouse, Xp54 in *Xenopus*, Me31b in *Drosophila*, and CGH1 in *Caenorhabditis elegans*, is a very abundant, evolutionarily highly conserved, cytoplasmic DEAD-box ATPase. In budding yeast, Dhh1 was first functionally characterized as a regulator of mRNA turnover (160, 161). By contrast, its orthologs, e.g., in *Xenopus*, were identified as components of translationally silenced mRNPs and shown to be required for the repression of translation (162, 163). A role for Dhh1 in translational repression was subsequently also confirmed in yeast (164), and the effects of Dhh1 on translation can be uncoupled from its effects on mRNA decay (165, 166). Nonetheless, the enhancement of mRNA turnover by Dhh1 was proposed to be a consequence of its function as a translational repressor since the processes of translation and decay are tightly coupled in cells, and the inhibition or slowing down of translation enhances mRNA turnover (167–170). However, at present how the ATPase DDX6 represses translation and how the processes of mRNA translation and turnover are linked remain mechanistically poorly explored. Yet, the effects of DDX6 on mRNA fate and translation have a widespread physiological impact, since DDX6 and its orthologs are involved in numerous cellular and developmental processes, including the regulation of meiosis, the antiviral response, and the renewal and differentiation of stem cells (163, 171–175).

An important cellular role of DDX6 lies in the control of mRNP condensation, and DDX6/Dhh1 is a critical regulator of processing body (P-body) formation. P-bodies are membraneless organelles that form via phase separation in the cytoplasm in the presence of untranslated mRNAs (149, 151, 176–178). Whereas the function of P-bodies is still debated, it is likely linked to the physiological effects of DDX6 described earlier in this section and DDX6's role in regulating the cytoplasmic fate of mRNAs by controlling their translation, storage, and decay. The activity of DDX6/Dhh1 is required for efficient P-body formation, and DDX6/Dhh1 can multimerize and coat mRNAs along their length; also, mutants that prevent ATP hydrolysis in yeast lead to formation of constitutive P-bodies (49, 165, 179). In addition to the ATP- and RNA-binding activity of DDX6/Dhh1, the N- and C-terminal extensions that flank its RecA core also play a critical role in P-body assembly (50). These tails are predicted to be intrinsically disordered

and have a low amino acid sequence complexity, one hallmark of protein domains that can undergo liquid–liquid phase separation. Accordingly, these termini promote phase separation in vitro (50, 69). Interestingly, in the presence of RNA and ATP, recombinant Dhh1 forms dynamic phase-separated condensates that can be dissolved upon stimulation of Dhh1's ATPase activity by its MIF4G-containing activator Not1 (49, 180). Since a large number of DEAD-box ATPases in organisms from bacteria to humans contain tails with low sequence complexity, Dhh1 has served as a general paradigm of how DEAD-box ATPases can enhance RNP condensation in cells (50, 52).

DDX3/Ded1

DDX3/Ded1 is another abundant, cytoplasmic DEAD-box ATPase that functions at the interphase of mRNA translation and storage. DDX3/Ded1 is highly conserved across evolution, and all eukaryotic organisms seem to express at least one member of this DEAD-box subfamily (181). Budding yeast contains two paralogs, DED1 and DBP1, that are differentially expressed but at least partially redundant (145, 181). The biochemical characteristics of DDX3/Ded1 have been examined in great detail. It can act as an RNA-dependent ATPase and ATP-dependent RNA helicase (182), but it can also promote RNA strand annealing (183), function as an RNPase that displaces proteins from RNA (38, 39), and form stable RNA clamps (34). Importantly, DDX3 also has clinical relevance, since mutations in DDX3 are commonly found in cancers, most notably medulloblastoma, a highly aggressive cerebellar tumor (184–187), and are linked to intellectual disability (188). Furthermore, DDX3 is the target of multiple viruses (181, 189, 190).

DDX3/Ded1 plays an essential role in the initiation of translation, where it functions together with eIF4A (141, 142, 144, 146, 181). Loss of function of DED1 in yeast leads to the inhibition of global protein synthesis and causes a reduction of polysomes with a concomitant accumulation of nontranslating 80S ribosomes (191, 192). In addition, Ded1 displays physical and genetic interactions with the translation initiation machinery (181). Several recent transcriptome-wide studies have provided important additional insight into the mechanism of DDX3/Ded1 function during translation initiation (71, 143, 145, 193). First, DDX3/Ded1 associates with the translation preinitiation complex via interactions with the ribosomal rRNA right next to the mRNA entry channel (145, 193). Second, whereas DDX3/Ded1 has a general role in the translation of many housekeeping genes, its inactivation particularly affects mRNAs with long and structure-prone 5′ UTRs (71, 145, 193, 194), which can be recapitulated in in vitro reconstitution experiments (195). Together, this suggests that the DDX3/Ded1 ATPase activity plays an important role in resolving secondary structures during preinitiation complex assembly and scanning. Interestingly, in the absence of DDX3/Ded1 function, translation can initiate from near-cognate start codons that are found immediately upstream of unresolved RNA structures (145).

In addition to its function in translational initiation, DDX3/Ded1 was also implicated in translational repression (181, 187, 196). The repressive role of DDX3/Ded1 seems to be tightly linked to its localization to stress granules, membraneless organelles that form through the condensation of mRNPs in stressful situations when general translation is attenuated. Mutations in DDX3/Ded1 that prevent or slow down ATP hydrolysis lead to the formation of bona fide stress granules even in the absence of stress (47, 50, 187). Furthermore, DDX3/Ded1 has intrinsically disordered N- and C-terminal extensions that contain domains of low sequence complexity, and DDX3/Ded1 can form dynamic, RNA-containing condensates in vitro (50, 71). In yeast, it was proposed that in the absence of ATP hydrolysis, a stable Ded1–eIF4F initiation complex assembles on the mRNA, which accumulates in stress granules. Upon ATP hydrolysis, this stalled complex is resolved, leading to the reactivation of translation and the disassembly of stress granules (47). In addition to its role in 5′ UTR scanning, it is conceivable that the

DDX3/Ded1 ATPase also unwinds intermolecular RNA–RNA interactions that occur in stress granules. Since RNA–RNA interactions promote stress granule assembly (152, 153), this would further enhance stress granule dynamics and dissolution.

Not surprisingly, the function of DDX3/Ded1 in translation and stress granule assembly is regulated and, for example, is under the control of the TORC1 pathway, a key regulator of cellular growth in eukaryotes (196). Furthermore, Ded1 itself seems to function as a stress sensor (71). Heat stress directly triggers condensation of Ded1, which causes its functional inactivation. Without Ded1 function, the translation of housekeeping genes is attenuated, whereas the production of stress proteins is stimulated, promoting a switch in the cellular translation program (71).

A COMMON ROLE FOR DEAD-BOX ATPASES AS REGULATORS OF CELLULAR CONDENSATES

The fate of an mRNA molecule from its birth at the site of transcription to its ultimate demise in the cytoplasm is by and large controlled by changes in the associated protein coat. mRNA processing can therefore be viewed as a carefully orchestrated assembly line in which specific sets of proteins get recruited at one step and are subsequently replaced by those required for the next (5, 8). Proteins recruited to the mRNA can modify and read out the nucleotide sequence, target the RNP complex to its next cellular localization, or determine the next processing step. In consequence, the orderly assembly of an RNP complex is critical for all mRNA-processing steps, and a major focus in RNA biology has been to define the mRNP composition during mRNA processing and to characterize the enzymatic machinery that brings about the molecular wardrobe changes that occur during the life of an mRNA.

Due to their biochemical activities as (*a*) ATP-dependent RNA helicases, (*b*) RNPases that remove proteins from RNA, and (*c*) RNA clamps that recruit protein complexes to RNA, DEAD-box proteins are ideal candidates to facilitate these molecular wardrobe changes by both removing old proteins and recruiting new ones. Indeed, as summarized in the section titled DEAD-Box ATPases Involved in the Regulation of Gene Expression, individual members of this family have been implicated in every step of gene expression. However, the molecular and mechanistic details of how these enzymes function to remodel and restructure specific mRNPs at distinct stages of their biogenesis often remain poorly understood.

In the last few years, it has become apparent that a very large number of proteins, especially RNA-binding proteins, can undergo liquid–liquid phase separation, often through weak, multivalent interactions, and form condensates either by themselves or with additional binding partners and RNA (197, 198). RNA can promote phase separation by acting as a flexible scaffold that recruits such RNA-binding proteins, thereby coordinating the formation of higher-order RNP assemblies. Furthermore, RNA–RNA interactions can enhance RNP granule assembly, likely by increasing the valency of the condensation reaction. In addition, RNA alone can phase separate, even without the help of proteins (3, 152–154, 156, 199–202). Thus, many RNPs have a high propensity to form multivalent interactions and cross-link into large networks that are thought to be the basis for the formation of cellular RNP condensates or RNP-containing membraneless organelles (203). As a consequence, a large number of RNA-processing steps either occur in or are associated with membraneless organelles (204–206). Interestingly, all characterized cellular RNP condensates were shown to contain at least one DEAD-box protein (50, 84, 207–216) (**Figure 2**), and there is increasing evidence that DEAD-box ATPases regulate these condensates (52).

One example is eIF4A, which limits stress granule formation in cells by restricting the formation of intermolecular RNA–RNA interactions (51, 154). In this case, the DEAD-box ATPase plays a role akin to the function of protein chaperones, such as HSP70, preventing unwanted

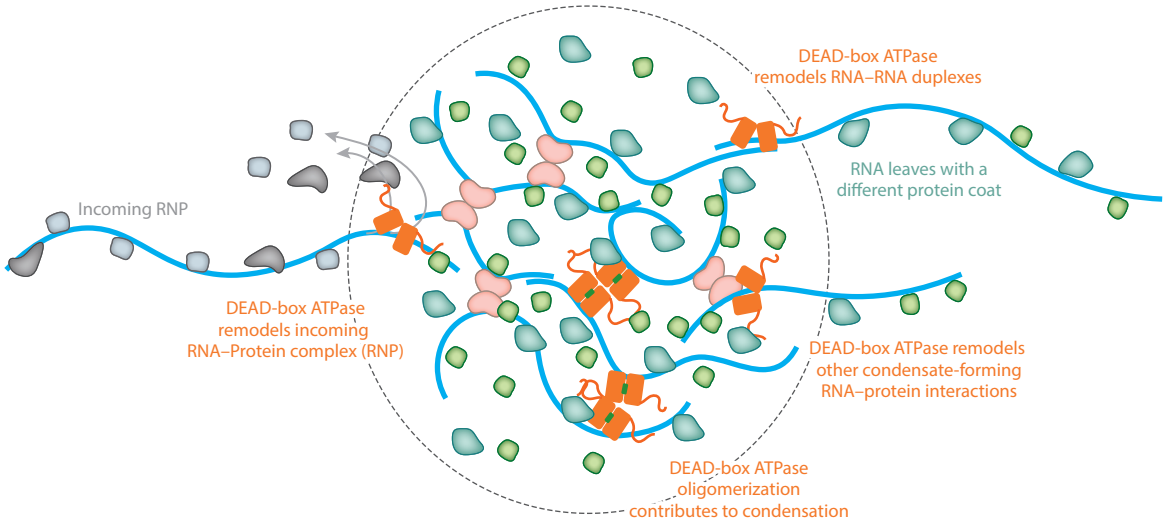

Figure 3

Model for DEAD-box ATPase function in biomolecular condensates. ATP-dependent RNA binding of a DEAD-box protein together with additional cofactors leads to the formation of multivalent, higher-order RNP complexes that can undergo condensation. ATPase activity can lead to the removal of proteins and RNA–RNA interactions. New RNA-binding proteins are recruited and the modified RNP is released. Together this leads to a remodeling of the mRNP, and the mRNP that leaves the compartment is distinct from the one that entered. Abbreviation: RNP, ribonucleoprotein.

aggregation. Given the ability of most DEAD-box proteins to melt short RNA duplexes, it is likely that other DEAD-box proteins can similarly interfere with RNA-mediated phase separation.

However, DEAD-box proteins also enhance condensate formation, since they can remain stably bound to their target. In this case, they become an integral part of the RNP and create additional multivalent interactions either through their core or their intrinsically disordered tails, which are often characterized by low amino acid sequence complexity. Such low-complexity sequences can promote weak homo- and heterotypic interactions but can also bind to RNA (217–219). A general model was proposed to explain how DEAD-box ATPases promote RNP condensation: In their ATP-and-RNA-bound state, DEAD-box proteins form multivalent interactions with RNA and themselves or other phase-separating proteins to trigger condensation. Upon ATP hydrolysis, often stimulated by cofactors such as Not1 for DDX6/Dhh1, RNA clients are released from the DEAD-box protein, thus lowering the valency of the complex. As a consequence, RNA is released from the condensate, and RNA-containing membraneless organelles are disassembled (50) (**Figure 3**).

Hence, DEAD-box ATPases can be viewed as rheostats that allow cells to control the amount of RNA condensation and to fine-tune between assembly and disassembly of RNP-containing membraneless organelles. In a state of low or no ATPase activity, RNA–protein interactions form and promote assembly, whereas high ATPase activity disrupts protein–RNA and RNA–RNA interactions in *cis* and in *trans*, enhancing disassembly (**Figures 1** and **3**) (47–51, 187). We have introduced the term condensase to describe this enzymatic activity of DEAD-box ATPases in regulating the dynamics of biomolecular condensates (52). DEAD-box ATPases work together with other regulators, including chaperones, heat shock proteins, or protein degradation machineries, to control the dynamics of biomolecular condensates. The condensase activity involves all previously ascribed biochemical functions of DEAD-box proteins as RNA helicases, RNA clamps,

and RNPases. There is now evidence from many RNP-containing membraneless organelles that they are regulated by DEAD-box ATPases, and given the high propensity of many mRNPs to form higher-order granules, it is not unlikely that all DEAD-box ATPases directly or indirectly regulate mRNP condensation in cells.

By coupling the ATP hydrolysis cycle of DEAD-box proteins to the dynamics and RNA flux of membraneless organelles, they are kept active and away from equilibrium. Thus, these organelles can generate biochemical work. In principle, they could even create directional fluxes of mRNP molecules between compartments in the absence of membrane boundaries (220). To explain some of the cellular functions of DEAD-box ATPases, we had therefore previously proposed that the various cellular RNP condensates, through their associated DEAD-box proteins, define a network of biochemical reaction centers that help to promote the molecular wardrobe changes that occur during various RNA-processing steps (50). Considering that DEAD-box ATPases are nonprocessive, high enzyme concentrations in such a center could set up a scenario in which many enzymes at the same time would enable the unwinding of more complex target substrates by mass action. Especially compelling is the fact that, upon entry into a condensate, mRNPs are exposed to a biochemically selective environment in which only a subset of the cellular proteome is present. Proteins that were removed by the activity of DEAD-box proteins are likely to be replaced by specific RNA-processing factors or RNA-binding proteins that are enriched in the condensate, since they have a much higher likelihood of modifying or loading onto the target RNA than factors outside of the condensate. The remodeled mRNP that leaves the compartment is distinct from the one that entered and can move to the next reaction center (**Figure 3**). This simplified model would be comparable to the membrane-enclosed secretory pathway, where proteins undergo an ordered set of maturation steps as they travel through the ER and distinct Golgi stacks. Clearly, additional work is needed to better understand the function of DEAD-box proteins in mRNP processing and condensation, and we expect many additional exciting discoveries from studies on this protein family.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors would like to apologize to all colleagues whose primary work could not be cited due to space limitations. We would like to thank all members of our laboratories for fruitful discussions. K.W. acknowledges support from the Swiss National Science Foundation (grants SNF 31003A_179275 and CRSII5_193740). M.H. is supported by the Swiss National Science Foundation (PCEFP3_187052) and the European Research Council (ERC-ST2020 950262).

LITERATURE CITED

1. Parsyan A, Svitkin Y, Shahbazian D, Gkogkas C, Lasko P, et al. 2011. mRNA helicases: the tacticians of translational control. *Nat. Rev. Mol. Cell Bio.* 12(4):235–45
2. Hinnebusch AG, Ivanov IP, Sonenberg N. 2016. Translational control by 5′-untranslated regions of eukaryotic mRNAs. *Science* 352(6292):1413–16
3. Van Treeck B, Parker R. 2018. Emerging roles for intermolecular RNA-RNA interactions in RNP assemblies. *Cell* 174(4):791–802
4. Buchan JR. 2014. mRNP granules: assembly, function, and connections with disease. *RNA Biol.* 11(8):1019–30

5. Singh G, Pratt G, Yeo GW, Moore MJ. 2015. The clothes make the mRNA: past and present trends in mRNP fashion. *Annu. Rev. Biochem.* 84:325–54
6. Gehring NH, Wahle E, Fischer U. 2017. Deciphering the mRNP code: RNA-bound determinants of post-transcriptional gene regulation. *Trends Biochem. Sci.* 42(5):369–82
7. Khong A, Parker R. 2020. The landscape of eukaryotic mRNPs. *RNA* 26(3):229–39
8. Kelly SM, Corbett AH. 2009. Messenger RNA export from the nucleus: a series of molecular wardrobe changes. *Traffic* 10(9):1199–208
9. Bourgeois CF, Mortreux F, Auboeuf D. 2016. The multiple functions of RNA helicases as drivers and regulators of gene expression. *Nat. Rev. Mol. Cell Bio.* 17(7):426–38
10. Björk P, Wieslander L. 2017. Integration of mRNP formation and export. *Cell Mol. Life Sci.* 74(16):2875–97
11. Eliscovich C, Singer RH. 2017. RNP transport in cell biology: the long and winding road. *Curr. Opin. Cell Biol.* 45:38–46
12. Wende W, Friedhoff P, Sträßer K. 2019. *The Biology of mRNA: Structure and Function*. Adv. Exp. Med. Biol. 1203. Cham, Switz.: Springer
13. Zarnack K, Balasubramanian S, Gantier MP, Kunetsky V, Kracht M, et al. 2020. Dynamic mRNP remodeling in response to internal and external stimuli. *Biomolecules* 10(9):1310
14. Das S, Vera M, Gandin V, Singer RH, Tutucci E. 2021. Intracellular mRNA transport and localized translation. *Nat. Rev. Mol. Cell Bio.* 22(7):483–504
15. Hilbert M, Karow AR, Klostermeier D. 2009. The mechanism of ATP-dependent RNA unwinding by DEAD box proteins. *Biol. Chem.* 390(12):1237–50
16. Linder P, Jankowsky E. 2011. From unwinding to clamping—the DEAD box RNA helicase family. *Nat. Rev. Mol. Cell Bio.* 12(8):505–16
17. Jarmoskaite I, Russell R. 2014. RNA helicase proteins as chaperones and remodelers. *Annu. Rev. Biochem.* 83:697–725
18. Fairman-Williams ME, Guenther U-P, Jankowsky E. 2010. SF1 and SF2 helicases: family matters. *Curr. Opin. Struc. Biol.* 20(3):313–24
19. Russon MP, Westerhouse KM, Tran EJ. 2021. Transcription, translation, and DNA repair: new insights from emerging noncanonical substrates of RNA helicases. *Biol. Chem.* 402(5):637–44
20. Caruthers JM, McKay DB. 2002. Helicase structure and mechanism. *Curr. Opin. Struc. Biol.* 12(1):123–33
21. Martina V, Patrick L. 2021. Happy birthday: 30 years of RNA helicases. In *RNA Remodeling Proteins: Methods and Protocols*, ed. M Boudvillain, pp. 17–34. Methods Mol. Biol. 2209. New York: Humana Press Inc.
22. Ozgur S, Buchwald G, Falk S, Chakrabarti S, Prabu JR, Conti E. 2015. The conformational plasticity of eukaryotic RNA-dependent ATPases. *FEBS J.* 282(5):850–63
23. Linder P, Lasko PF, Ashburner M, Leroy P, Nielsen PJ, et al. 1989. Birth of the D-E-A-D box. *Nature* 337(6203):121–22
24. Linder P, Fuller-Pace F. 2015. Happy birthday: 25 years of DEAD-box proteins. In *RNA Remodeling Proteins: Methods and Protocols*, ed. M Boudvillain, pp. 17–33. Methods Mol. Biol. 1259. New York: Humana Press Inc.
25. Caruthers JM, Johnson ER, McKay DB. 2000. Crystal structure of yeast initiation factor 4A, a DEAD-box RNA helicase. *PNAS* 97(24):13080–85
26. Bono F, Ebert J, Lorentzen E, Conti E. 2006. The crystal structure of the exon junction complex reveals how it maintains a stable grip on mRNA. *Cell* 126(4):713–25
27. Andersen CBF, Ballut L, Johansen JS, Chamieh H, Nielsen KH, et al. 2006. Structure of the exon junction core complex with a trapped DEAD-box ATPase bound to RNA. *Science* 313(5795):1968–72
28. Mallam AL, Campo MD, Gilman B, Sidote DJ, Lambowitz AM. 2012. Structural basis for RNA-duplex recognition and unwinding by the DEAD-box helicase Mss116p. *Nature* 490(7418):121–25
29. Sengoku T, Nureki O, Nakamura A, Kobayashi S, Yokoyama S. 2006. Structural basis for RNA unwinding by the DEAD-box protein *Drosophila* Vasa. *Cell* 125(2):287–300
30. Yang Q, Campo MD, Lambowitz AM, Jankowsky E. 2007. DEAD-box proteins unwind duplexes by local strand separation. *Mol. Cell* 28(2):253–63

31. Campo MD, Lambowitz AM. 2009. Structure of the yeast DEAD box protein Mss116p reveals two wedges that crimp RNA. *Mol. Cell* 35(5):598–609
32. Steimer L, Klostermeier D. 2012. RNA helicases in infection and disease. *RNA Biol*. 9(6):751–71
33. Gilman B, Tijerina P, Russell R. 2017. Distinct RNA-unwinding mechanisms of DEAD-box and DEAH-box RNA helicase proteins in remodeling structured RNAs and RNPs. *Biochem. Soc. Trans.* 45(6):1313–21
34. Liu F, Putnam AA, Jankowsky E. 2014. DEAD-box helicases form nucleotide-dependent, long-lived complexes with RNA. *Biochemistry* 53(2):423–33
35. Rogers GW, Richter NJ, Merrick WC. 1999. Biochemical and kinetic characterization of the RNA helicase activity of eukaryotic initiation factor 4A. *J. Biol. Chem.* 274(18):12236–44
36. Chamot D, Colvin KR, Kujat-Choy SL, Owttrim GW. 2005. RNA structural rearrangement via unwinding and annealing by the cyanobacterial RNA helicase, CrhR. *J. Biol. Chem.* 280(3):2036–44
37. Pérez-Calero C, Bayona-Feliu A, Xue X, Barroso SI, Muñoz S, et al. 2020. UAP56/DDX39B is a major cotranscriptional RNA–DNA helicase that unwinds harmful R loops genome-wide. *Gene Dev.* 34(13–14):898–912
38. Fairman ME, Maroney PA, Wang W, Bowers HA, Gollnick P, et al. 2004. Protein displacement by DExH/D "RNA helicases" without duplex unwinding. *Science* 304(5671):730–34
39. Bowers HA, Maroney PA, Fairman ME, Kastner B, Lührmann R, et al. 2006. Discriminatory RNP remodeling by the DEAD-box protein DED1. *RNA* 12(5):903–12
40. Hilbert M, Kebbel F, Gubaev A, Klostermeier D. 2011. eIF4G stimulates the activity of the DEAD box protein eIF4A by a conformational guidance mechanism. *Nucleic Acids Res.* 39(6):2260–70
41. Montpetit B, Thomsen ND, Helmke KJ, Seeliger MA, Berger JM, Weis K. 2011. A conserved mechanism of DEAD-box ATPase activation by nucleoporins and InsP6 in mRNA export. *Nature* 472(7342):238–42
42. Mathys H, Basquin J, Ozgur S, Czarnocki-Cieciura M, Bonneau F, et al. 2014. Structural and biochemical insights to the role of the CCR4-NOT complex and DDX6 ATPase in microRNA repression. *Mol. Cell* 54(5):751–65
43. Chen Y, Boland A, Kuzuoğlu-Öztürk D, Bawankar P, Loh B, et al. 2014. A DDX6-CNOT1 complex and W-binding pockets in CNOT9 reveal direct links between miRNA target recognition and silencing. *Mol. Cell* 54(5):737–50
44. Lin DH, Correia AR, Cai SW, Huber FM, Jette CA, Hoelz A. 2018. Structural and functional analysis of mRNA export regulation by the nuclear pore complex. *Nat. Commun.* 9(1):2319
45. Buchwald G, Schüssler S, Basquin C, Hir HL, Conti E. 2013. Crystal structure of the human eIF4AIII-CWC22 complex shows how a DEAD-box protein is inhibited by a MIF4G domain. *PNAS* 110(48):E4611–18
46. Ballut L, Marchadier B, Baguet A, Tomasetto C, Séraphin B, Hir HL. 2005. The exon junction core complex is locked onto RNA by inhibition of eIF4AIII ATPase activity. *Nat. Struct. Mol. Biol.* 12(10):861–69
47. Hilliker A, Gao Z, Jankowsky E, Parker R. 2011. The DEAD-box protein Ded1 modulates translation by the formation and resolution of an eIF4F-mRNA complex. *Mol. Cell* 43(6):962–72
48. Xiol J, Spinelli P, Laussmann MA, Homolka D, Yang Z, et al. 2014. RNA clamping by Vasa assembles a piRNA amplifier complex on transposon transcripts. *Cell* 157(7):1698–711
49. Mugler CF, Hondele M, Heinrich S, Sachdev R, Vallotton P, et al. 2016. ATPase activity of the DEAD-box protein Dhh1 controls processing body formation. *eLife* 5:e18746
50. Hondele M, Sachdev R, Heinrich S, Wang J, Vallotton P, et al. 2019. DEAD-box ATPases are global regulators of phase-separated organelles. *Nature* 573(7772):144–48
51. Tauber D, Tauber G, Khong A, Van Treeck B, Pelletier J, Parker R. 2020. Modulation of RNA condensation by the DEAD-box protein eIF4A. *Cell* 180(3):411–26.e16
52. Weis K. 2021. Dead or alive: DEAD-box ATPases as regulators of ribonucleoprotein complex condensation. *Biol. Chem.* 402(5):653–61
53. Tanner NK, Cordin O, Banroques J, Doère M, Linder P. 2003. The Q motif: A newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. *Mol. Cell* 11(1):127–38

54. Cordin O, Tanner NK, Doère M, Linder P, Banroques J. 2004. The newly discovered Q motif of DEAD-box RNA helicases regulates RNA-binding and helicase activity. *EMBO J.* 23(13):2478–87
55. Pause A, Méthot N, Sonenberg N. 1993. The HRIGRXXR region of the DEAD box RNA helicase eukaryotic translation initiation factor 4A is required for RNA binding and ATP hydrolysis. *Mol. Cell. Biol.* 13(11):6789–98
56. Mohr G, Campo MD, Mohr S, Yang Q, Jia H, et al. 2008. Function of the C-terminal domain of the DEAD-box protein Mss116p analyzed in vivo and in vitro. *J. Mol. Biol.* 375(5):1344–64
57. Collins R, Karlberg T, Lehtiö L, Schütz P, van den Berg S, et al. 2009. The DEXD/H-box RNA helicase DDX19 is regulated by an α-helical switch. *J. Biol. Chem.* 284(16):10296–300
58. Floor SN, Condon KJ, Sharma D, Jankowsky E, Doudna JA. 2016. Autoinhibitory interdomain interactions and subfamily-specific extensions redefine the catalytic core of the human DEAD-box protein DDX3. *J. Biol. Chem.* 291(5):2412–21
59. Ngo TD, Partin AC, Nam Y. 2019. RNA specificity and autoregulation of DDX17, a modulator of microRNA biogenesis. *Cell Rep.* 29(12):4024–35.e5
60. Kossen K, Karginov FV, Uhlenbeck OC. 2002. The carboxy-terminal domain of the DExDH protein YxiN is sufficient to confer specificity for 23S rRNA. *J. Mol. Biol.* 324(4):625–36
61. Mallam AL, Jarmoskaite I, Tijerina P, Campo MD, Seifert S, et al. 2011. Solution structures of DEAD-box RNA chaperones reveal conformational changes and nucleic acid tethering by a basic tail. *PNAS* 108(30):12254–59
62. Steimer L, Wurm JP, Linden MH, Rudolph MG, Wöhnert J, Klostermeier D. 2013. Recognition of two distinct elements in the RNA substrate by the RNA-binding domain of the *T. thermophilus* DEAD box helicase Hera. *Nucleic Acids Res.* 41(12):6259–72
63. Giraud C, Hausmann S, Lemeille S, Prados J, Redder P, Linder P. 2015. The C-terminal region of the RNA helicase CshA is required for the interaction with the degradosome and turnover of bulk RNA in the opportunistic pathogen *Staphylococcus aureus*. *RNA Biol.* 12(6):658–74
64. Rudolph MG, Klostermeier D. 2015. When core competence is not enough: functional interplay of the DEAD-box helicase core with ancillary domains and auxiliary factors in RNA binding and unwinding. *Biol. Chem.* 396(8):849–65
65. Busa VF, Rector MJ, Russell R. 2017. The DEAD-box protein CYT-19 uses arginine residues in its C-tail to tether RNA substrates. *Biochemistry* 56(28):3571–78
66. Nott TJ, Petsalaki E, Farber P, Jervis D, Fussner E, et al. 2015. Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles. *Mol. Cell* 57(5):936–47
67. Elbaum-Garfinkle S, Kim Y, Szczepaniak K, Chen CC-H, Eckmann CR, et al. 2015. The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics. *PNAS* 112(23):7189–94
68. Nott TJ, Craggs TD, Baldwin AJ. 2016. Membraneless organelles can melt nucleic acid duplexes and act as biomolecular filters. *Nat. Chem.* 8(6):569–75
69. Faltova L, Küffner AM, Hondele M, Weis K, Arosio P. 2018. Multifunctional protein materials and microreactors using low complexity domains as molecular adhesives. *ACS Nano* 12(10):9991–99
70. Saito M, Hess D, Eglinger J, Fritsch AW, Kreysing M, et al. 2019. Acetylation of intrinsically disordered regions regulates phase separation. *Nat. Chem. Biol.* 15(1):51–61
71. Iserman C, Altamirano CD, Jegers C, Friedrich U, Zarin T, et al. 2020. Condensation of Ded1p promotes a translational switch from housekeeping to stress protein production. *Cell* 181(4):818–31.e19
72. Crabtree MD, Holland J, Kompella P, Babl L, Turner N, et al. 2021. Repulsive electrostatic interactions modulate dense and dilute phase properties of biomolecular condensates. bioRxiv 2020.10.29.357863. **https://doi.org/10.1101/2020.10.29.357863**
73. Zhang M, Green MR. 2001. Identification and characterization of yUAP/Sub2p, a yeast homolog of the essential human pre-mRNA splicing factor hUAP56. *Gene Dev.* 15(1):30–35
74. Shi H, Cordin O, Minder CM, Linder P, Xu R-M. 2004. Crystal structure of the human ATP-dependent splicing and export factor UAP56. *PNAS* 101(51):17628–33
75. Libri D, Graziani N, Saguez C, Boulay J. 2001. Multiple roles for the yeast SUB2/yUAP56 gene in splicing. *Gene Dev.* 15(1):36–41

76. Kistler AL, Guthrie C. 2001. Deletion of MUD2, the yeast homolog of U2AF65, can bypass the requirement for Sub2, an essential spliceosomal ATPase. *Gene Dev*. 15(1):42–49
77. Sträßer K, Hurt E. 2001. Splicing factor Sub2p is required for nuclear mRNA export through its interaction with Yra1p. *Nature* 413(6856):648–52
78. Jensen TH, Boulay J, Rosbash M, Libri D. 2001. The DECD box putative ATPase Sub2p is an early mRNA export factor. *Curr. Biol.* 11(21):1711–15
79. Luo M-J, Zhou Z, Magni K, Christoforides C, Rappsilber J, et al. 2001. Pre-mRNA splicing and mRNA export linked by direct interactions between UAP56 and Aly. *Nature* 413(6856):644–47
80. Heath CG, Viphakone N, Wilson SA. 2016. The role of TREX in gene expression and disease. *Biochem. J.* 473(19):2911–35
81. Köhler A, Hurt E. 2007. Exporting RNA from the nucleus to the cytoplasm. *Nat. Rev. Mol. Cell Bio.* 8(10):761–73
82. Taniguchi I, Ohno M. 2007. ATP-dependent recruitment of export factor Aly/REF onto intronless mRNAs by RNA helicase UAP56. *Mol. Cell. Biol.* 28(2):601–8
83. Hautbergue GM, Hung M-L, Golovanov AP, Lian L-Y, Wilson SA. 2008. Mutually exclusive interactions drive handover of mRNA from export adaptors to TAP. *PNAS* 105(13):5154–59
84. Galganski L, Urbanek MO, Krzyzosiak WJ. 2017. Nuclear speckles: molecular organization, biological function and role in disease. *Nucleic Acids Res*. 45(18):10350–68
85. Sträßer K, Masuda S, Mason P, Pfannstiel J, Oppizzi M, et al. 2002. TREX is a conserved complex coupling transcription with messenger RNA export. *Nature* 417(6886):304–8
86. Masuda S, Das R, Cheng H, Hurt E, Dorman N, Reed R. 2005. Recruitment of the human TREX complex to mRNA during splicing. *Gene Dev*. 19(13):1512–17
87. Cheng H, Dufu K, Lee C-S, Hsu JL, Dias A, Reed R. 2006. Human mRNA export machinery recruited to the 5′ end of mRNA. *Cell* 127(7):1389–400
88. Rougemaille M, Dieppois G, Kisseleva-Romanova E, Gudipati RK, Lemoine S, et al. 2008. THO/Sub2p functions to coordinate 3′-end processing with gene-nuclear pore association. *Cell* 135(2):308–21
89. Domínguez-Sánchez MS, Barroso S, Gómez-González B, Luna R, Aguilera A. 2011. Genome instability and transcription elongation impairment in human cells depleted of THO/TREX. *PLOS Genet*. 7(12):e1002386
90. Saguez C, Gonzales FA, Schmid M, Bøggild A, Latrick CM, et al. 2013. Mutational analysis of the yeast RNA helicase Sub2p reveals conserved domains required for growth, mRNA export, and genomic stability. *RNA* 19(10):1363–71
91. Luna R, Rondón AG, Pérez-Calero C, Salas-Armenteros I, Aguilera A. 2020. The THO complex as a paradigm for the prevention of cotranscriptional R-loops. *Cold Spring Harb. Symp.* 84:039594
92. Schuller SK, Schuller JM, Prabu JR, Baumgärtner M, Bonneau F, et al. 2020. Structural insights into the nucleic acid remodeling mechanisms of the yeast THO-Sub2 complex. *eLife* 9:e61467
93. Pühringer T, Hohmann U, Fin L, Pacheco-Fiallos B, Schellhaas U, et al. 2020. Structure of the human core transcription-export complex reveals a hub for multivalent interactions. *eLife* 9:e61503
94. Xie Y, Clarke BP, Kim YJ, Ivey AL, Hill PS, et al. 2021. Cryo-EM structure of the yeast TREX complex and coordination with the SR-like protein Gbp2. *eLife* 10:e65699
95. Ren Y, Schmiege P, Blobel G. 2017. Structural and biochemical analyses of the DEAD-box ATPase Sub2 in association with THO or Yra1. *eLife* 6:e20070
96. Ilık İA, Aktaş T. 2021. Nuclear speckles: dynamic hubs of gene expression regulation. *FEBS J*. **https://doi.org/10.1111/febs.16117**
97. Ford MJ, Anton IA, Lane DP. 1988. Nuclear protein with sequence homology to translation initiation factor eIF-4A. *Nature* 332(6166):736–38
98. Xing Z, Wang S, Tran EJ. 2017. Characterization of the mammalian DEAD-box protein DDX5 reveals functional conservation with *S. cerevisiae* ortholog Dbp2 in transcriptional control and glucose metabolism. *RNA* 23(7):1125–38
99. Xing Z, Ma WK, Tran EJ. 2019. The DDX5/Dbp2 subfamily of DEAD-box RNA helicases. *WIREs RNA* 10(2):e1519

100. Gao J, Byrd AK, Zybailov BL, Marecki JC, Guderyon MJ, et al. 2019. DEAD-box RNA helicases Dbp2, Ded1 and Mss116 bind to G-quadruplex nucleic acids and destabilize G-quadruplex RNA. *Chem. Commun.* 55(31):4467–70
101. Yan KK-P, Obi I, Sabouri N. 2021. The RGG domain in the C-terminus of the DEAD box helicases Dbp2 and Ded1 is necessary for G-quadruplex destabilization. *Nucleic Acids Res.* 49(14):8339–54
102. Tedeschi FA, Cloutier SC, Tran EJ, Jankowsky E. 2018. The DEAD-box protein Dbp2p is linked to noncoding RNAs, the helicase Sen1p, and R-loops. *RNA* 24(12):1693–705
103. Hodroj D, Recolin B, Serhal K, Martinez S, Tsanov N, et al. 2017. An ATR-dependent function for the Ddx19 RNA helicase in nuclear R-loop metabolism. *EMBO J.* 36(9):1182–98
104. Ma WK, Cloutier SC, Tran EJ. 2013. The DEAD-box protein Dbp2 functions with the RNA-binding protein Yra1 to promote mRNP assembly. *J. Mol. Biol.* 425(20):3824–38
105. Tseng SS-I, Weaver PL, Liu Y, Hitomi M, Tartakoff AM, Chang T. 1998. Dbp5p, a cytosolic RNA helicase, is required for poly(A)+ RNA export. *EMBO J.* 17(9):2651–62
106. Snay-Hodge CA, Colot HV, Goldstein AL, Cole CN. 1998. Dbp5p/Rat8p is a yeast nuclear pore-associated DEAD-box protein essential for RNA export. *EMBO J.* 17(9):2663–76
107. Tran EJ, Zhou Y, Corbett AH, Wente SR. 2007. The DEAD-box protein Dbp5 controls mRNA export by triggering specific RNA:protein remodeling events. *Mol. Cell* 28(5):850–59
108. Lund MK, Guthrie C. 2005. The DEAD-box protein Dbp5p is required to dissociate Mex67p from exported mRNPs at the nuclear rim. *Mol. Cell* 20(4):645–51
109. Rajan AAN, Montpetit B. 2021. Emerging molecular functions and novel roles for the DEAD-box protein Dbp5/DDX19 in gene expression. *Cell Mol. Life Sci.* 78(5):2019–30
110. Weirich CS, Erzberger JP, Flick JS, Berger JM, Thorner J, Weis K. 2006. Activation of the DExD/H-box protein Dbp5 by the nuclear-pore protein Gle1 and its coactivator InsP6 is required for mRNA export. *Nat. Cell Biol.* 8(7):668–76
111. Alcazar-Roman AR, Tran EJ, Guo S, Wente SR. 2006. Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. *Nat. Cell Biol.* 8(7):711–16
112. Hodge CA, Colot HV, Stafford P, Cole CN. 1999. Rat8p/Dbp5p is a shuttling transport factor that interacts with Rat7p/Nup159p and Gle1p and suppresses the mRNA export defect of *xpo1-1* cells. *EMBO J.* 18(20):5778–88
113. Schmitt C, von Kobbe C, Bachi A, Panté N, Rodrigues JP, et al. 1999. Dbp5, a DEAD-box protein required for mRNA export, is recruited to the cytoplasmic fibrils of nuclear pore complex via a conserved interaction with CAN/Nup159p. *EMBO J.* 18(15):4332–47
114. Weirich CS, Erzberger JP, Berger JM, Weis K. 2004. The N-terminal domain of Nup159 forms a β-propeller that functions in mRNA export by tethering the helicase Dbp5 to the nuclear pore. *Mol. Cell* 16(5):749–60
115. Folkmann AW, Noble KN, Cole CN, Wente SR. 2011. Dbp5, Gle1-IP6 and Nup159. *Nucleus* 2(6):540–48
116. Noble KN, Tran EJ, Alcázar-Román AR, Hodge CA, Cole CN, Wente SR. 2011. The Dbp5 cycle at the nuclear pore complex during mRNA export II: Nucleotide cycling and mRNP remodeling by Dbp5 are controlled by Nup159 and Gle1. *Genes Dev.* 25(10):1065–77
117. Derrer CP, Mancini R, Vallotton P, Huet S, Weis K, Dultz E. 2019. The RNA export factor Mex67 functions as a mobile nucleoporin. *J. Cell Biol.* 218(12):3967–76
118. York JD, Odom AR, Murphy R, Ives EB, Wente SR. 1999. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science* 285(5424):96–100
119. Takemura R, Inoue Y, Izawa S. 2004. Stress response in yeast mRNA export factor: Reversible changes in Rat8p localization are caused by ethanol stress but not heat shock. *J. Cell Sci.* 117(18):4189–97
120. Izawa S, Takemura R, Ikeda K, Fukuda K, Wakai Y, Inoue Y. 2005. Characterization of Rat8 localization and mRNA export in *Saccharomyces cerevisiae* during the brewing of Japanese sake. *Appl. Microbiol. Biotechnol.* 69(1):86–91
121. Adams RL, Wente SR. 2020. Dbp5 associates with RNA-bound Mex67 and Nab2 and its localization at the nuclear pore complex is sufficient for mRNP export and cell viability. *PLOS Genet.* 16(10):e1009033
122. Wu H, Becker D, Krebber H. 2014. Telomerase RNA TLC1 shuttling to the cytoplasm requires mRNA export factors and is important for telomere maintenance. *Cell Rep.* 8(6):1630–38

123. Neumann B, Wu H, Hackmann A, Krebber H. 2016. Nuclear export of pre-ribosomal subunits requires Dbp5, but not as an RNA-helicase as for mRNA export. *PLOS ONE* 11(2):e0149571
124. Lari A, Rajan AAN, Sandhu R, Reiter T, Montpetit R, et al. 2019. A nuclear role for the DEAD-box protein Dbp5 in tRNA export. *eLife* 8:e48410
125. Serpeloni M, Vidal NM, Goldenberg S, Ávila AR, Hoffmann FG. 2011. Comparative genomics of proteins involved in RNA nucleocytoplasmic export. *BMC Evol. Biol.* 11(1):7
126. Obado SO, Field MC, Rout MP. 2017. Comparative interactomics provides evidence for functional specialization of the nuclear pore complex. *Nucleus* 8(4):340–52
127. Gatfield D, Hir HL, Schmitt C, Braun IC, Köcher T, et al. 2001. The DExH/D box protein HEL/UAP56 is essential for mRNA nuclear export in *Drosophila*. *Curr. Biol.* 11(21):1716–21
128. Hampoelz B, Andres-Pons A, Kastritis P, Beck M. 2019. Structure and assembly of the nuclear pore complex. *Annu. Rev. Biophys.* 48:515–36
129. Nielsen PJ, McMaster GK, Trachsel H. 1985. Cloning of eukaryotic protein synthesis initiation factor genes: isolation and characterization of cDNA clones encoding factor eIF-4A. *Nucleic Acids Res.* 13(19):6867–80
130. Rogers GW, Komar AA, Merrick WC. 2002. eIF4A: the godfather of the DEAD box helicases. *Prog. Nucleic Acid Res.* 72:307–31
131. Gingras A-C, Raught B, Sonenberg N. 1999. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* 68:913–63
132. Andreou AZ, Klostermeier D. 2012. The DEAD-box helicase eIF4A. *RNA Biol.* 10(1):19–32
133. Grifo JA, Abramson RD, Satler CA, Merrick WC. 1984. RNA-stimulated ATPase activity of eukaryotic initiation factors. *J. Biol. Chem.* 259(13):8648–54
134. Ray BK, Lawson TG, Kramer JC, Cladaras MH, Grifo JA, et al. 1985. ATP-dependent unwinding of messenger RNA structure by eukaryotic initiation factors. *J. Biol. Chem.* 260(12):7651–58
135. Lorsch JR, Herschlag D. 1998. The DEAD box protein eIF4A. 1. A minimal kinetic and thermodynamic framework reveals coupled binding of RNA and nucleotide. *Biochemistry* 37(8):2180–93
136. Lorsch JR, Herschlag D. 1998. The DEAD box protein eIF4A. 2. A cycle of nucleotide and RNA-dependent conformational changes. *Biochemistry* 37(8):2194–206
137. Rozen F, Edery I, Meerovitch K, Dever TE, Merrick WC, Sonenberg N. 1990. Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. *Mol. Cell. Biol.* 10(3):1134–44
138. Johnson ER, McKay DB. 1999. Crystallographic structure of the amino terminal domain of yeast initiation factor 4A, a representative DEAD-box RNA helicase. *RNA* 5(12):1526–34
139. Benz J, Trachsel H, Baumann U. 1999. Crystal structure of the ATPase domain of translation initiation factor 4A from *Saccharomyces cerevisiae*—the prototype of the DEAD box protein family. *Structure* 7(6):671–79
140. Svitkin YV, Pause A, Haghighat A, Pyronnet S, Witherell G, et al. 2001. The requirement for eukaryotic initiation factor 4A (eIF4A) in translation is in direct proportion to the degree of mRNA 5′ secondary structure. *RNA* 7(3):382–94
141. Linder P. 2003. Yeast RNA helicases of the DEAD-box family involved in translation initiation. *Biol. Cell* 95(3–4):157–67
142. Marsden S, Nardelli M, Linder P, McCarthy JEG. 2006. Unwinding single RNA molecules using helicases involved in eukaryotic translation initiation. *J. Mol. Biol.* 361(2):327–35
143. Sen ND, Zhou F, Ingolia NT, Hinnebusch AG. 2015. Genome-wide analysis of translational efficiency reveals distinct but overlapping functions of yeast DEAD-box RNA helicases Ded1 and eIF4A. *Genome Res.* 25(8):1196–205
144. Gao Z, Putnam AA, Bowers HA, Guenther U-P, Ye X, et al. 2016. Coupling between the DEAD-box RNA helicases Ded1p and eIF4A. *eLife* 5:e16408
145. Guenther U-P, Weinberg DE, Zubradt MM, Tedeschi FA, Stawicki BN, et al. 2018. The helicase Ded1p controls use of near-cognate translation initiation codons in 5′ UTRs. *Nature* 559(7712):130–34
146. Gulay S, Gupta N, Lorsch JR, Hinnebusch AG. 2020. Distinct interactions of eIF4A and eIF4E with RNA helicase Ded1 stimulate translation in vivo. *eLife* 9:e58243
147. Yourik P, Aitken CE, Zhou F, Gupta N, Hinnebusch AG, Lorsch JR. 2017. Yeast eIF4A enhances recruitment of mRNAs regardless of their structural complexity. *eLife* 6:e31476

148. Sokabe M, Fraser CS. 2017. A helicase-independent activity of eIF4A in promoting mRNA recruitment to the human ribosome. *PNAS* 114(24):6304–9
149. Balagopal V, Parker R. 2009. Polysomes, P bodies and stress granules: states and fates of eukaryotic mRNAs. *Curr. Opin. Cell Biol.* 21(3):403–8
150. Buchan JR, Parker R. 2009. Eukaryotic stress granules: the ins and outs of translation. *Mol. Cell* 36(6):932–41
151. Ivanov P, Kedersha N, Anderson P. 2018. Stress granules and processing bodies in translational control. *Cold Spring Harb. Perspect. Biol.* 11(5):a032813
152. Van Treeck B, Protter DSW, Matheny T, Khong A, Link CD, Parker R. 2018. RNA self-assembly contributes to stress granule formation and defining the stress granule transcriptome. *PNAS* 115(11):201800038
153. Begovich K, Wilhelm JE. 2020. An in vitro assembly system identifies roles for RNA nucleation and ATP in yeast stress granule formation. *Mol. Cell* 79(6):991–1007.e4
154. Tauber D, Tauber G, Parker R. 2020. Mechanisms and regulation of RNA condensation in RNP granule formation. *Trends Biochem. Sci.* 45(9):764–78
155. Matheny T, Van Treeck B, Huynh TN, Parker R. 2020. RNA partitioning into stress granules is based on the summation of multiple interactions. *RNA* 27(2):174–89
156. Tian S, Curnutte HA, Trcek T. 2020. RNA granules: a view from the RNA perspective. *Molecules* 25(14):3130
157. Hir HL, Saulière J, Wang Z. 2016. The exon junction complex as a node of post-transcriptional networks. *Nat. Rev. Mol. Cell Bio.* 17(1):41–54
158. Boehm V, Gehring NH. 2016. Exon junction complexes: supervising the gene expression assembly line. *Trends Genet.* 32(11):724–35
159. Nielsen KH, Chamieh H, Andersen CBF, Fredslund F, Hamborg K, et al. 2009. Mechanism of ATP turnover inhibition in the EJC. *RNA* 15(1):67–75
160. Coller J, Tucker M, Sheth U, Valencia-Sanchez MA, Parker R. 2001. The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. *RNA* 7(12):1717–27
161. Fischer N, Weis K. 2002. The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. *EMBO J.* 21(11):2788–97
162. Ladomery M, Wade E, Sommerville J. 1997. Xp54, the *Xenopus* homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. *Nucleic Acids Res.* 25(5):965–73
163. Weston A, Sommerville J. 2006. Xp54 and related (DDX6-like) RNA helicases: roles in messenger RNP assembly, translation regulation and RNA degradation. *Nucleic Acids Res.* 34(10):3082–94
164. Coller J, Parker R. 2005. General translational repression by activators of mRNA decapping. *Cell* 122(6):875–86
165. Carroll JS, Munchel SE, Weis K. 2011. The DExD/H box ATPase Dhh1 functions in translational repression, mRNA decay, and processing body dynamics. *J. Cell Biol.* 194(4):527–37
166. Sweet T, Kovalak C, Coller J. 2012. The DEAD-box protein Dhh1 promotes decapping by slowing ribosome movement. *PLOS Biol.* 10(6):e1001342
167. Hu W, Sweet TJ, Chamnongpol S, Baker KE, Coller J. 2009. Co-translational mRNA decay in *Saccharomyces cerevisiae*. *Nature* 461(7261):225–29
168. Pelechano V, Wei W, Steinmetz LM. 2015. Widespread co-translational RNA decay reveals ribosome dynamics. *Cell* 161(6):1400–12
169. Radhakrishnan A, Chen Y-H, Martin S, Alhusaini N, Green R, Coller J. 2016. The DEAD-box protein Dhh1p couples mRNA decay and translation by monitoring codon optimality. *Cell* 167(1):122–32.e9
170. Chan LY, Mugler CF, Heinrich S, Vallotton P, Weis K. 2018. Non-invasive measurement of mRNA decay reveals translation initiation as the major determinant of mRNA stability. *eLife* 7:e32536
171. Ostareck DH, Vries ISN, Ostareck-Lederer A. 2014. DDX6 and its orthologs as modulators of cellular and viral RNA expression. *WIREs RNA* 5(5):659–78
172. Jung D, Ahn J, Rhee B, Kim J. 2017. Mutational analysis of the RNA helicase Dhh1 in Ste12 expression and yeast mating. *J. Microbiol.* 55(5):373–78

173. Núñez RD, Budt M, Saenger S, Paki K, Arnold U, et al. 2018. The RNA helicase DDX6 associates with RIG-I to augment induction of antiviral signaling. *Int. J. Mol. Sci.* 19(7):1877
174. Freimer JW, Hu T, Blelloch R. 2018. Decoupling the impact of microRNAs on translational repression versus RNA degradation in embryonic stem cells. *eLife* 7:e38014
175. Stefano BD, Luo E-C, Haggerty C, Aigner S, Charlton J, et al. 2019. The RNA helicase DDX6 controls cellular plasticity by modulating P-body homeostasis. *Cell Stem Cell* 25(5):622–38.e13
176. Sheth U, Parker R. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* 300(5620):805–8
177. Teixeira D, Sheth U, Valencia-Sanchez MA, Brengues M, Parker R. 2005. Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA* 11(4):371–82
178. Parker R, Sheth U. 2007. P bodies and the control of mRNA translation and degradation. *Mol. Cell* 25(5):635–46
179. Ernoult-Lange M, Baconnais S, Harper M, Minshall N, Souquere S, et al. 2012. Multiple binding of repressed mRNAs by the P-body protein Rck/p54. *RNA* 18(9):1702–15
180. Linsenmeier M, Hondele M, Grigolato F, Secchi E, Weis K, Arosio P. 2021. Dynamic arrest and aging of biomolecular condensates are regulated by low-complexity domains, RNA and biochemical activity. *bioRxiv* 2021.02.26.433003. **https://doi.org/10.1101/2021.02.26.433003**
181. Sharma D, Jankowsky E. 2014. The Ded1/DDX3 subfamily of DEAD-box RNA helicases. *Crit. Rev. Biochem. Mol.* 49(4):343–60
182. Iost I, Dreyfus M, Linder P. 1999. Ded1p, a DEAD-box protein required for translation initiation in *Saccharomyces cerevisiae*, is an RNA helicase. *J. Biol. Chem.* 274(25):17677–83
183. Yang Q, Jankowsky E. 2005. ATP- and ADP-dependent modulation of RNA unwinding and strand annealing activities by the DEAD-box protein DED1. *Biochemistry* 44(41):13591–601
184. Pugh TJ, Weeraratne SD, Archer TC, Krummel DAP, Auclair D, et al. 2012. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* 488(7409):106–10
185. Bol GM, Xie M, Raman V. 2015. DDX3, a potential target for cancer treatment. *Mol. Cancer* 14(1):188
186. Oh S, Flynn RA, Floor SN, Purzner J, Martin L, et al. 2016. Medulloblastoma-associated DDX3 variant selectively alters the translational response to stress. *Oncotarget* 7(19):28169–82
187. Valentin-Vega YA, Wang Y-D, Parker M, Patmore DM, Kanagaraj A, et al. 2016. Cancer-associated DDX3X mutations drive stress granule assembly and impair global translation. *Sci. Rep.* 6(1):25996
188. Blok LS, Madsen E, Juusola J, Gilissen C, Baralle D, et al. 2015. Mutations in DDX3X are a common cause of unexplained intellectual disability with gender-specific effects on Wnt signaling. *Am. J. Hum. Genet.* 97(2):343–52
189. Valiente-Echeverría F, Hermoso MA, Soto-Rifo R. 2015. RNA helicase DDX3: at the crossroad of viral replication and antiviral immunity. *Rev. Med. Virol.* 25(5):286–99
190. Hernández-Díaz T, Valiente-Echeverría F, Soto-Rifo R. 2021. RNA helicase DDX3: a double-edged sword for viral replication and immune signaling. *Microorganisms* 9(6):1206
191. Chuang R-Y, Weaver PL, Liu Z, Chang T-H. 1997. Requirement of the DEAD-box protein Ded1p for messenger RNA translation. *Science* 275(5305):1468–71
192. de la Cruz J, Iost I, Kressler D, Linder P. 1997. The p20 and Ded1 proteins have antagonistic roles in eIF4E-dependent translation in *Saccharomyces cerevisiae*. *PNAS* 94(10):5201–6
193. Calviello L, Venkataramanan S, Rogowski KJ, Wyler E, Wilkins K, et al. 2021. DDX3 depletion represses translation of mRNAs with complex 5′ UTRs. *Nucleic Acids Res.* 49(9):5336–50
194. Sen ND, Gupta N, Archer SK, Preiss T, Lorsch JR, Hinnebusch AG. 2019. Functional interplay between DEAD-box RNA helicases Ded1 and Dbp1 in preinitiation complex attachment and scanning on structured mRNAs in vivo. *Nucleic Acids Res.* 47(16):8785–806
195. Gupta N, Lorsch JR, Hinnebusch AG. 2018. Yeast Ded1 promotes 48S translation pre-initiation complex assembly in an mRNA-specific and eIF4F-dependent manner. *eLife* 7:e38892
196. Aryanpur PP, Renner DM, Rodela E, Mittelmeier TM, Byrd A, Bolger TA. 2019. The DEAD-box RNA helicase Ded1 has a role in the translational response to TORC1 inhibition. *Mol. Biol. Cell* 30(17):2171–84
197. Shin Y, Brangwynne CP. 2017. Liquid phase condensation in cell physiology and disease. *Science* 357(6357):eaaf4382

198. Banani SF, Lee HO, Hyman AA, Rosen MK. 2017. Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Bio.* 18(5):285–98
199. Jain A, Vale RD. 2017. RNA phase transitions in repeat expansion disorders. *Nature* 546(7657):243–47
200. Langdon EM, Qiu Y, Niaki AG, McLaughlin GA, Weidmann C, et al. 2018. mRNA structure determines specificity of a polyQ-driven phase separation. *Science* 360(6391):eaar7432
201. Ma W, Zheng G, Xie W, Mayr C. 2021. In vivo reconstitution finds multivalent RNA–RNA interactions as drivers of mesh-like condensates. *eLife* 10:e64252
202. Roden C, Gladfelter AS. 2021. RNA contributions to the form and function of biomolecular condensates. *Nat. Rev. Mol. Cell Bio.* 22(3):183–95
203. Wiedner HJ, Giudice J. 2021. It's not just a phase: function and characteristics of RNA-binding proteins in phase separation. *Nat. Struct. Mol. Biol.* 28(6):465–73
204. Kato M, McKnight SL. 2016. A solid-state conceptualization of information transfer from gene to message to protein. *Annu. Rev. Biochem.* 87:351–90
205. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA. 2017. A phase separation model for transcriptional control. *Cell* 169(1):13–23
206. McKnight SL. 2019. Passing through. *Trends Biochem. Sci.* 44(11):899–901
207. Charroux B, Pellizzoni L, Perkinson RA, Shevchenko A, Mann M, Dreyfuss G. 1999. Gemin3: a novel dead box protein that interacts with SMN, the spinal muscular atrophy gene product, and is a component of gems. *J. Cell Biol.* 147(6):1181–94
208. Andersen JS, Lyon CE, Fox AH, Leung AKL, Lam YW, et al. 2002. Directed proteomic analysis of the human nucleolus. *Curr. Biol.* 12(1):1–11
209. Saitoh N, Spahr CS, Patterson SD, Bubulya P, Neuwald AF, Spector DL. 2004. Proteomic analysis of interchromatin granule clusters. *Mol. Biol. Cell* 15(8):3876–90
210. Dias AP, Dufu K, Lei H, Reed R. 2010. A role for TREX components in the release of spliced mRNA from nuclear speckle domains. *Nat. Commun.* 1(1):97
211. Tu Y-T, Barrientos A. 2015. The human mitochondrial DEAD-box protein DDX28 resides in RNA granules and functions in mitoribosome assembly. *Cell Rep.* 10(6):854–64
212. Calo E, Flynn RA, Martin L, Spitale RC, Chang HY, Wysocka J. 2015. RNA helicase DDX21 coordinates transcription and ribosomal RNA processing. *Nature* 518(7538):249–53
213. Jain S, Wheeler JR, Walters RW, Agrawal A, Barsic A, Parker R. 2016. ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* 164(3):487–98
214. Hubstenberger A, Courel M, Bénard M, Souquere S, Ernoult-Lange M, et al. 2017. P-Body purification reveals the condensation of repressed mRNA regulons. *Mol. Cell* 68(1):144–57.e5
215. Markmiller S, Soltanieh S, Server KL, Mak R, Jin W, et al. 2018. Context-dependent and disease-specific diversity in protein interactions within stress granules. *Cell* 172(3):590–604.e13
216. Youn J-Y, Dunham WH, Hong SJ, Knight JDR, Bashkurov M, et al. 2018. High-density proximity mapping reveals the subcellular organization of mRNA-associated granules and bodies. *Mol. Cell* 69(3):517–32.e11
217. Mier P, Paladin L, Tamana S, Petrosian S, Hajdu-Soltész B, et al. 2019. Disentangling the complexity of low complexity proteins. *Brief. Bioinform.* 21(2):bbz007
218. Balcerak A, Trebinska-Stryjewska A, Konopinski R, Wakula M, Grzybowska EA. 2019. RNA–protein interactions: disorder, moonlighting and junk contribute to eukaryotic complexity. *Open. Biol.* 9(6):190096
219. Franzmann TM, Alberti S. 2019. Prion-like low-complexity sequences: key regulators of protein solubility and phase behavior. *J. Biol. Chem.* 294(18):7128–36
220. Hondele M, Heinrich S, Rios PDL, Weis K. 2020. Membraneless organelles: phasing out of equilibrium. *Emerg. Top. Life Sci.* 4(3):343–54