The translational landscape as regulated by the RNA helicase DDX3

Joon Tae Park¹ & Sekyung Oh²,*

¹Division of Life Sciences, Incheon National University, Incheon 22012, ²Department of Medical Science, Catholic Kwandong University College of Medicine, Incheon 22711, Korea

CONTINUOUSLY RENEWING THE PROTEOME, TRANSLATION IS EXQUISITELY CONTROLLED BY A NUMBER OF DEDICATED FACTORS THAT INTERACT WITH THE RIBOSOME. THE RNA HELICASE DDX3 BELONGING TO THE DEAD BOX FAMILY HAS EMERGED AS ONE OF THE CRITICAL REGULATORS OF TRANSLATION, THE FAILURE OF WHICH IS FREQUENTLY OBSERVED IN A WIDE RANGE OF PROLIFERATIVE, Degenerative, AND INFECTIOUS DISEASES IN HUMANS. DDX3 UNWINDS DOUBLE-STRANDED RNA MOLECULES WITH COUPLED ATP HYDROLYSIS AND THEREBY REMODELS COMPLEX RNA STRUCTURES PRESENT IN VARIOUS PROTEIN-CODING AND NONCODING RNAs. By interacting with specific features on messenger RNAs (mRNAs) and 18S ribosomal RNA (rRNA), DDX3 facilitates translation, while repressing it under certain conditions. We review recent findings underlying these properties of DDX3 in diverse modes of translation, such as cap-dependent and cap-independent translation initiation, usage of upstream open reading frames, and stress-induced ribonucleoprotein granule formation. We further discuss how disease-associated DDX3 variants alter the translation landscape in the cell. [BMB Reports 2022; 55(3): 125-135]

INTRODUCTION

Gene expression occurs via sequential and coordinated mechanisms, ranging from pre-mRNA transcription and splicing in the nucleus to translation and decay of processed mRNA in the cytosol. While transcription has been established as an initial event in gene expression, post-transcriptional processes play an equally crucial role in shaping the gene expression landscape in response to a variety of cell-intrinsic and extrinsic stimuli. Notably, the levels of mRNA and protein products of “protein-coding” genes correlate loosely with each other (1), indicating distinct regulation of gene expression at the translation level. The importance of appropriate translational control of gene expression is reflected in numerous proliferative and degenerative diseases characterized by abnormal protein synthesis (2, 3). Thus, elucidating the gene expression at the translation level provides insight into the pathophysiological response to proteomic alteration.

The process of translation requires immense energy, with ~20% of cellular ATP hydrolysis devoted solely to protein synthesis at the ribosome (4). Besides the intricate interplay between the mRNA and the aminoacyl tRNAs on the binding sites of the ribosome, translation requires coordinated actions of a dedicated set of translation factors to facilitate the initiation, elongation, and termination phases of translation (5). Moreover, the mRNA sequence and secondary structure also greatly influences translation (5). Molecular recognition of these cis elements on mRNA by trans-acting factors leads to critical control of various steps in translation. RNA helicases represent one such class of trans-acting factors binding to double-stranded regions in RNA molecules and unwinding them into single strands (6). Notably, DDX3, a member of the DEAD-box family RNA helicases, is gaining increasing attention. DDX3 has been implicated in human malignancies and neuronal diseases as well as antiviral immunity and intellectual disability (7-10). Notably, a big share of these pathologies appears to have their roots in altered translation response to stress mediated by disease-linked DDX3 variants (9).

We summarize the role of DDX3 in cellular translation mechanisms under normal and stress conditions. We discuss the biochemical properties and the biological roles of DDX3 and our current understanding of various modes of translation. We also highlight recent findings of DDX3 shaping the translational landscape in various biological contexts.

DDX3, AN ATP-DEPENDENT RNA HELICASE OF THE DEAD-BOX FAMILY

A large family of RNA helicases that share the signature Asp-Glu-Ala-Asp (DEAD) motif in their protein sequences is universally found in eukaryotes, suggesting their evolutionarily conserved role in RNA metabolism and regulation (11). These so-called DEAD-box helicases are characterized by shared catalysis, namely strand unwinding and separation of double-stranded RNA molecules with coupled ATP hydrolysis (11). The human DDX3X (also

ISSN: 1976-670X (electronic edition)
Copyright © 2022 by the The Korean Society for Biochemistry and Molecular Biology
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
known as DBX and DDX3) is a member of the DEAD-box family. Encoded by the DDX3X gene on p11.3-11.23 of the X chromosome, DDX3X is expressed ubiquitously in our body (12, 13). A paralogous gene on the non-recombining region of the Y chromosome, DDX3Y, encodes a highly homologous protein, DDX3Y (14, 15), which is thought to be expressed mostly in the male germline, thus presumably underlying male fertility (15). Since DDX3X and DDX3Y show a high protein sequence similarity (92%) (14) and can functionally complement each other (16), we designate both paralogs as DDX3 in aggregate.

Almost all sequenced eukaryotes encode DDX3 orthologs, and the Saccharomyces cerevisiae Ded1p is one of the most intensively studied (17). Other notable DDX3 homologs include the Xenopus laevis An3, Mus Musculus Pt10, and Drosophila melanogaster Belle proteins (17). These DDX3 orthologs and homologs belonging to the DEAD-box family display distinctive similarity in domain organization and structure and thus constitute the Ded1p/DDX3 subfamily, which also includes other closely related DEAD-box helicases, the Vasa/DDX4 homologs (17).

The human DDX3 protein is a ∼73-kDa polypeptide composed of either 661 or 662-amino acid residues, resulting from alternative splicing (Fig. 1) (8). As a member of the DEAD-box helicase family, DDX3 carries a characteristic helicase core, which is composed of two RecA-like domains in tandem (Fig. 1) (18). Twelve signature helicase motifs responsible for ATP binding and hydrolysis (motifs I, II, DEAD, VI), RNA binding (Ia, Ib, Ic, IVa, Va, VI), and communication between RNA and ATP binding sites (II, Iva) are speckled across the helicase core (Fig. 1) (17, 18). The helicase core is flanked by structurally flexible, low complexity domains (LCD) at both the amino (N) and the carboxyl (C) termini (Fig. 1A) (11, 19, 20). Both LCDs are thought to play an extracatalytic, regulatory role, thereby functionally distinguishing DDX3 from other DEAD-box family members. For instance, The N- and the C-terminal LCDs carry CRM-1-dependent and TAP-dependent nuclear export signals, respectively (Fig. 1) (21-23). The N-terminal LCD also harbors a binding site for the key translation factor, elf4E, which is absent in other DEAD-box family proteins, suggesting a role for DDX3 in translation regulation (Fig. 1) (24, 25).

A recent X-ray crystallographic study has redefined the functional core of the Ded1/DDX3 subfamily of proteins (26). The updated functional core not only contains the central, RecA-like domains, but extends into the contiguous LCDs including the so-called N-terminal extension (NTE) and the C-terminal extension (CTE) that abut the helicase core (Fig. 1) (26). The NTE and the CTE appear to contribute differentially to DDX3-mediated catalysis, with the NTE and the CTE engaged in ATP hydrolysis and in RNA duplex unwinding, respectively (26, 27). A recent X-ray crystallographic study of the pre-unwound state of the molecular complex between DDX3 and the substrate double-stranded RNA (dsRNA) further elucidated the catalytic mechanism (28). Thus, DDX3 acts as a dimer recognizing a 2-turn of dsRNA, with each DDX3 molecule recognizing a single strand of the RNA duplex. Conformational changes induced by ATP binding to DDX3 were proposed to unwind the dsRNA substrate, while the subsequent ATP hydrolysis mediates the release of the reaction products, single-stranded, unwound RNAs (28).

Since the discovery of human DDX3X and DDX3Y genes in 1990s (13, 14), DDX3 has been implicated in most aspects of RNA metabolism, ranging from transcription, pre-mRNA splicing, RNA export, and translation (9). An early, pioneering work unveiled the role of DDX3 in binding and exporting of the HIV-1 genome from the nucleus to the cytosol (22). Based on this pro-viral RNA nuclear export, subsequent virological studies reported that DDX3 binds to numerous RNA viral and retroviral RNA molecules, inducing either pro-viral response or antiviral innate immunity depending on the context, which does not always require ATP-binding or strand-separating activity of DDX3 (29, 30). However, few studies reported a systematic analysis of cellular RNA substrates of DDX3 until recently, which examined either model RNA substrate or select cellular transcripts in analyzing the activity of DDX3 (17, 31).

Recent transcriptome-wide cross-linking immunoprecipitation (CLIP)-seq experiments have just started to reveal the extent and nature of cellular RNA substrates of DDX3 (32). Various research groups established that DDX3 binds to virtually all

Fig. 1. Domain structure of DDX3. Two tandem RecA-like domains (red) are flanked by the low complexity domains at the N and C terminals (gray). Twelve signature motifs shared by DEAD-box family proteins are shown inside the RecA-like domains. The motif II contains the motif DEAD that defines the family. Two nuclear export signals (NES) and an elf4E-binding region are also shown. The newly defined functional core (Ref 26) not only contains the central RecA-like domains but also includes abutting NTE and CTE. See the text for detailed description. Numbers indicate positions of amino acid residues from the N to the C terminus.
transcribed mRNAs (19, 25, 33, 34), preferentially to their 5’ untranslated regions (19, 25, 33, 34) and guanine (G)-rich sequences (25), as well as specific sites on 18S ribosomal RNA (rRNA) (25, 33, 34). DDX3 was also complexed exclusively with translation-related proteins (25, 31). All these findings suggest that DDX3 mainly promotes translation on the ribosome by physically associating with specific features on mRNA and rRNA as well as complexing with key translation-related proteins (19, 25, 33-35). While DDX3 affects the global translation landscape, the degree of translation induced varies with individual genes (19, 25, 33-35), as revealed by ribosome profiling (also known as ribo-seq) studies (36, 37). Indeed, the translation of some mRNAs can be downregulated by DDX3 expression (24, 38). In this regard, we highlight recent studies elucidating the role of DDX3 in various types of translation to alter the proteomic landscape in the cell in the presence of diverse intrinsic and extrinsic signals.

**ALTERED EXPRESSION AND GENETIC VARIATIONS OF HUMAN DDX3**

As mentioned above, DDX3 has been implicated in proviral mechanisms or antiviral innate immunity in response to infection by RNA viruses and retroviruses (29, 30). However, altered expression and genetic variations of DDX3 has mostly been documented by studies on human malignancies, and more recently on hereditary defects associated with human hindbrain and cortical development and intellectual disability (7-10, 39). It is unclear how alterations in DDX3 structure and expression and genetic variations of DDX3 has mostly been documented by studies on human malignancies, and more recently on hereditary defects associated with human hindbrain and cortical development and intellectual disability (7-10, 39).

Most human organs universally express DDX3 principally from the DDX3X gene, while the expression of the DDX3Y paralog appears to be restricted to the male germline (14). Notably, the DDX3 expression in cancer cells is aberrant relative to that of neighboring normal cells. These observations from cancer studies, however, appear inconsistent due to conflicting observations of either upregulated or downregulated DDX3 expression (9). Further, most of these clinical studies did not investigate whether the altered DDX3 expression is a cause or a consequence of the malignant phenotypes. Even so, considering the primary role of DDX3 in translation, it is speculated that the accelerated protein production by the increased DDX3 expression in cancer cells may contribute to rapid cell growth (40). However, cancer cells may also need to attenuate translation to moderate the toxic effects of misfolded and aggregated proteins in tumor cells (41-43), mediated via decreased DDX3 expression.

To date, elevated DDX3 expression has been reported in a wide range of cancers, including glioma, medulloblastoma (MB), meningioma, head and neck squamous cell carcinoma (HNSCC), lung cancer, breast cancer, hepatocellular carcinoma (HCC), gallbladder carcinoma, pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), prostate cancer, and sarcoma (9). Patients with enhanced DDX3 expression in lung cancer, gallbladder carcinoma, glioma, meningioma, PDAC, or in some cases of smoking-associated HNSSC are likely to have poor prognosis. However, reduced DDX3 expression has also been reported in patients with CRC, non-smoking-related HNSSC, or virus-infected lung cancer and HCC, who are also likely to have poor prognosis.

In addition to deviant expression, malignant cells in diverse cancers tend to develop genetic variations of the DDX3X gene, which fall into one of the two broad categories. First, the elimination of a functional copy of the DDX3X gene is frequently observed in various cancers. For example, a study investigating chronic lymphoid leukemia (CLL) revealed nonsense mutations and frameshift indels of the DDX3X gene in ~10% of the patients, who express truncated DDX3 proteins (44). Similarly, a study of melanoma uncovered ~5.8% of the patients with DDX3X mutations, including 35% of them carrying truncations (45). Truncating mutations affecting the DDX3X gene are also frequently observed in natural killer-T-cell lymphoma (NKTCL), in which DDX3X truncations represent the most recurring mutations (46). On the other hand, the loss of a functional DDX3X copy can also occur due to gene-inactivating translocations. In T-cell acute lymphoblastic leukemia (T-ALL), for instance, the DDX3X gene appears to be one of the frequently inactivated genes caused by insertion of the translocating MLLT10 gene (47).

Another large share of DDX3X variations occurring in human malignancies takes the form of missense mutations, most of which affect the central, RecA-like domains, thereby inactivating the catalytic competency of the affected DDX3 proteins. For example, 65% of DDX3X-mutated melanoma patients were found to have missense mutations (45). Likewise, roughly half of the DDX3X variations occurring in NKTCL are missense mutations (46). Recurring DDX3 A404P and E348K variants in this cancer have been shown to decrease RNA unwinding activity and are associated with poor prognosis (46).

Recent whole genome and exome sequencing studies of medulloblastoma (MB), a malignant tumor occurring in the cerebellum and the brain stem of children and adolescents (48), revealed a plethora of DDX3X mutations, which are exclusively missense or small in-frame deletions affecting the central, RecA-like domains of DDX3X (48). Notably, DDX3X mutations were exclusively identified in the WNT and SHH subgroups among the four molecular subgroups of MB including WNT, SHH, Group 3, and Group 4 (48). Approximately 50% of patients with WNT MB were found to harbor mutations in the DDX3X gene, which is thus the second most frequently mutated gene after the CTNNB1 gene encoding β-catenin (48). While DDX3X mutations were overall less frequently observed in SHH MB (11%) than in WNT MB, they accumulated further in adult patients with SHH MB (54%) than in pediatric cases (7.2%) (49). It is unclear why only the WNT and the SHH subgroups of MB, but not Groups 3 and 4, develop DDX3X mutations. However, a recent mouse genetic study revealed that mouse Ddx3 mediates Hox gene expression and pyroptotic tumor suppression, which restricts WNT and SHH pathway.
activation and tumor formation in the lower and the upper rhombic lips, respectively (50). Loss of Ddx3x alleviates these restrictions in lineage and stress signaling, contributing to spread of WNT and SHH tumors (50).

Studies of MB provide us with a rich source of natural DDX3X alleles, which can help elucidate the roles of key catalytic residues in the RNA helicase DDX3 (48, 49). In vitro catalysis of purified DDX3 variants such as G302V, G325E, R276K, and R534H in combination with functional complementation of yeast ded1 demonstrated that these MB-associated variants are catalytically compromised (25-27). These missense DDX3X variants have further been shown to facilitate stress granule assembly (see below) and adjust normal stress-induced translation downregulation, suggesting an adaptive role of the catalytically compromised DDX3X variants in cells under stress (19, 25). These recent studies provide insight into the role of DDX3X in a variety of translation mechanisms.

OVERVIEW OF VARIOUS TRANSLATION MODES

The mRNA translation efficiency and fidelity is critically affected by sequence features and secondary structures (5). The presence of the m'GpppN-modified 5' cap (m, a methyl group; p, a phosphate group; and N, any nucleotide) and the poly-adenylated 3' poly(A) tail distinguishes mRNA from all the other RNA classes (5). These features not only stabilize the mRNA molecule but also facilitate translation via exquisite interaction between mRNA and translation factors (5). Binding to the 5' cap, eukaryotic Initiation Factor 4E (eIF4E) forms a complex called eIF4F by assembling the DEAD-box helicase eIF4A and the scaffold protein eIF4G (5). The eIF4F complex in turn recruits the 43S pre-initiation complex (PIC), which consists of the 40S ribosomal small subunit and the ternary complex (TC, consisting of eIF1, eIF1A, eIF3, and eIF5). On the other hand, the poly(A)-binding protein (PABP) binds to the 3' poly(A) tail (5). The PABP-bound 3' poly(A) tail and the eIF4F-bound 5' cap are linked via binding between PABP and eIF4G (5). As a result, the mRNA exhibits a circularized, closed loop conformation, which is thought to facilitate multiple rounds of translation via recycling of ribosomal subunits (5).

The mRNA translation entails initiation, elongation, and termination, followed by ribosomal recycling (5). Translation factors at each translation stage interact differentially with respect to regional divisions along the length of the engaged mRNA, namely the 5' untranslated region (5' UTR, also known as the 5' leader), the coding sequence (CDS), and the 3' untranslated region (3' UTR) (5). Translation is initiated at the 5' UTR, which is the section between the 5' cap and the translation initiation site (TIS, also known as the start codon) (5). The 43S PIC formed on the 5' cap (see above) moves in the 5'-to-3' direction along the 5' UTR, searching for the optimum TIS (5). The 43S PIC, on its arrival at the TIS, releases all the factors other than 40S ribosomal subunit, to which the 60S ribosomal large subunit attaches to form the complete 80S ribosome, concluding the initiation stage (5).

Once assembled, the 80S ribosome moves towards the 3' end along the CDS (the section between the TIS and the stop codon), synthesizing an elongating chain of polypeptide by accommodating the codon-anticodon interaction between the mRNA and the aminoacyl tRNAs (5). This stage requires eukaryotic Elongation Factor 1 (eEF1), eEF2, and eEF5A, and continues until the 80S ribosome reaches the stop codon (5). The 80S ribosome arriving at the stop codon on the CDS terminates the new peptide bond formation and releases the resulting nascent polypeptide, which requires two main factors, eukaryotic Release Factor 1 (eRF1) and eRF3 (5). Coupled to this termination stage, the ATP-binding cassette family protein ABCE1 induces conformational changes in the 80S ribosome, releasing the 60S ribosome large subunit and subsequently dissociating the deacylated tRNA from the 40S ribosome small subunit (5). This ribosome recycling stage completes the cycle of translation, and the dissociated ribosomal subunits are available for further rounds of translation (51). Flanking the CDS, the 3' UTR is associated with numerous RNA-binding proteins and microRNAs (miRNAs), which play a diverse role in controlling the level of mRNA translation yield (52).

While all the regional divisions of mRNA are delicately regulated to meet the translational demands of the cell, the translation initiation on the 5' UTR is the best characterized step (5). Notably, complex secondary structures with high GC content in 5' UTRs can impede the scanning process by the 43S PIC (5). An extreme example of such a complex structure called G-quadruplex (a G-rich structure stabilized by stacked G-G-G-G tetrads) is frequently found in oncogene mRNAs (53), suggesting its role in moderating tumor-promoting gene expression. Remodeling of such complex secondary structures is thought to be crucial in efficient translation initiation. Indeed, the DEAD-box helicase eIF4A, a core constituent of the eIF4F complex (5), is well known for its role in promoting translation by unwinding bases on G-quadruplexes and other complex secondary structures in the 5' UTR (54). Importantly, elf4A per se appears to be a weak helicase (55), thereby requiring other RNA helicases for efficient translation of mRNAs with structured 5' UTRs.

Another well-known 5' UTR regulation mechanism involves the translational response to the activation of the growth-promoting mTORC1 pathway (56), leading to the translation of such mRNAs containing a specific nucleotide sequence motif called terminal oligopyrimidine (TOP) (57). The activation of mTORC1 pathway inactivates the 4E-BP family of eIF4E repressors, leading to the binding of elf4E to the 5' cap, thereby promoting translation of the TOP-containing mRNAs (57).

Other characteristics of the 5' UTR sequence can lead to alternative modes of translation initiation. For example, both translation initiator of short 5' UTR (TISU) (58) and cap-independent translation enhancer (CTE) elements (59) on 5'UTRs of select mRNAs promote translation initiation in a cap-dependent, but scanning-independent manner, via direct interaction...
between ribosomal proteins S3 and S10e and the TISU element (60) and between the 18S rRNA in the 40S ribosome small subunit and the CITE element (59), respectively. While TISU-mediated initiation promotes continuous translation of mRNAs for mitochondrial proteins under stress such as nutrient deprivation (61), CITE-mediated initiation stimulates translation of several viral RNAs as well as select cellular mRNAs (62, 63).

Noncanonical, alternative translation initiation can occur even without recognition of the 5' cap by the elf4f complex. One such cap-independent initiation involves peculiarly structured elements known as internal ribosomal entry sites (IRES), which are present in many viral RNAs and an estimated 5-10% of cellular mRNAs (64). Translation of these IRES-containing RNAs requires a dedicated set of trans-acting proteins called IRES-acting factors (ITAFs) (64). While viral ITAFs promote translation of the viral RNAs with IRESs for reproduction, cellular ITAF-IRES interactions appear to play an important role in a variety of biological processes, ranging from myogenesis, neurogenesis, oncogenesis, and stress response (64).

Another notable sequence feature that mediates cap-independent translation initiation includes m^6^A (N^6^-methyl adenosine) modification, which has recently gained enormous attention (65). In fact, m^6^A is one of the most prevalent modifications of both coding and noncoding RNAs, and is found in more than 7,000 mRNAs in humans and mice (65). Importantly, many trans-acting factors called "readers" recognize m^6^A modifications on RNAs, leading to specific responses (65). While the majority of m^6^A modifications are associated with the 3' UTR near the stop codon (66), the level of 5' UTR m^6^A appears to be significant enough to promote a cap-independent translation initiation, especially in response to stress (67, 68). Notably, m^6^A modifications present in the 5' UTR can simulate translation of TOP-containing mRNAs, regardless of the 5' cap and IRESs (69).

While the CDS represents the main open reading frame (ORF) for the 80S ribosome to synthesize a polypeptide chain, the 5' UTRs of nearly half of all human mRNAs contain at least one upstream ORF (uORF) (70). Short in length, uORFs are often out-of-frame and overlap with the main ORF downstream. Therefore, early engagement of the 80S ribosome at the start codon of a uORF is likely to result in abortive or attenuated translation of the main downstream ORF (5). The mechanism of uORF regulation is best understood when cells engage in various modes of translation (Fig. 2). DDX3 plays a crucial role not only in cap-dependent but also in cap-independent translation, promoting translation in general while repressing it in some conditions. While the ability of DDX3 to resolve complex secondary structures in the 5' UTR facilitates appropriate translation initiation, we have also found that DDX3 facilitates the elongation of nascent polypeptide chain in the CDS by the 80S ribosome. Moreover, we have begun to understand the mechanism by which DDX3 participates in stress granule (SG) formation to downregulate translation globally in response to stress, while upholding translation of select mRNAs.

The role of the DDX3 orthologs in translation was suggested by early studies, showing that mutational inactivation of Ddx7 in yeast results in global translation downregulation accompanied by polysome collapse (74, 75). A recent immunoprecipitation-
Translational regulation by the RNA helicase DDX3
Joon Tae Park and Sekyung Oh

How can this property of DDX3 bind G-rich sequences affect translation? In the yeast, repression of Ded1p activity leads to accumulation of organized RNA structures in 5' UTRs (33). Using ribosome profiling, the same study further showed that the compromised Ded1p activity leads to an unconventional translation initiation from near-cognate start codons occurring immediately upstream of these induced structures, while protein production from the main ORFs is diminished (33), indicating that unravelling of constraining mRNA structures by Ded1p is crucial for accurate translation initiation. Of note, whereas the yeast Ded1p was shown to control translation of most mRNAs (33), the range of the translation response altered by the yeast DDX3, as measured by ribosome profiling, appears to be rather narrow (19, 25, 33), which, however, varies in cells under stress (25).

Although less prevalent than in 5' UTR, significant levels of DDX3 binding to mRNAs were detected in the CDS and the 3' UTR as well (Fig. 2) (25), suggesting a role for DDX3 beyond translation initiation in the 5' UTR. A comparison between DDX3 binding and ribosome footprints on mRNAs further revealed that DDX3 is located immediately 5' upstream of the ribosome (25), suggesting a role for DDX3 in resolving obstructing structures in the CDS. Indeed, a recent study of the DDX3 orthologs in Leishmania infantum showed that loss of DDX3 decelerates ribosome movement along the CDS, causing the emergence of elongation-stalled ribosomes and, consequently,

In addition to proteomics analysis of the biological role of DDX3, recent CLIP-seq studies have provided us with unprecedented information regarding the interaction between cellular RNAs and DDX3, and the specific RNA features underlying the interaction (19, 25, 33, 34). Both the human DDX3 and the yeast Ded1p orthologs bind to almost all transcribed mRNAs (19, 25, 33, 34) as well as 18S rRNA (25, 33, 34), supporting their involvement in mRNA translation. Notably, DDX3 and Ded1p interact with mRNAs by binding predominantly to 5' UTRs (Fig. 2) (19, 25, 33, 34), with the interaction particularly accumulated near the TIS (25). DDX3 and Ded1p were also shown to interact with 18S rRNA at specific locations (Fig. 2) (25, 33, 34), most notably, those mapped adjacent to the mRNA entry channel of the ribosome (33, 34). Thus, both the proteomics and the CLIP-seq approaches suggest that DDX3 orthologs play a major role in translation initiation.

A CLIP-seq study further demonstrated that DDX3 favors binding to guanine nucleotide (G-rich sequences) (25), suggesting the requirement of the RNA helicase DDX3 in unwinding rigidly organized structures with high melting temperatures. This finding at the transcriptome level is consistent with a previous study showing that DDX3 facilitates translation of selected mRNAs with complex 5' UTR secondary structures (Fig. 2) (31). Using ribosome profiling, the same study further showed that the compromised Ded1p activity leads to an unconventional translation initiation from near-cognate start codons occurring immediately upstream of these induced structures, while protein production from the main ORFs is diminished (33), indicating that unravelling of constraining mRNA structures by Ded1p is crucial for accurate translation initiation. Of note, whereas the yeast Ded1p was shown to control translation of most mRNAs (33), the range of the translation response altered by the human DDX3, as measured by ribosome profiling, appears to be rather narrow (19, 25, 33), which, however, varies in cells under stress (25).

Although less prevalent than in 5' UTR, significant levels of DDX3 binding to mRNAs were detected in the CDS and the 3' UTR as well (Fig. 2) (25), suggesting a role for DDX3 beyond translation initiation in the 5' UTR. A comparison between DDX3 binding and ribosome footprints on mRNAs further revealed that DDX3 is located immediately 5' upstream of the ribosome (25), suggesting a role for DDX3 in resolving obstructing structures in the CDS. Indeed, a recent study of the DDX3 ortholog of Leishmania infantum showed that loss of DDX3 decelerates ribosome movement along the CDS, causing the emergence of elongation-stalled ribosomes and, consequently,
Translational regulation by the RNA helicase DDX3
Joon Tae Park and Sekyung Oh

reduced protein production (Fig. 2B) (38). Loss of DDX3 in Leishmania also triggered defective recruitment of the ribosome recycling factor ABCE1 and the termination factors eRF3 and GTPBP1, suggesting inefficient ribosome dissociation and recycling in the absence of DDX3 (38). Interestingly, prolonged ribosome stalling induced by DDX3 loss leads to ubiquitination of the nascent polypeptide chain with accompanying recruitment of E3 ubiquitin ligases (Fig. 2B) (38). Cotranslational ubiquitination is considered as one of the pervasive proteome quality control mechanisms and is intimately associated with translation (80-82). Thus, DDX3 appears to contribute to the maintenance of proteome quality via translation elongation and termination by binding to CDSs and 3' UTRs of mRNAs (Fig. 2) (25, 38).

Contrary to the translation-promoting role, translation-suppressing roles for DDX3 have also been suggested. On one hand, an early study using a bi-cistronic translation reporter demonstrated that DDX3 can repress cap-dependent translation by titrating out eIF4E from the interaction with eIF4G and demonstrated that DDX3 can repress cap-dependent translation in the context of amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) (83). The expansion of the hexanucleotide GGGGCC (G4C2) is one of the most common genetic causes of both ALS and FTD (84, 85). Translation of the G4C2-containing mRNAs such as the one from the C9ORF72 gene generates toxic, dipeptide repeat (DPR) proteins, via the process called the via repeat-associated non-AUG (RAN) translation (86). In this neuropathological context, DDX3 was shown to bind directly to the sense strand of G4C2-repeat RNAs, suppressing the RAN translation from all three reading frames (83). The helicase activity of DDX3 appears to be essential for the suppression of the RAN translation, suggesting that DDX3-mediated unwinding of the repeat-associated RNA structure attenuates the RAN translation. In this regard, increasing DDX3 expression and activity may have therapeutic utility in treating ALS and FTD (83).

On the other hand, DDX3 has long been known as a constituent of SGs in cells exposed to stress, thereby mediating stress-induced translation downregulation (Fig. 2C) (8, 23, 31, 87, 88). While the forced expression of wild-type DDX3 per se can drive spontaneous SG formation in the absence of stress (23, 89, 90), the expression of medulloblastoma-associated, catalytically impaired DDX3 variants was shown to intensify the SG formation (Fig. 2C) (19), suggesting an association between the failure of DDX3 in mounting proper translation and SG formation. SG formation is perceived as a survival strategy of stress-exposed cells by triaging mRNAs (91). In this regard, a recent study underscored a pivotal biological role for DDX3 in making decisions between survival and death under stress, demonstrating that DDX3 interacts with NLPR3 to activate inflammasomes (92), which ultimately leads to pyroptotic cell death (93). Induction of SG formation was proposed to hinder DDX3-mediated NLPR3 inflammasome formation, thereby promoting cell survival (92).

While the DDX3-induced SG formation remains unclear, a recent study elucidated a mechanism by which the acetylation status of DDX3 regulates SG maturation (94). The deacetylase HDAC6 was shown to target the N-terminal LCD of DDX3, with the resulting deacetylated DDX3 mediating liquid-liquid phase separation (LLPS) (94), the key process underlying the establishment of membrane-less subcellular structures such as SGs (95, 96). Notably, DDX3 significantly gains binding to the CDS and the 3' UTR in response to stress, while withdrawing its otherwise, predominant occupancy in the 3' UTR (25), suggesting a new, yet still unclear role for DDX3 in coating broader regions of the translation-unengaged mRNAs in SGs. Further studies are needed to elucidate whether and how this spread of binding and the deacetylation of DDX3 are related in cells under stress.

Not all mRNAs undergo translational downregulation when cells are exposed to stress. A ribosome profiling experiment revealed varied, gene-specific responses in cells expressing either wild-type or the medulloblastoma-associated, catalytically compromised R534H variant of DDX3 (25). Whereas wild-type DDX3 expression under stress augments translation downregulation, DDX3 R534H expression specifically ensures translation of mRNAs related to chromatin organization and maintenance such as those of histone proteins, accompanied by increased binding of DDX3 R334H to histone mRNAs (Fig. 2C) (25), suggesting a cellular adaptation mechanism to maintain the integrity of the genome under stress. In fact, histone mRNAs are molecularly idiosyncratic in that they contain short UTRs, few introns, and highly repetitive sequences with high GC content, and lack the 3’ poly(A) tail. Notably, the translation of histone mRNAs was shown to rely on their binding to a region on the 18S rRNA called helix 16 (h16), which is not only adjacent to the mRNA entry channel of the ribosome (97) but also binds with DDX3 and Ded1p (33, 34). Thus, a potential competition between DDX3 proteins and histone mRNAs for binding to h16 was proposed (34), which might also explain the factors sustaining histone mRNA translation in cells either expressing the catalytically impaired DDX3 R534H variant (25) or lacking DDX3 (34).

The yeast Ded1p appears to alter the gene-level translation responses more widely than the human ortholog (33, 98). Heat shock and pH stress induce Ded1p condensation, switching off translation of housekeeping mRNAs but simultaneously switching on stress-response mRNAs, as a stress-adaptive strategy (98). Similarly, the human DDX3 was shown to induce the translation of the integrated stress response pathway (ISR) target ATF4 (42), via phosphorylated eIF2α-mediated skipping of the second uORF (see above) (99). Given the well-perceived role for ATF4 in coordinating gene expression under stressful environment (73), this human DDX3 regulation of ATF4 expression in cells under stress (99) is consistent with the results of yeast Ded1p in translational switching (98), suggesting a shared stress-
CONCLUSIONS

While the DEAD-box RNA helicase DDX3 plays a pleiotropic role in RNA metabolism, our understanding of the DDX3-regulated translation response has rapidly advanced in recent years. With the availability of transcriptome and proteome-wide atlases of the DDX3-interacting molecules, we have begun to understand how DDX3 shapes the translational landscape at a finer and more comprehensive level (19, 25, 33, 34), which was enabled by recent advances in transcriptomics and transcriptomics technologies such as advanced mass spectrometry, RNA-seq, CLIP-seq, and ribosome profiling (32, 36). These new research tools have led us to appreciate the fundamental roles for the DDX3 orthologs not only in promoting translation globally but also in varying the degree of genespecific translational outputs. Studies have also revealed a translation-coordinating role of DDX3 in coping with various cellular stresses (25), which is encountered in diverse human pathologies, including viral infection, neurodegeneration, intellectual disability, and cancers. In addition to advances in basic biological investigation, the active pursuit of the development of small molecule inhibitors targeting DDX3 (e.g., RK-33) has a huge potential for therapeutic success (100). These innovative tools and advances contribute to ongoing studies elucidating biologically meaningful and clinically controllable translation landscape regulated by DDX3.

ACKNOWLEDGEMENTS

The authors thank members of the Park and the Oh laboratories for critical reading of the manuscript. This research was funded by The National Research Foundation of Korea (NRF-2018R1D1A1B07045410 and NRF-2021R1A2C1011293 to S.O.).

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Schwarnhaüsser B, Busse D, Li N et al (2011) Global quantification of mammalian gene expression control. Nature 473, 337-342.
2. Tahmasebi S, Khoutorsky A, Mathews MB and Sonenberg N (2018) Translation deregulation in human disease. Nat Rev Mol Cell Biol 19, 791-807.
3. Ruggiero D (2013) Translational control in cancer etiology. Cold Spring Harb Perspect Biol 5, a012336.
4. Buttgerid F and Brand MD (1995) A hierarchy of ATP-consuming processes in mammalian cells. Biochem J 312, 163-167.
5. Jackson RJ, Hellen CUT and Pestova TV (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. Nat Rev Mol Cell Biol 11, 113-127.
6. Jankowsky A, Guerther UP and Jankowsky E (2011) The RNA helicase database. Nucleic Acids Res 39, D338-D341.
7. Valiente-Echeverría F, Hemoso MA and Soto-Rifo R (2015) RNA helicase DDX3: at the crossroad of viral replication and antiviral immunity. Rev Med Virol 25, 286-299.
8. Soto-Rifo R and Ohlmann T (2013) The role of the DEAD-box RNA helicase DDX3 in mRNA metabolism. Wiley interdisciplinary reviews. RNA 4, 369-385.
9. Mo J, Liang H, Su C et al (2021) DDX3X: structure, physiologic functions and cancer. Mol Cancer 20, 38.
10. Kellaris G, Khan K, Baig SM et al (2018) A hypomorphic inherited pathogenic variant in DDX3X causes male intellectual disability with additional neurodevelopmental and neurodegenerative features. Hum Genomics 12, 11.
11. Linder P and Jankowsky E (2011) From unwinding to clamping - the DEAD box RNA helicase family. Nat Rev Mol Cell Biol 12, 505-516.
12. Kim YS, Lee SG, Park SH and Song K (2001) Gene structure of the human DDX3X and chromosome mapping of its related sequences. Mol Cell 12, 209-214.
13. Park SH, Lee SG, Kim Y and Song K (1998) Assignment 1 of a human putative RNA helicase gene, DDX3X, to human X chromosome bands p11.3–p11.23. Cytogenet Genome Res 81, 178-179.
14. Lahn BT and Page DC (1997) Functional coherence of the human Y chromosome. Science 278, 675-680.
15. Foresta C, Ferlin A and Moro E (2000) Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. Hum Mol Genet 9, 1161-1169.
16. Venkataramanan S, Gadek M, Calviello L, Wilkins K and Floor S (2021) DDX3X and DDX3Y are redundant in protein synthesis. RNA 27, ma078926.121.
17. Sharma D and Jankowsky E (2014) The Ded1/DDX3 subfamily of DEAD-box RNA helicases. Crit Rev Biochem Mol Biol 49, 343-360.
18. Högbom M, Collins R, Berg S van den et al (2007) Crystal structure of conserved domains 1 and 2 of the human DEAD-box helicase DDX3X in complex with the mononucleotide AMP. J Mol Biol 372, 150-159.
19. Valentin-Vega YA, Wang YD, Parker M et al (2016) Cancer-associated DDX3X mutations drive stress granule assembly and impair global translation. Sci Rep 6, 25996.
20. Valentini M and Linder P (2020) RNA remodeling proteins, methods and protocols. Methods Mol Biol 2209, 17-34.
21. Brennan R, Haap-Hoff A, Gu L et al (2018) Investigating nucleocytoplasmic shuttling of the human DEAD-box helicase DDX3. Eur J Cell Biol 97, 501-516.
22. Yedavalli, VSRK, Neuveut C, Chi YH, Kleiman L and Jeang KT (2004) Requirement of DDX3 DEAD box RNA helicase DDX3. Eur J Cell Biol 97, 501-516.
23. Lai MC, Lee YHW and Tarn WY (2008) The DEAD-box RNA helicase DDX3 associates with export messenger ribonucleoproteins as well as tip-associated protein and participates in translational control. Mol Biol Cell 19, 3847-3858.
24. Shih JW, Tsai TY, Chao CH and Lee YHW (2008) Candidate tumor suppressor DDX3 RNA helicase specifically represses cap-dependent translation by acting as an elf4E inhibitory protein. Oncogene 27, 700-714.
25. Oh S, Flynn RA, Floor SN et al (2016) Medulloblastoma-associated DDX3X variant selectively alters the translational response to stress. Oncotarget 7, 28169-28182
26. Floor SN, Condon KJ, Sharma D, Jankowsky E and 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, 2412-2421
27. Epling LB, Grace CR, Lowe BR, Partridge JF and Enemark EJ (2015) Cancer-associated mutants of RNA helicase DDX3X are defective in RNA-stimulated ATP hydrolysis. J Mol Biol 427, 1779-1796
28. Song H and Ji X (2019) The mechanism of RNA duplex recognition and unwinding by DEAD-box helicase DDX3X. Nat Commun 10, 2085
29. Hernández-Díaz T, Valiente-Echeverría F and Soto-Rifo R (2021) RNA helicase DDX3: a double-edged sword for viral replication and immune signaling. Microorganisms 9, 1206
30. Schröder M (2011) Viruses and the human DEAD-box helicase DDX3X: inhibition or exploitation? Biochem Soc Trans 39, 679-683
31. Soto-Rifo R, Rubilar PS, Limousin T et al (2012) DEAD-box protein DDX3 associates with elf4F to promote translation of selected mRNAs. EMBO J 31, 3745-3756
32. Darnell RB (2010) HITS-CLIP: panoramic views of protein-RNA regulation in living cells. Wiley interdisciplinary reviews. RNA 1, 266-286
33. Guenther UP, Weinberg DE, Zubratti MM et al (2018) The helicase Ded1p controls use of near-cognate translation initiation codons in 5′ UTRs. Nature 559, 130-134
34. Calviello L, Venkataramanan S, Rogowski KJ et al (2021) DDX3 depletion represses translation of mRNAs with complex 5′ UTRs. Nucleic Acids Res 49, 5336-5350
35. Jankowsky E and Guenther UP (2018) A helicase links upstream ORFs and RNA structure. Curr Genet 65, 453-456
36. Ingolia NT, Ghirmanthi S, Newman JRS and Weissman JS (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science (New York, NY) 324, 218-223
37. Ingolia NT (2014) Ribosome profiling: new views of translation, from single codons to genome scale. Nat Rev Genet 15, 205-213
38. Padmanabhan PK, Ferreira GR, Zghidi-Abouzid O et al (2021) Genetic depletion of the RNA helicase DDX3 leads to impaired elongation of translating ribosomes triggering co-translational quality control of newly synthesized polypeptides. Nucleic Acids Res 49, 9459-9478
39. Lennox AL, Hoye ML, Jiang R et al (2020) Pathogenic DDX3X mutations impair RNA metabolism and neurogenesis during fetal cortical development. Neuron 106, 404-420.e8
40. Robichaud N, Sonenberg N, Ruggero D and Schneider RJ (2019) Translational control in cancer. Cold Spring Harb Perspect Biol 11, a032896
41. Sherman MY and Qian SB (2013) Less is more: improving protein synthesis by translation slow down. Trends Biochem Sci 38, 585-591
42. Liu B and Qian SB (2014) Translational reprogramming in cellular stress response. Wiley interdisciplinary reviews. RNA 5, 301-305
43. Conn CS and Qian SB (2013) Nutrient signaling in protein homeostasis: an increase in quantity at the expense of quality. Sci Signal 6, ra24-ra24
44. Ojha J, Secretro CR, Rabe KG et al (2014) Identification of recurrent truncated DDX3X mutations in chronic lymphocytic leukaemia. Br J Haematol 169, 443-448
45. Phung B, Cieslak M, Sanna A et al (2019) The X-linked DDX3X RNA helicase dictates translation reprogramming and metastasis in melanoma. Cell Rep 27, 3573-3586.e7
46. Jiang L, Gu ZH, Yan ZX et al (2015) Exome sequencing identifies somatic mutations of DDX3X in natural killer/T-cell lymphoma. Nat Genet 47, 1061-1066
47. Brandimarte L, Starza RJ, Gianfelici V et al (2014) DDX3X-MLLT10 fusion in adults with NOTCH1 positive T-cell acute lymphoblastic leukaemia. Haematologica 99, 64-66
48. Northcott PA, Jones DTW, Kool M et al (2012) Medulloblastomas: the end of the beginning. Nat Rev Cancer 12, 818-834
49. Kool M, Jones DTW, Jäger N et al (2014) Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothed inhibition. Cancer Cell 25, 393-405
50. Patmore DM, Jassim A, Nathan E et al (2020) DDX3X suppresses the susceptibility of hindbrain lineages to medulloblastoma. Dev Cell 54, 435-470.e5
51. Schuller AP and Green R (2018) Roadblocks and resolutions in eukaryotic translation. Nat Rev Mol Cell Biol 19, 526-541
52. Mayr C (2017) Regulation by 3′-untranslated regions. Annu Rev Genet 51, 171-194
53. Kwok CK and Merrick CJ (2017) G-quadruplexes: prediction, characterization, and biological application. Trends in Biotechnol 35, 997-1013
54. Wolfe AL, Singh K, Zhong Y et al (2014) RNA G-quadruplexes cause elf4A-dependent oncogene translation in cancer. Nature 513, 65-70
55. Rogers GW, Richter NJ and Merrick WC (1999) Biochemical and kinetic characterization of the RNA helicase activity of eukaryotic initiation factor 4A*. J Biol Chem 274, 12236-12244
56. Sexton RA and Sabatini DM (2017) mTOR signaling in growth, metabolism, and disease. Cell 168, 960-976
57. Thoren CC, Chantranupong L, Keys HR et al (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 485, 109-113
58. Elbakki S, Sinvani H, Haimov O et al (2011) Unique translation initiation of mRNAs-containing TISU element. Nucleic Acids Res 39, 7598-7609
59. Yueh A and Schneider RJ (2018) Translation by ribosome shunting on adenovirus and hsp70 mRNAs facilitated by complementarity to 18S rRNA. Genes Dev 14, 414-421
60. Haimov O, Sinvani H, Martin F et al (2017) Efficient and accurate translation initiation directed by TISU involves RPS5 and RPS10e binding and differential eukaryotic initiation factor 1A regulation. Mol Cell Biol 37, e00150-17
Translational regulation by the RNA helicase DDX3
Joon Tae Park and Sekyung Oh

61. Sirvani H, Haimov O, Svitkin Y et al. (2015) Translational tolerance of mitochondrial genes to metabolic energy stress involves TISU and elf1-elf4G1 cooperation in start codon selection. Cell Metab 21, 479-492
62. Koh DC, Edelman GM and Mauro VP. (2013) Physical evidence supporting a ribosomal shunting mechanism of translation initiation for BACE1 mRNA. Translation 1, e24400
63. Nicholson J, Jevons SJ, Grosje B et al. (2017) 3′ ligase cIAP2 mediates downregulation of MRE11 and radiosensitivity in response to HDAC inhibition in bladder cancer. Cancer Res 77, 3027-3039
64. Yamamoto H, Unbehaun A and Spahn CMT. (2017) Ribosomal chamber music: toward an understanding of IRES mechanisms. Trends Biochem Sci 42, 655-668
65. Meyer KD and Jaffrey SR. (2017) Rethinking m6A readers, writers, and erasers. Annu Rev Cell Dev Biol 33, 319-342
66. Meyer KD, Saletore Y, Zumbo P et al. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell 149, 1635-1646
67. Meyer KD, Patil DP, Mao Y et al. (2017) m6A facilitates translation. Mol Cell 68, 504-514
68. Zhou J, Wan J, Gao X et al. (2015) Dynamic m6A promotes cap-independent translation. Cell 163, 999-1010
69. Zhou J, Wen J, Gao X et al. (2015) Dynamic m6A mRNA methylation directly translocates control of heat shock response. Nature 526, 591-594
70. Coots RA, Liu XM, Yao Y et al. (2017) m6A facilitates eIF4F-independent mRNA translation. Mol Cell 68, 504-514
71. Calvo SE, Pagliarini DJ and Mootha VK. (2009) Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. Proc Natl Acad Sci U S A 106, 7507-7512
72. Gao X, Wen J, Liu B et al. (2015) Quantitative profiling of the RNA helicase DDX3X as a repressor of C9ORF72 (GGGGCC)n repeat-associated Non-ATG (RAN) translation. Nature 104, 885-898.e8
73. DeJesus-Hernandez M, Mackenzie IR, Boeve BF et al. (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTLD and ALS. Nature 72, 245-256
74. Rutherford H, Majouine E, Walte A et al. (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Nature 27, 257-268
75. Cleary JD, Pattamatta A and Rana LPW. (2018) Repeat-associated non-ATG (RAN) translation. J Biol Chem 293, 16127-16141
76. Shih JW, Wang WT, Tsai TY et al. (2012) Critical roles of RNA helicase DDX3X and its interactions with elf1-eIF4G1 in stress granule assembly and stress response. Bio Chem J 441, 119-129
77. Soto-Rifo R, Rubilar PS and Ohlmann T. (2013) The DEAD-box helicase DDX3 substitutes for the cap-binding protein elf4E to promote compartmentalized translation initiation of the HIV-1 genomic RNA. Nucleic Acids Res 41, 6286-6299
78. Gilks N, Kedersha N, Ayodele M et al. (2004) Stress granule assembly is mediated by prior-like aggregation of TIA-1. Mol Biol Cell 15, 5383-5398
79. Tourrière H, Chebli K, Zokri L et al. (2003) The RasGAP-associated endoribonuclease G3BP assemblies stress granules. J Cell Biol 160, 823-831
80. Corbet GA and Parker R. (2020) RNP granule formation: lessons from P-bodies and stress granules. Cold Spring Harb Symp Quant Biol 84, 040329
81. Samir P, Kesavardhana S, Patmore DM et al. (2019) DDX3X acts as a live-or-die checkpoint in stressed cells by regulating NLRP3 inflammasome. Nature 573, 590-594
82. Shi J, Gao W and Shao F. (2017) Pyropotosis: gasdermin-mediated programmed necrotic cell death. Trends in Biochem Sci 42, 245-254
83. Saito M, Hess D, Eglinger J et al. (2019) Acetylation of intrinsically disordered regions regulates phase separation. Nat Chem Biol 15, 51-61
84. Zhou HX, Nguemaha V, Mazarakos K and Qin S. (2018) Why do disordered and structured proteins behave differently in phase separation? Trends Biochem Sci 43, 499-516
85. Courchaine EM, Lu A and Neugebauer KM. (2016) Droplet organelles? EMBO J 35, 1603-1612
86. Martin F, Ménétret JF, Simonetti A et al. (2016) The RGG domain in the C-terminus of the DEAD box helicases Dhp2 and Ded1 is necessary for G-quadruplex destabilization. Nucleic Acids Res 49, 8339-8354
87. Comyn SA, Chan GT and Mayor T. (2014) False start: Cotranslational protein ubiquitination and cytosolic protein quality control. J Proteomics 100, 92-101
88. Wang F, Durfee LA and Hübner JM. (2013) A cotranslational ubiquitination pathway for quality control of misfolded proteins. Mol Cell 50, 368-378
89. pelletier DSW and Parker R. (2016) Principles of stress granule assembly is mediated by prion-like aggregation of TIA-1. Mol Biol Cell 15, 5383-5398
90. Corbet GA and Parker R. (2020) RNP granule formation: lessons from P-bodies and stress granules. Cold Spring Harb Symp Quant Biol 84, 040329
91. Samir P, Kesavardhana S, Patmore DM et al. (2019) DDX3X acts as a live-or-die checkpoint in stressed cells by regulating NLRP3 inflammasome. Nature 573, 590-594
92. Shi J, Gao W and Shao F. (2017) Pyropotosis: gasdermin-mediated programmed necrotic cell death. Trends in Biochem Sci 42, 245-254
93. Saito M, Hess D, Eglinger J et al. (2019) Acetylation of intrinsically disordered regions regulates phase separation. Nat Chem Biol 15, 51-61
94. Zhou HX, Nguemaha V, Mazarakos K and Qin S. (2018) Why do disordered and structured proteins behave differently in phase separation? Trends Biochem Sci 43, 499-516
95. Courchaine EM, Lu A and Neugebauer KM. (2016) Droplet organelles? EMBO J 35, 1603-1612
96. Martin F, Ménétret JF, Simonetti A et al. (2016) The RGG domain...
somal 18S rRNA base pairs with mRNA during eukaryotic translation initiation. Nat Commun 7, 12622.

98. Iserman C, Almirano CD, Jegers C et al (2020) Condensation of Ded1p promotes a translational switch from housekeeping to stress protein production. Cell 181, 818-831.e19

99. Adjibade P, St-Sauveur VG, Bergeman J et al (2017) DDX3 regulates endoplasmic reticulum stress-induced ATF4 expression. Sci Rep 7, 13832

100. Voss MRH van, Diest PJ van and Raman V (2017) Targeting RNA helicases in cancer: the translation trap. Biochimica et biophysica acta. Rev Cancer 1868, 510-520