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The translational landscape as regulated by the RNA helicase DDX3

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ABSTRACT

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.

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 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* Pl10, 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 (Figure 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 (Figure 1) (18). Twelve signature helicase motifs responsible

for ATP binding and hydrolysis (motifs Q, I, II/DEAD, VI), RNA binding (Ia, Ib, Ic, IV, Iva, V, VI), and communication between RNA and ATP binding sites (III, Iva) are speckled across the helicase core (Figure 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 (Figure 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 (Figure 1) (21–23). The N-terminal LCD also harbors a binding site for the key translation factor, eIF4E, which is absent in other DEAD-box family proteins, suggesting a role for DDX3 in translation regulation (Figure 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 (Figure 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 RNA 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 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 modify the translation landscape of the affected cells and contribute to disease phenotypes.

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 (HNSSC), 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 DDX3 (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 DDX3 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 DDX3 variants in cells under stress (19, 25). These recent studies provide insight into the role of DDX3 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^7GpppN-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 eIF2, initiator methionyl-transfer RNA ($tRNAi^{Met}$), and GTP) along with 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 eIF5A, 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, eIF4A 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 eIF4E 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 (CITE) 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 eIF4F 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-transacting 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^6A (N^6-methyl adenosine) modification, which has recently gained enormous attention (65). In fact, m^6A 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^6A modifications on RNAs, leading to specific responses (65). While the majority of m^6A modifications are associated with the 3' UTR near the stop codon (66), the level of 5' UTR m^6A appears to be significant enough to promote a cap-independent translation initiation, especially in response to stress (67, 68). Notably, m^6A

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 an 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 the integrated stress response (IRS) pathway (42), which is initiated by activation of one of the four major ISR kinases under stress: GCN2 by uncharged tRNA resulting from amino acid starvation and other stresses, PERK by unfolded proteins in the endoplasmic reticulum, PKR by the double-stranded RNAs in virus-infected cells, and HRI in the presence of low levels of heme and other stresses (42). Importantly, a common substrate of all the four kinases is eIF2α, in which Ser-51 is specifically phosphorylated. As a core constituent of the TC, the GDP bound on eIF2α must be replenished with a new GTP by the guanine nucleotide exchange factor (GEF) eIF2B the TC to participate in the formation of the 43S PIC (5). The phosphorylated eIF2α, however, inhibits eIF2B and therefore remains bound to GDP, resulting in repression of the translation initiation process (42).

The translationally repressed mRNAs in response to stress are known to partition into non-membrane-bound compartments called stress granules (SGs), which are aggregates of translation-disengaged mRNAs with 40S ribosomal subunits and other initiation factors (71). SG formation not only halts the translation of the mRNAs but also protects them from hostile environment. SG formation is reversible, and SG-associated mRNAs return to the normal

translation once the cell resolves the stress and eIF2α is dephosphorylated by the protein phosphatase 1 (PP1) complex (71).

While most protein synthesis is greatly diminished with eIF2α phosphorylation, the translation of privileged, stress-responsive mRNAs can be induced (42). Importantly, this eIF2α phosphorylation-induced translation almost always involves uORFs, as revealed by recent ribosome profiling studies (36, 72). The master transcriptional effector in stress response, ATF4 is best known for its expression regulation at the translation level involving two uORFs in the 5' UTR of its mRNA (73). Notably, the second uORF (uORF2) of ATF4 mRNA overlaps out-of-frame with the downstream main ORF (73). In the absence of stress, uORF2 is translated, suppressing the translation initiation on the TIS of the main ORF. Under stress, however, the phosphorylated eIF2α decreases the assembly of a new PIC at the TIS of uORF2 and instead promotes assembly at the TIS of the main ORF, leading to ATF4 protein production. Similar mechanisms to turn on main ORFs via the ISR-eIF2α pathway have been reported for other mRNAs using ribosome profiling experiments (36, 72). More strikingly, these transcriptome-wide studies have also revealed that uORFs not only uses the canonical AUG codon but also a variety of non-AUG codons as alternative TISs (36, 72).

TRANSLATION REGULATED BY DDX3

With this basic understanding of protein-producing mechanisms in mind, we now review recent studies discussing how DDX3 participates in various modes of translation (Figure 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 *Ded1* in yeast results in global translation downregulation accompanied by polysome collapse (74, 75). A recent immunoprecipitation-mass spectrometry analysis indeed revealed that the major class of DDX3-interacting proteins represented translation-related proteins, most of which were implicated in the initiation step such as components of the eIF4F and the eIF3 complexes (25). This proteome-level observation is consistent with previous, smaller-scale studies that also showed the association of DDX3 and Ded1p with the eIF4F and eIF3 complexes (31, 76–78), suggesting that translation initiation be the major function of the DDX3 orthologs.

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 (Figure 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 (Figure 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 (Figure 2) (31). A recent study similarly reported that the RGG domain in the C-terminal region of the yeast Ded1p binds and destabilizes G-quadruplexes (79), a key feature of G-rich sequences (53).

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 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 (Figure 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, reduced protein production (Figure 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 (Figure 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 (Figure 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 instead promote an IRES-dependent, cap-independent translation regardless of the DDX3 helicase activity (24). While how confidently this reporter analysis mirrors natural translation response remains unclear, a recent study unveiled a specific, translation-suppressing role for DDX3 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 (Figure 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 (Figure 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 NLRP3 to activate inflammasomes (92), which ultimately leads to pyroptotic cell death (93). Induction of SG formation was proposed to hinder DDX3-mediated NLRP3 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 5' 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 R534H to histone mRNAs (Figure 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-adaptive translation response involving the DDX3 orthologs.

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 proteomics 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 gene-specific 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.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 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 (NESs) and an eIF4E-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.

Figure 2. DDX3 regulates diverse translation responses. Three regional divisions of mRNA are shown from left to right, 5' UTR, CDS, and 3' UTR as well as the 5' cap and the poly(A) tail. (A) Normal translation. DDX3 (blue-filled circles) binds preferentially to the 5' UTR and complicated secondary structures (pin-shaped dark gray lines). DDX3 promotes mostly translation initiation in the 5'UTR and also elongation in the CDS. (B). Either DDX3 in cells under stress or catalytically compromised DDX3 (red-filled circles) represses translation at the initiation and the elongation stages, resulting not only in the accumulation of secondary structures (pin-shaped dark gray lines) and but also in translation initiations from uORFs (yellow box) (e.g., ATF4; note the blue line from an uORF-associated ribosome). Ribosome stalling due to induced secondary structures can also increase the level of cotranslationally ubiquitinated nascent polypeptides (red line attached to the ribosome). (C) Stress induces stress granules (SGs) of ribonucleoprotein complex via complexation of translation-unengaged mRNAs and translation initiation factors with DDX3. Under SG-forming conditions, DDX3 binding spreads to the CDS and the 3' UTR (note the spread of the red-filled circles to the CDS and the 3' UTR). While most of the translation is halted, some privileged mRNAs, e.g., histone mRNAs, still undergo translation.

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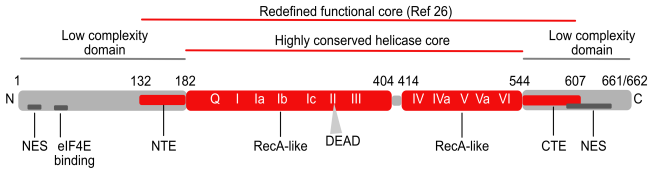

Fig. 1.

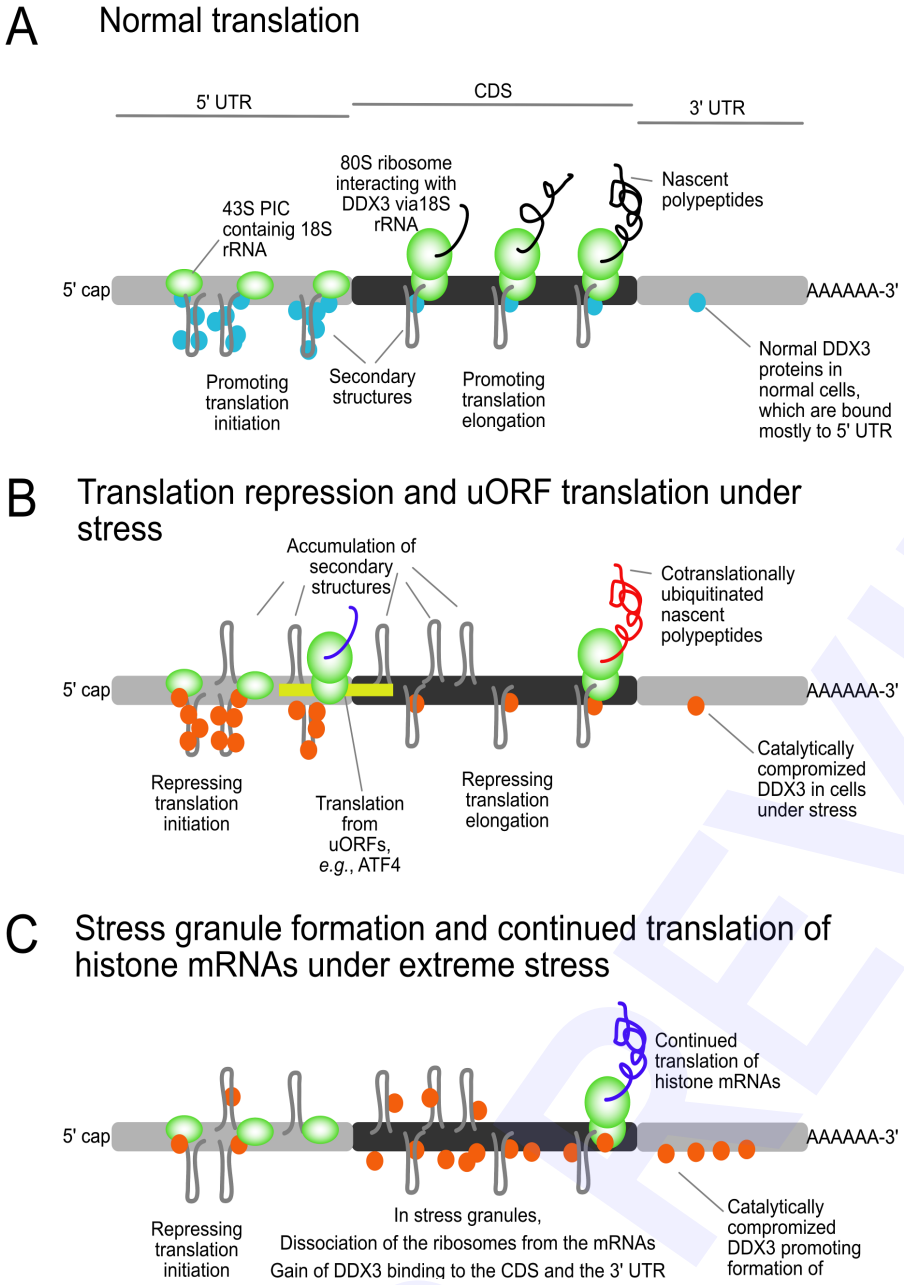

Fig. 2.