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ADVANCED REVIEW

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The RGG motif proteins: Interactions, functions, and regulations

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Abstract

Proteins with motifs rich in arginines and glycines were discovered decades ago and are functionally involved in a staggering range of essential processes in the cell. Versatile, specific, yet adaptable molecular interactions enabled by the unique combination of arginine and glycine, combined with multiplicity of molecular recognition conferred by repeated di-, tri-, and multiple peptide motifs, allow RGG motif proteins to interact with a broad range of proteins and nucleic acids. Furthermore, posttranslational modifications at the arginines in the motif extend the RGG protein's capacity for a fine-tuned regulation. In this review, we focus on the biochemical properties of the RGG motif, its molecular interactions with RNAs and proteins, and roles of the posttranslational modification in modulating their interactions. We discuss current knowledge of the RGG motif proteins involved in mRNA transport and translation, highlight our merging understanding of their molecular functions in translational regulation and summarize areas of research in the future critical in understanding this important family of proteins.

This article is categorized under:

RNA Interactions with Proteins and Other Molecules > Protein-RNA Recognition

Abbreviations: 53BP1, p53 binding protein 1; aDMA, ω-N^G, N^G-asymmetric dimethylarginines; ALS, amyotrophic lateral sclerosis; Aven, apoptosis and caspase activation inhibitor; APC, adenomatous polyposis coli; ASF, A-site finger; BAZ1A, bromodomain adjacent to zinc finger domain 1A; BRD4, bromodomain-containing protein 4; CIRBP, Cold-inducible RNA-binding protein; CTD, C-terminal domain; Ded1, defines essential domain 1; EMSA, electrophoretic mobility shift assay; ERα, estrogen receptor α; EWS, Ewing's sarcoma protein; FET, FUS, EWS and TAF15; FGF, fibroblast growth factor; FMRP, Fragile X mental retardation protein; FUS, Fused in sarcoma (protein); G3BP1, Ras-GAP SH3-binding protein 1; GAR, glycine-arginine-rich; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; HITS-CLIP, high-throughput sequencing coupled with crosslinking immunoprecipitation; hnRNP, heterogeneous nuclear ribonucleoprotein; HSP70, heat shock protein 70; IDR, intrinsically disordered region; IRES, internal ribosome entry site; Jmjd6, Jumonji-domain containing protein 6; KH, K-homology; Lsm14, Sm-like protein 14; MMA, ω-N^G monomethylarginines; NES, nuclear export signal; NLS, nuclear localization signal; NMD, nonsense mediated decay; Npl3, nucleolar protein 3; NTD, N-terminal domain; PABP, Poly A binding protein; P-bodies, cytoplasmic processing bodies; PIC, pre-initiation complex; PGM, proline-glycine-methionine; PRMTs, protein arginine methyltransferases; PTM, posttranslational modification; PY, proline-tyrosine; QGSY, glutamine-glycine-serine-tyrosine; RanBPM, Ran-binding protein microtubule; RBP, RNA-binding protein; RHA, RNA helicase A; RGG, arginine–glycine–glycine; RRM, RNA recognition motif; SAM, S-adenosyl methionine; Scd6, suppressor of clathrin deficiency 6; sDMA, ω-N^G,N'^G-symmetric dimethylarginines; Sho, Shadoo protein; siRNA, small interfering RNA; SMN, survival of motor neurons (complex); snRNA, small nuclear RNA; TRAF6, factor receptor 6.

RNA Interactions with Proteins and Other Molecules > Protein-RNA Interactions: Functional Implications
Translation > Mechanisms

KEYWORDS
arginine-glycine-glycine/arginine-glycine motif, mRNA transport, RGG/RG motif, RNA-binding proteins, translation/protein biosynthesis

1 | INTRODUCTION

RNA-binding proteins (RBPs) play versatile and critical roles in regulating gene expression (Gebauer et al., 2021; Gerstberger et al., 2014; Hentze et al., 2018). Among the many known RBPs, RGG-containing proteins are the second most common RBPs in the human genome (Ozdilek et al., 2017; Thandapani et al., 2013). This family of proteins is broadly defined as proteins that contain relatively closely spaced RGG and/or RG repeats of varying lengths, interspersed with different amino acids (Corley & Gready, 2008; Kiledjian & Dreyfuss, 1992; Thandapani et al., 2013). For different proteins in the family, the length of spacing and the identity of amino acids between the adjacent RGG and/or RG repeats can vary, giving the RGG proteins another layer of compositional complexity.

The RGG (Figure 1) motif, initially named the RGG box, was found in the C-terminal region of the heterogeneous nuclear ribonucleoprotein U (hnRNPU; Kiledjian & Dreyfuss, 1992). The motif confers binding with RNA homopolymers polyG, ployA, and polyU (Kiledjian & Dreyfuss, 1992). An alignment of the RGG and XRG (X represents any other amino acids) regions of hnRNPU with other RBPs from different eukaryotic species provided the first indication that

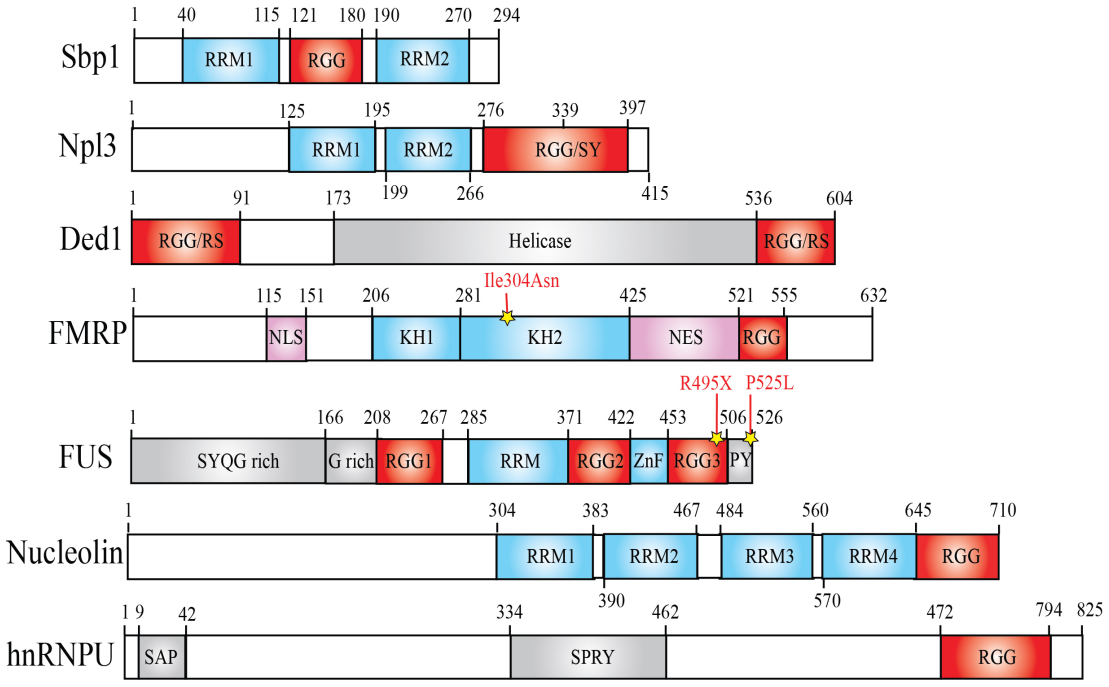


FIGURE 1 Domain architecture of several representative RGG motif proteins. The domain architecture of mammalian FMRP, FUS, nucleolin and hnRNPU as well as yeast Sbp1, Npl3 and Ded1 that contain RGG domains. The RGG regions are shown in red and other RNA binding domains are shown in blue. The nuclear export signal and nuclear localization signals in FMRP are shown in pink. A region is considered RGG if it carries two or more RG/RGG sequences within 30 amino acids. RGG domains often contain other amino acid-rich sequences within, such as proline-tyrosine (PY) in FUS, arginine-serine (RS) in Ded1 and serine-tyrosine (SY) in Npl3. Ded1 contains a helicase domain for RNA unwinding activity. hnRNPU contains a DNA binding domain (SAP) and a domain of unknown function (SPRY). FUS contains glutamine-glycine-serine-tyrosine (QGSY) rich and glycine (G) rich regions that are intrinsically disordered. Point mutations in FMRP and FUS that cause human diseases due to dys-regulation are highlighted in red (I am able to edit).

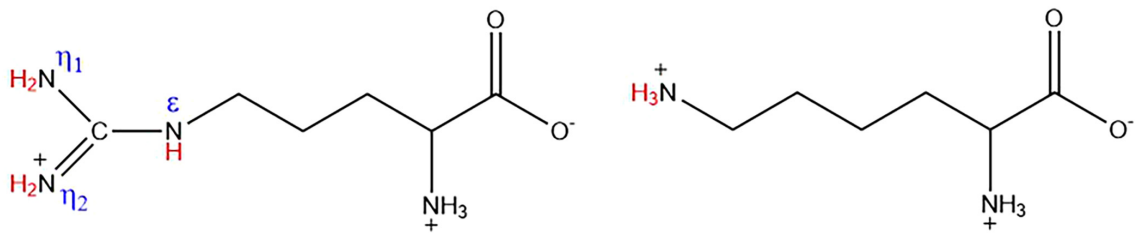
the RGG motif was likely to be conserved (Kiledjian & Dreyfuss, 1992). Later, evolutionary conservation of this motif was confirmed by an alignment of the RGGs in the N-terminal region of the Shadoo protein (Sho) from fish to mammals (Corley & Gready, 2008).

Because of their unique molecular features, proteins harboring the RGG motif are functionally involved in a staggering range of cellular processes such as DNA repair (Déry et al., 2008; Mastrocola et al., 2013; Yu et al., 2012), modulation of chromatin structure (Erard et al., 1988; Yan et al., 2021), transcription (Mowen et al., 2004; Rickards et al., 2007), pre-mRNA splicing (Lee et al., 2018; Zhou et al., 2019), RNA transport (Singleton et al., 1995), translation (Athar & Joseph, 2020; Chen et al., 2014), ribonucleoprotein biogenesis (Ginisty et al., 1998), and more. The fact that arginines and glycines are exclusively utilized in this group of proteins indicates the distinctive functions of these two amino acids (Corley & Gready, 2008). In addition to the general positive charge density, the composition and chemical structure of the arginine sidechain must be important, as other positively charged amino acids, such as lysine, are not found in the motif. Compared with lysine, arginine has more hydrogen bonding potential. It contains three amino groups in a planar geometry: two primary amino groups (NH2) at the η position and a secondary amine (NH) at the ε position. All three amino groups can function as hydrogen bond donors (Figure 2). In contrast, lysine contains one single terminal amino group (NH2) with tetrahedral geometry. Furthermore, the positively charged guanidino group with planer geometry allows arginine to interact with aromatic residues in both proteins and nucleic acids through cation–π interactions (Tripsianes et al., 2011). Notably, the two primary amino groups at the tip of the guanidino group can be posttransnationally modified by protein arginine methyltransferases (PRMTs; Guccione & Richard, 2019), providing another fine-tuned layer of interactions and regulations.

Glycines residues in the RGG motif, on the other hand, confer a larger degree of backbone flexibility compared with other natural amino acids. Furthermore, while the RGG repeats can be generally characterized as an intrinsically disordered region (IDRs; Dyson & Wright, 2005), proteins containing these repeats often have other modular domains that are well-defined structurally (Loughlin et al., 2019; Valverde et al., 2007). Therefore, with a combination of specificity, versatility and flexibility, RGG motif proteins interact with a diverse range of proteins and nucleic acids (Thandapani et al., 2013), executing indispensable functions for cell physiology.

Furthermore, the RGG motif proteins are interesting because of their relevance to human disease and related therapeutic applications (Thandapani et al., 2013). Owing to near-ubiquitous, yet specific interactions conferred by the RGG motif, deletion (Kazdoba et al., 2014) or overexpression (Fonseca et al., 2015) of the RGG proteins, as well as alteration of their posttranslational modification (PTM) states (Hofweber et al., 2018), often disrupt the delicate balance of their complex interaction network, leading to human diseases. Therefore, understanding the molecular mechanisms important for interactions and regulation of the RGG motif proteins will provide insights into how its mis-regulation may manifest as disease. Finally, due to their implication in disease, RGG motif proteins could be potential targets for drug design, an exciting area to explore with promising results (Hammoudeh et al., 2009; Krishnan et al., 2014).

The cellular functions of many RGG motif proteins and their relevance to human disease have been expertly reviewed earlier (Gebauer et al., 2021; Rajyaguru & Parker, 2012; Thandapani et al., 2013). In this review, we discuss the biochemical properties of the RGG proteins, their molecular interactions and regulations, and the current knowledge of several select RGG proteins in mRNA transport and translation.



Arginine Lysine

FIGURE 2 Comparison of arginine and lysine structures. Hydrogen bond donors of arginine and lysine residues are shown in red. Arginine has five hydrogen bond donors while lysine has three.

2 | MOLECULAR INTERACTIONS

While intrinsically disordered by nature, the unique amino acid composition of the RGG motif allows for both specific and nonspecific interactions with many proteins and nucleic acids. As discussed above, arginine and glycine residues in the motif confer broad yet specific molecular interactions. In addition, many hydrophobic and aromatic amino acids present between the RGG repeats are likely to contribute to the hydrophobic interactions involved in the protein-nucleic acid and protein–protein interactions.

2.1 | RGG–RNA interactions

Following the study of hnRNPU RGG and RNA homopolymer interaction (Kiledjian & Dreyfuss, 1992), it was reported that the RGG motif of nucleolin (Figure 1), an abundant nucleolar protein that shuttles host proteins and RNA between the nucleus and cytoplasm (Terrier et al., 2016), bound to G-quadruplex (G-quartet) RNA (Hanakahi et al., 1999; Izumi & Funa, 2019), and that the RGG region at the C-terminus of Ewing's sarcoma (EWS) bound to both G-quartet DNA and RNA (Takahama et al., 2011). Furthermore, the stability of G-quartet RNA was enhanced upon binding of Fragile X mental retardation protein (FMRP) RGG region (Figure 1; Ramos et al., 2003; Schaeffer et al., 2001; Zanotti et al., 2006).

What is the molecular basis for RGG–RNA interactions? Is it an electrostatic interaction, an amino acid-specific molecular interaction, or both? In an early study focused on nucleolin, which consists of four RNA recognition motif (RRM) domains and one RGG-rich domain, the specific protein–RNA interaction requires the four RRM domains. In contrast, the RGG-domain binds to RNAs in a nonspecific manner, but its presence increases the affinity of hnRNPU for its target RNAs by about 10-fold (Ghisolfi, Kharrat, et al., 1992). This result indicates that the RGG domain binds to RNA in a sequence nonspecific way, and the motif facilitates RNA binding through the interaction of its positively charged arginine sidechain with the negatively charged RNA backbone. On the other hand, the RGG region of hnRNPU only binds to polyA, ployG, and polyU, leaving polyC unbound (Kiledjian & Dreyfuss, 1992), suggesting that a sequence-specific RGG–RNA interaction is possible. Furthermore, the arginine in the RGG motif is critical for the recognition of specific nucleic acid structures. An R to K mutation or methylation of the arginine of RGG repeats resulted in decreased binding affinity of the EWS RGG region to the G-quartet DNA and RNA tested (Takahama et al., 2011).

RGG proteins often contain other types of RNA binding domains such as RRM and K-homology (KH) domains (Burd & Dreyfuss, 1994), making the RGG-specific RNA interactions challenging to characterize. For example, RNA sequences bound by hnRNPU in vivo were identified using optimized crosslinking immunoprecipitation coupled with high-throughput sequencing (HITS-CLIP; Yugami et al., 2020), although how the RGG domain interacts with those target sequences needs to be further characterized. The RNA-binding properties of an entire RGG-region, or individual RGG repeats within this domain, were systematically investigated in two recent biochemical studies (Brandariz-Nunez et al., 2018; Ozdilek et al., 2017). The binding between an A-rich RNA sequence with individual RRM and RGG domains of protein Sbp1 are relatively weak, but the interaction is considerably enhanced when all of the Sbp1 protein domains (Figure 1) are present (Brandariz-Nunez et al., 2018). Furthermore, an individual RGG region in fused in sarcoma protein (FUS; Figure 1) shows weak RNA-binding affinities, which are enhanced when all RGG regions are present (Ozdilek et al., 2017).

What does an RGG motif look like in three-dimensions? In an early investigation, the nucleolin C-terminal domain (CTD) RGG region bound to an RNA was suggested to contain repeated β-turns using circular dichroic and infrared spectroscopic analyses combined with computer modeling (Ghisolfi, Joseph, et al., 1992). The formation of the β-turn structure of a short FMRP RGG peptide with an in vitro selected G-rich RNA was later visualized by a crystallographic study (Vasilyev et al., 2015).

Despite these efforts, variations in the number of RGG repeats (Chong, Vernon, & Forman-Kay, 2018) and the heterogeneity of the amino acids intervening them (Corley & Gready, 2008) pose challenges to obtaining three-dimensional structures of physiologically relevant RGG motifs. To date, structures of short RGG-containing peptides bound to synthetic G-rich RNAs (Phan et al., 2011; Vasilyev et al., 2015) or proteins (Tunnicliffe et al., 2019) were obtained. Nevertheless, these structures, combined with the knowledge gained about protein–RNA interactions involving arginine and glycine residues from other proteins, provide a framework to understand the nature of RGG–RNA interactions. Glycine residues indeed confer backbone flexibility to enhance conformational adaptability for the intrinsically discorded RGG motif. The arginine, on the other hand, plays two general roles in RNA recognition. First, the positive charge of the

arginine increases electrostatic interactions with RNA in a nonspecific manner. Second, arginine can make highly specific hydrogen bonding networks with the RNA backbone, recognizing specific RNA tertiary structures such as stem-loops, internal loops, bulges, and purine-quartets (Calnan et al., 1991; Churcher et al., 1993; Tan et al., 1993; Weeks & Crothers, 1991). In addition to the RNA tertiary structure, and given the diversity of the RGG repeats, specific interactions involving the RNA bases and the arginine side chain are also possible. Indeed, binding of arginine residues to the atoms in the major groove of guanosines was observed in structures of either an RGGGGR peptide fragment or a short stretch of the FMRP RGG peptide bound to a G-rich synthetic RNA (Phan et al., 2011; Vasilyev et al., 2015).

2.2 | RGG–protein interactions

The RGG motif mediates many protein–protein interactions that are important for cellular functions. For example, the FMRP RGG region interacts with Ran-binding protein RanBPM/RanBP9 in the microtubule-organizing center (Menon et al., 2004). The RNA helicase A (RHA) RGG region interacts with the N-terminus of β-actin, an interaction important for the recruitment of RNA polymerase II to the transcription pre-initiation complex (PIC; Tang et al., 2009). The RGG-rich region of the cold-induced RNA-binding protein (CIRBP) forms a nonclassical nuclear localization signal and is recognized by the importin Transportin-1 (TNPO1; Bourgeois et al., 2020), establishing an important interaction for nuclear import of CIRBP. The Tudor domain encoded by survival motor neuron (SMN) in exon 3 interacts with EWS RGG repeats (Young et al., 2003), and the nucleolin RGG region binds ribosomal proteins in vitro (Bouvet, Diaz, Kindbeiter, Madjar, & Amalric, 1998), although the exact physiological functions of these interactions remain to be delineated. The RGG region in the yeast protein Scd6 was reported to interact with eukaryotic initiation factor 4G (eIF4G) and repressed translation initiation (Rajyaguru et al., 2012), whereas the Sbp1 RGG region binds to cytoplasmic polyA-binding protein and modulates translation activity of mRNAs in an RNA and methylation dependent manner (Brandariz-Nunez et al., 2018).

Many physiologically important host–viral interactions involve an RGG region. The nucleolin RGG repeats interact with hepatitis C virus (HCV) nonstructural protein NS5B (Kusakawa et al., 2007), a viral RNA-dependent polymerase of the viral replication complex (Ishii et al., 1999; Kusakawa et al., 2007). In vitro replication functionality of NS5B was inhibited in the presence of the nucleolin RGG motif (Hirano et al., 2003). The RGG repeats in the host hnRNPs including hnRNP A1, Q, R, U, and K interact with the N-terminal Rev protein (Hadian et al., 2009), an important adaptor protein for nuclear export of viral RNAs of human immunodeficiency virus type 1 (HIV-1; Felber et al., 1989; Hadzopoulou-Cladaras et al., 1989). The arginine residues in hnRNPA1, a nuclear-cytoplasmic shuttling protein (Piñol-Roma & Dreyfuss, 1992), are important for the Rev–hnRNPA1 interaction because this interaction is lost when all six arginines in the hnRNPA1 RGG were substituted with alanine residues. The Rev–hnRNPA1 interaction is important for HIV replication, as overexpression of hnRNPA1 in HIV-infected cells increases the amount of HIV capsid protein. Likewise, the production of HIV protein is reduced when hnRNPA1 is knocked down (Hadian et al., 2009).

Protein–protein interactions involving RGG regions were studied using either two-hybrid analysis (Fridell et al., 1996; Menon et al., 2004) or pulldown assays (Gao et al., 2016; Hadian et al., 2009; Senger et al., 1998) in most of the investigations. As many proteins contain domains other than RGG that interact with proteins or nucleic acids, careful control experiments are required to establish an RGG-specific interaction. For example, FMRP harbors a nuclear localization signal and RGG motifs in its C-terminal region (Eberhart et al., 1996), and FUS has overlapping PY and RGG regions (Crozat et al., 1993; Eberhart et al., 1996; Yoshizawa et al., 2018). The extent to which neighboring domains or overlapping non-RGG peptide motifs contribute to the protein–protein interactions is a critical question to answer to develop a mechanistic understanding of the RGG-specific interactions.

In addition to the specific molecular features and functionalities derived from arginine and glycine, three underlying principles of the RGG-specific interactions are likely shared among different RGG motif proteins at the molecular level. First, the RGG motif itself, as a short peptide recognition element, is most likely to be unstructured in its free form but become more structured upon binding to its target using coupled folding with binding (Dyson & Wright, 2002; Spolar & Record, 1994), a mechanism by which disordered motifs fold upon interacting with their targets. Second, structural polymorphism is expected from the RGG motif due to its diverse interacting partners, as seen in many proteins with IDRs (Dyson & Wright, 2005). Third, because of their conformational adaptability and their ability to bind multiple targets with high specificity and moderate affinity, short motifs as well as protein domains within the RGG proteins are likely to function synergistically and exploit allosteric coupling to regulate their diverse interactions (Brandariz-Nunez et al., 2018; Ozdilek et al., 2017).

3 | POSTTRANSLATIONAL MODIFICATION OF ARGININES IN THE RGG MOTIF

Arginine methylation is an abundant modification in the cell. Many RNA and DNA binding proteins have been identified to contain methyl-arginine residues (Gary & Clarke, 1998). These proteins are functionally involved in important cellular processes including DNA repair, signal transduction, transcription, translation, apoptosis pathways and more (Boisvert et al., 2003). For reviews on arginine methylation, please see (Boisvert et al., 2005; Wu et al., 2021).

Arginine methylation refers to the posttranslational addition of monomethyl or dimethyl groups to the guanidino nitrogen atoms by evolutionarily conserved protein arginine methyltransferases (PRMTs; Figure 3; Gary & Clarke, 1998). In eukaryotes, four forms of arginine methylation have been identified: ω-N^G monomethylarginines (MMA); ω-N^G,N^G-asymmetric dimethylarginines (aDMA); ω-N^G,N'^G-symmetric dimethylarginines (sDMA); and δ-N^G monomethylarginines (Figure 3; Kakimoto & Akazawa, 1970). PRMTs transfer a methyl group from S-adenosyl methionine (SAM) to a nitrogen atom of the guanidium group of the arginine. Although methylation does not change the positive charge of the arginine, it introduces steric constraints, increases the sidechain bulkiness, enhances sidechain hydrophobicity, and reduces or even blocks its hydrogen bonding potential.

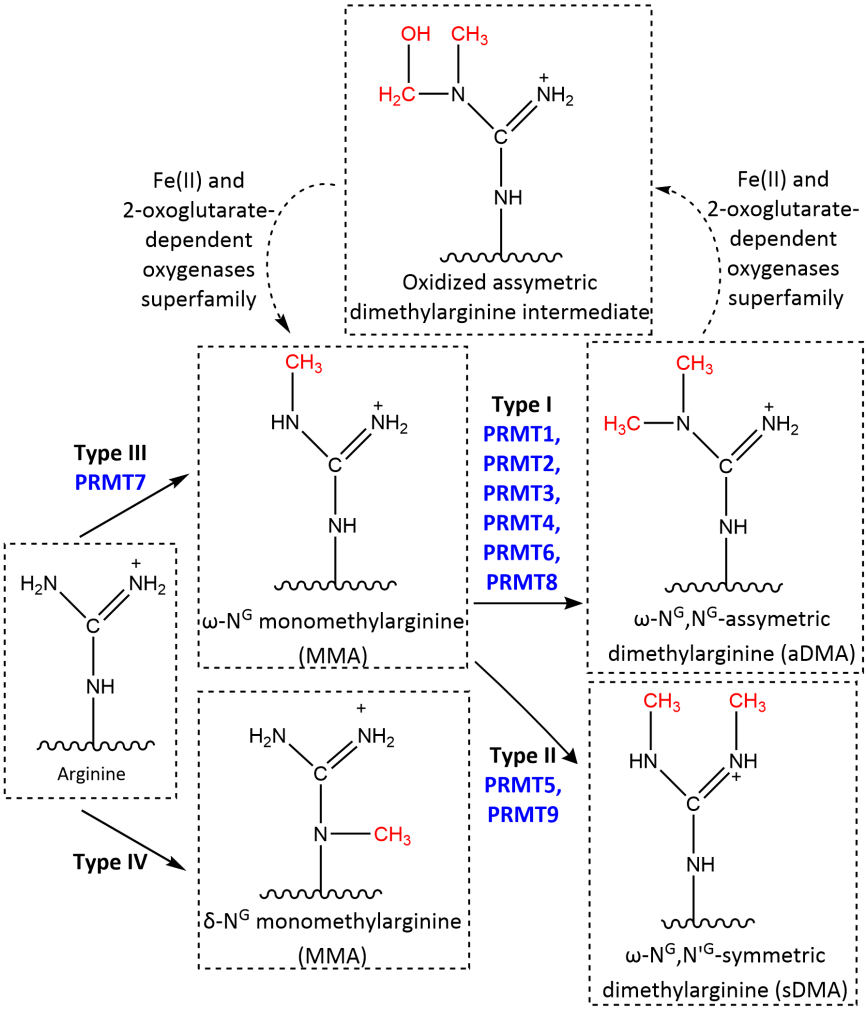


FIGURE 3 Methylation derivatives of arginine by PRMTs and arginine demethylases. Nine PRMTs have been identified in human and are divided into four types (Wu et al., 2021). Type I, II and III modifies the ω-N of the guanidium group, while type IV, identified in yeast, modifies the δ-N of the guanidium. Type I PRMTs catalyze asymmetric dimethylation and this family of proteins includes PRMT1, PRMT2, PRMT3, PRMT4 (CARM1), PRMT6 and PRMT8. Type II PRMTs, including PRMT5 and PRMT9, catalyze symmetric dimethylations. PRMT7 belongs to the type III PRMT and catalyzes monomethylation. The enzyme superfamily Fe (II) and 2-oxoglutarate- dependent oxygenases, such as Jmjd6, demethylates arginine in a two-step reaction.

Arginine residues in RGG regions were first reported to act as substrates for protein arginine methyltransferases in 1985 (Lischwe et al., 1985). This study reported dimethylated arginines in the peptide fragment generated from trypsin digestion in the C terminus of nucleolin (Lischwe et al., 1985). Arginines in the glycine-arginine-rich (GAR) regions are preferred substrates for many PRMTs (Najbauer et al., 1993), although arginines outside of the GAR can also be methylated (Boisvert et al., 2005; Wu et al., 2021). It is yet to be determined molecular mechanisms of how each type of PRMT selects its substrate. For instance, PRMT4 (CARM1) recognizes proline-glycine-methionine (PGM) rich motifs found in the proteins SmB (Cheng et al., 2007) and PABP (Lee & Bedford, 2002), and performs asymmetric dimethylation on the RGG motifs within PGM (Figure 3). However, PRMT5 also recognizes PGMs to catalyze symmetric dimethylation. Moreover, PRMT5 has shown to methylate substrates for PRMT1, PRMT3, PRMT6, and PRMT8 (Cheng et al., 2007). Substrate scavenging has been observed, where the absence of one type of PRMT increases the activity of another type to compensate and methylate its substrate. For example, two different protein arginine methyltransferases, PRMT1 and PRMT5, scavenge substrates specific to each other (Dhar et al., 2013).

Arginine methylation plays an important role in modulating RGG-specific interactions. First, arginine methylation alters RGG–RNA interactions in an RNA-dependent manner. For example, arginine methylation does not change RGG-specific interactions with short RNA sequences (Brandariz-Nunez et al., 2018; Xu & Henry, 2004), but does inhibit interactions between the FMRP RGG region and a highly structured G-rich RNA found by in vitro selection (Blackwell et al., 2010). In a recent study, methylation of residues R95 and R177 in the RGG regions of the Sars-CoV-2 nucleocapsid protein is critical for the nucleocapsid protein to interact with the 5′UTR of SARS-Cov-2 genomic RNA (Cai et al., 2021), an interaction required for viral packing.

Second, arginine methylation also changes protein–protein interactions to varying degrees, ranging from marginal to substantial. For example, symmetrically dimethylated arginines in the RG-rich peptide fragment of p53 binding protein (53BP1) do not alter its dimerization with the first Tudor domain of 53BP1 (Charier et al., 2004). However, methylation within the RGG motif slightly decreases SMN-EWS interaction (Young et al., 2003), and completely abolishes the interactions between TNPO1 and CIRBP (Bourgeois et al., 2020), as well as between Sbp1 and Pab1 (Brandariz-Nunez et al., 2018). Conversely, methylation can enhance protein–protein interactions, as seen in many Tudor domain–RGG interactions. A cluster of aromatic residues in the Tudor domain of the SND1 protein interacts with methylated arginines of PIWIL1 RGG motif protein via cation–π interactions (Liu et al., 2010). The Tudor domain in SMN interacts with the Sm RGG motif when its arginines are symmetrically dimethylated, and this molecular interaction is important for small nuclear RNP assembly (Brahms et al., 2000; Brahms et al., 2001).

Due to alterations in protein–protein (Dormann et al., 2012) and protein–RNA interactions (Dolzhanskaya et al., 2006; Lien et al., 2016), posttranslational modifications including methylation of RGG affect the localization of RGG proteins (de Leeuw et al., 2007; Gilbert et al., 2001; Matsumoto et al., 2012). Localization of representative RGG proteins involved in mRNA transport and translation is summarized in Figure 4, and the details will be discussed later in this review.

Third, adding another layer of complexity, methylation of the arginine residues in RGG motifs is reversible (Tsai et al., 2016), though the enzymes involved in demethylation are not well characterized (Wesche et al., 2017). Demethylation of arginine is shown to be carried out by the Fe(II) and 2-oxoglutarate-dependent oxygenase superfamily (Figure 3; Walport et al., 2016). Proteins that belong to this superfamily can either hydroxylate amino acids or demethylate lysine and arginine residues (Markolovic et al., 2015). For example, Jumonji-domain containing protein 6 (Jmjd6), one of the Fe(II) and 2-oxoglutarate-dependent oxygenases, was shown to demethylate arginines in Ras-GAP SH3 Binding Protein 1 (G3BP1), a stress granule-nucleating protein (Tsai et al., 2017).

Fourth, arginine methylation and de-methylation are dynamic processes that depend on physiological clues and environmental stimuli. For example, in vivo methylation status of the RGG regions of estrogen receptor α (ERα) and G3BP1 changes upon changes in their environment (le Romancer et al., 2008; Tsai et al., 2016). Methylation of the ERα RGG increases in the presence of estrogens in MCF-7 cells in 5 min, but the modification is rapidly lost after 10 min (Le Romancer et al., 2008). Similarly, the asymmetric di-methylation of arginines in G3BP1 is decreased by ∼20% after treating cells with arsenite for 30 min and is further decreased by ∼50% after 1 h under the same oxidative stress conditions, however, the methylation status recovered once the stress was removed. Moreover, the type of RGG demethylation can be stress-dependent. Demethylation of G3BP1 was studied by comparison of methylation status of the protein under stressed and unstressed conditions using mass spectrometry. Demethylation of G3BP1 asymmetric dimethylation and mono-methylation was only observed under oxidative stress, but not under heat shock or endoplasmic reticulum stresses, whereas demethylation of symmetric dimethylarginine was observed under endoplasmic reticulum stress (Tsai et al., 2016).

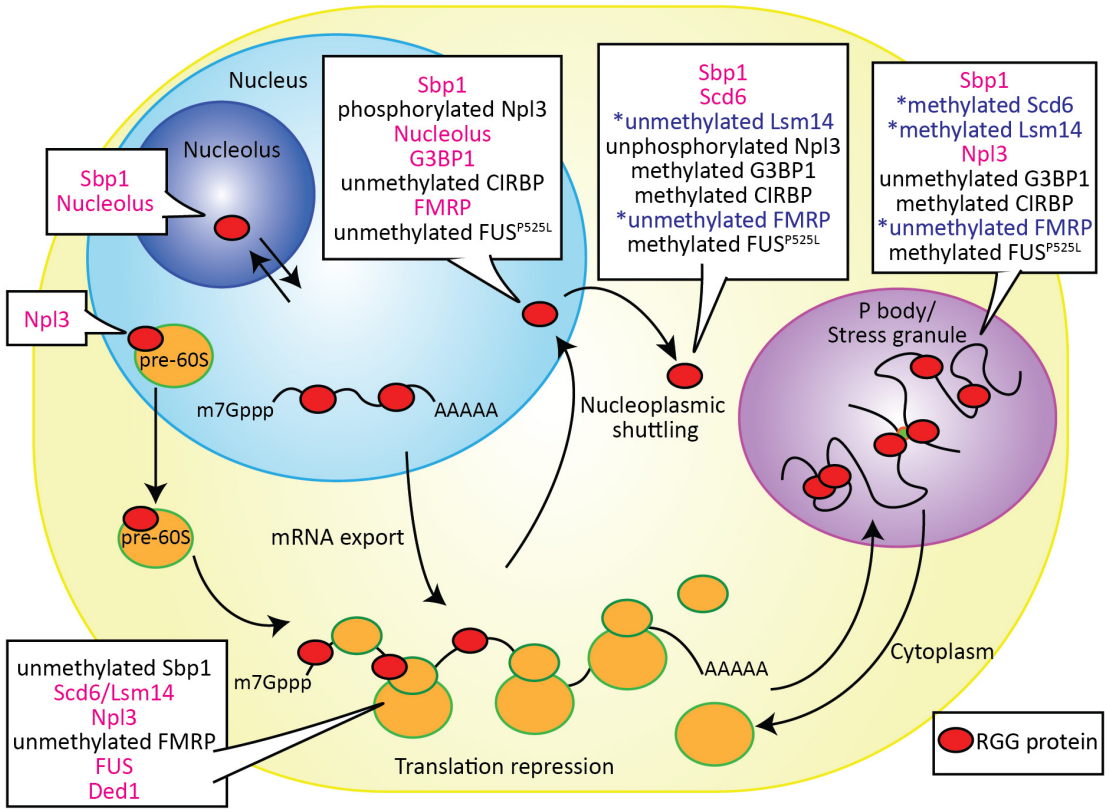


FIGURE 4 Posttranslational modifications of RGG proteins impact their cellular localization. Many nucleocytoplasmic shuttling RGG proteins localize in the nucleolus, the nucleus, cytoplasm, or cytoplasmic granules such as P bodies or stress granules. Localization of some RGG proteins such as FMRP depend on their NES and NLS for nuclear and cytoplasmic transport, while localization of other RGG proteins depend on their PTMs. Modifications such as methylation on the RGG motif or phosphorylation on specific aminos acids such as serine alter protein–protein and/or protein–RNA interactions. RGG motif proteins, effects of methylation on localization studied through PRMT knockout and site-directed mutagenesis of the PRMT-targeted arginine residues, were colored in black. RGG motif proteins, effects of methylation on localization have not been investigated, are shown in magenta. Blue with asterisk denotes the RGG motif proteins in which the direct effect of methylation has not been studied through site-directed mutagenesis of the specific arginine residues but studied via PRMT knockout.

In summary, methylation of the RGG motif fine-tunes the RGG-specific interaction, providing a valuable means for regulation. A better understanding of this process would likely provide foundations for developing valuable therapeutics. For example, PRMT inhibitors are used for cancer treatment (Fedoriw et al., 2019; see reviews (Hwang et al., 2021; Wu et al., 2021). Furthermore, substitution of R95 or R177 with lysine or inclusion of type I PRMT inhibitors prevents the interaction between the N protein and 5′UTR of SARS-CoV-2, indicating arginine methylation as a potential target for inhibition of viral genome assembly and packaging (Cai et al., 2021). It remains to be seen how other posttranslational modifications such as phosphorylation (Cobianchi et al., 1993) of residues close to the RGG motif would affect the function of the RGG region (Stetler et al., 2006) and RGG-containing proteins.

4 | RGG-DEPENDENT INTERACTION IN PROTEIN SELF-ASSOCIATION AND FORMATION OF MEMBRANELESS CELLULAR ORGANELLES

Many proteins self-associate through their RGG regions in vitro, and arginine methylations often weaken the observed self-association. Ewing's sarcoma protein (EWS), a nuclear protein that interacts with transcriptional and RNA splicing machinery, self-associates through its RGG region (Shaw et al., 2010). Likewise, Npl3 (Figure 1), a nuclear-cytoplasmic protein that is essential to RNA splicing (Kress et al., 2008), export (Lee et al., 1996), ribosome maturation (Baierlein et al., 2013), and translation (Estrella et al., 2009), homodimerizes via an unmethylated C-terminal RGG region in vitro.

The amino acid region from residue 276 to 339 within the RGG region of Npl3 (see Figure 1) is minimally required for the protein homodimerization (Baierlein et al., 2013). Moreover, dimerization of Npl3 was only observed in cells lacking the yeast arginine methyltransferase, Hmt1, but not in its presence, suggesting arginine methylation may inhibit Npl3 dimerization (McBride et al., 2005; Yu et al., 2004). As seen in Npl3, arginine methylation reduces the extent of RGG-dependent dimerization of Scd6 (Poornima et al., 2019).

Like other proteins with IDRs, RGG proteins promote phase separation to form membraneless organelles in the cyto- and nucleoplasm (Kato et al., 2012; Weber & Brangwynne, 2012), which are important for RNP compartmentalization. For example, CIRBP RGG regions are required for the recruitment of this protein to stress granules (Bourgeois et al., 2020). Furthermore, both the RRM and RGG domains of G3BP1 are essential for stress granule formation (Yang et al., 2020). How a self-assembly process in vitro and stress granule formation in vivo are related remains to be studied in more detail, but the current data seem to support, at least in part, a model that the two processes are somewhat related. As shown in a recent study, the dimerized G3BP1 provides a sufficient number of interfaces for multiple RNAs to bind, which drives stress granule formation when the RNA concentration is increased (Sanders et al., 2020). The ability of the RGG region to bind to both RNAs and proteins increases the degree of protein–RNA interactions in the network of protein–RNA complexes and facilitates the formation of stress granules (Sanders et al., 2020).

As seen with in vitro RGG-dependent dimerization, methylation is important to regulating RNA granule assembly (Figure 4). Hmt1 asymmetrically dimethylates Scd6 CTD RGG repeats in an RNA-independent manner, and the arginine methylation is important for Scd6 localization to P bodies under stress (Lien et al., 2016). Similarly, Lsm14, the human homolog of Scd6, plays a critical role in the assembly of cytoplasmic mRNP granules (Figure 4). Like Scd6, Lsm14 RGGs and their methylation states are essential to Lsm14 localization to cytoplasmic P-bodies. The CTD RGG region of Lsm14 is asymmetrically dimethylated by PRMT1 in vivo, as well as asymmetrically dimethylated and symmetrically dimethylated by PRMT1 and PRMT5, respectively, in vitro. PRMT1 knockdown by siRNA results in redistribution of Lsm14 in the cytoplasm, instead of accumulation in P-bodies (Matsumoto et al., 2012). Moreover, hnRNPA1 forms granules under oxidative stress upon methylation (Guil et al., 2006). An R to K substitution within the RGG region of G3BP1, which maintains the positive charge of the amino acid but eliminates the unique structure of the arginine sidechain and the possibility of arginine methylation, reduces the degree of stress granule formation. In contrast, methylation in the G3BP1 RGG region represses stress granule assembly, because a methylation mimetic, an R to F substitution, reduces stress granule formation. In addition, the presence of PRMT1 or PRMT5 represses stress granule formation, whereas demethylation of G3BP1 promotes granule formation (Tsai et al., 2016). Further experiments are required to characterize how RGG-dependent self-association, protein–protein interactions, and protein–RNA interactions contribute to the formation of dynamic cellular RNP bodies.

5 | RGG PROTEINS IN MRNA TRANSPORT AND TRANSLATION

RGG proteins play critical functions in the cell. Here we selectively review current knowledge on RGG proteins important for mRNA transport and translation (Figure 1), in the hope to highlight merging understanding of their molecular interactions and mechanisms of action.

5.1 | Sbp1

Sbp1 is a single-stranded RNA-binding protein that contains a central RGG region between two distal RNA recognition motifs (Jong et al., 1987). It binds to A-rich RNA sequences (Brandariz-Nunez et al., 2018), which explains an early observation of Sbp1 coimmunoprecipation with snR10 and snR11 (Jong et al., 1987), as both snoRNAs contain A-rich RNA regions. Overexpression of Sbp1 at least partially restores decapping activity that is defective in the temperature-sensitive dcp mutants (Segal et al., 2006; Tharun & Parker, 1999), suggesting a role for this protein in the cytoplasmic decapping pathway. Sbp1 co-sediments with both translating ribosomes and nontranslating RNPs (Brandariz-Nunez et al., 2018), but localizes to P-bodies under glucose starvation conditions (Segal et al., 2006).

At the molecular level, four aspects pertaining to the mechanism of translational control by Sbp1 are worthy of discussion. First, the individual domains, whether RRM or RGG, in Sbp1 bind to the A-rich sequence in Pab1 mRNA weakly, but the full-length Sbp1 protein binds to the same RNA sequence with much higher affinity, strongly suggesting that the three domains in Sbp1 bind to RNA synergistically. Second, the Sbp1 RGG region directly binds to

the eukaryotic initiation factor Pab1 protein and represses transcript-specific translation in an RNA and RGG-dependent manner (Brandariz-Nunez et al., 2018). Third, methylation of arginine residues in the RGG region abolishes this specific protein–protein interaction and the inhibitory effect of Sbp1 on Pab1 mRNA translation. It is noteworthy that, although Sbp1 was reported to interact with eIF4G earlier (Rajyaguru et al., 2012), this interaction was confirmed to be indirect and only takes place through Pab1 protein in an RNA dependent manner (Brandariz-Nunez et al., 2018).

Finally, we are accustomed to thinking that the protein alters the RNA function when considering the function of a specific RNA–protein interaction. However, the concept that the RNA may change the function of a protein upon binding, that is, "riboregulation" (Hentze et al., 2018), is relatively under-appreciated. Here, Brandariz-Nunez et al. (2018) showed that the translation function of the Sbp1 RGG domain is altered by its interaction with RNAs. Sbp1 inhibits both cap-dependent and cap-independent translation initiation of Pab1 mRNA via the RGG-Pab1 mRNA interaction. RGG methylation abolishes its binding to Pab1 mRNA, thereby abolishing the translation repression by Sbp1. This effect was only observed when the full-length Sbp1 is used, but not when the two RRM domains flanking the RGG domain were deleted, as inhibition of the translation on RNAs persists when the RGG domain alone is present, regardless of its methylation state. These results demonstrate the importance of RNA binding in modulating the interaction and function of RNA-binding proteins in the cell.

Given the diversity of mechanisms by which translation can be regulated, it remains to be seen how prevalent the aforementioned mechanisms are among RGG proteins. However, because of the shared chemical property of the RGG motif, we expect the principles underlying Sbp1-specific translational control to be shared among the RGG-motif proteins involved in translation.

5.2 | Npl3

Yeast Npl3 is a nuclear–cytoplasmic shuttling protein comprised of two RRMs and one RGG domain containing 15 RGG tripeptides (Bossie et al., 1992; Flach et al., 1994; Singleton et al., 1995). This protein plays essential functions in RNA splicing (Kress et al., 2008), RNA Pol II elongation control (Holmes et al., 2015), and translation regulation (Baierlein et al., 2013; Estrella et al., 2009).

Npl3 is required for the nuclear export of mRNA and pre-60S ribosomes. Deletion of Npl3 results in a decreased level of 60S ribosomal subunits in the cytoplasm (Stage-Zimmermann et al., 2000). Npl3 co-immunoprecipitated with the pre-60S export proteins Nmd3 and Arx1 in an RNA-independent manner, and nuclear export of pre-60S subunits was defective in ΔNpl3 cells (Hackmann et al., 2011). Mutations in one of the RRM domains of Npl3 results in stop-codon read-through on a dual-luciferase reporter construct, suggesting that Npl3 plays a role in maintaining the accuracy of translation termination (Estrella et al., 2009).

The integrity of the Npl3 RGG region affects cellular translation rates. Truncation of about half of the RGG region (Npl3Δ100, C-terminal 100 amino acid deletion, Figure 1) results in a nearly 60% decrease in cellular incorporation of [^{35}S] methionine after 30 min at 37°C and a reduction in the degree of global translation as shown by the polysome profile. Furthermore, 80S ribosome formation is defective in the Npl3Δ100 cell in vivo (Baierlein et al., 2013). No similar detrimental effect was observed in the same study using an Npl3 mutant lacking a quarter of the RGG region (Npl3Δ50, C-terminal 50 amino acid deletion, Figure 1). Other than these limited observations, mechanisms of Npl3-specific translational control remain to be elucidated.

While playing an important role in translation, the RGG region and its methylation state do not directly affect the subcellular localization of Npl3 (McBride et al., 2005). However, phosphorylation of S411 in the C-terminal serine-arginine (SR) repeat, which overlaps the RGG motif, is required for proper nuclear localization of Npl3. This explains the in vivo mislocalization of Npl3 caused by C-terminal truncation of the Npl3 RGG region (Baierlein et al., 2013; Flach et al., 1994). The kinase Sky1 is responsible for the S411 phosphorylation, as deletion of Sky1 results in cytoplasmic localization of Npl3 (Gilbert et al., 2001). On the other hand, nuclear localization of Npl3 is likely mediated by Mtr10 (Senger et al., 1998), a nuclear import receptor protein in yeast. Npl3 mislocalizes in the cytoplasm at 37°C in an Mtr10 mutant defective in nuclear import (Senger et al., 1998). Furthermore, Npl3 co-purified with Mtr10 when it was pulled down from cell lysate. However, no RNase was added in this study (Gilbert et al., 2001). As such, it is unclear whether the two proteins directly interact (Gilbert et al., 2001; Pemberton et al., 1997; Senger et al., 1998). Nevertheless, a decreased level of interaction between these two proteins in the ΔSky1 cell suggests a certain degree of specificity for the Npl3–Mtr10 interaction (Gilbert et al., 2001).

5.3 | Ded1

Yeast protein Ded1 (Figure 1) is an ATP-dependent DEAD-box RNA helicase that shuttles between the cytoplasm and the nucleus (Berthelot et al., 2004; Iost et al., 1999; Senissar et al., 2014). Ded1 carries an RGG region in both its C- and N-termini, which allow the protein to play dual functionalities in protein synthesis. On one hand, it accumulates in P-bodies in an ATPase-dependent manner under normal growth and glucose starvation, decreases the amount of translating ribosomes and represses 48S preinitiation complex upon overexpression, acting as a translation repressor (Beckham et al., 2007; Hilliker et al., 2011). On the other hand, Ded1 associates with the mRNA cap-binding proteins, unwinds 5′UTR secondary structures, and promotes assembly of the 48S pre-initiation complex in an RNA-structure and eIF4F-dependent manner in vitro (Berthelot et al., 2004; Gupta et al., 2018; Sen et al., 2015).

The presence of Ded1 promoted the formation of 48S PICs on mRNA constructs that were monitored using a native gel electrophoretic mobility shift assay (EMSA; Gupta et al., 2018). The N-terminal domain (NTD) of Ded1 interacts with eukaryotic initiation factor eIF4A (Gao et al., 2016). This interaction is required for the helicase unwinding activity of Ded1 (Gao et al., 2016; Senissar et al., 2014) and stimulates in vitro formation of 48S PICs on mRNAs (Gupta et al., 2018). The RGG region in the Ded1 CTD interacts with eIF4G (Hilliker et al., 2011), establishing a critical interaction for both Ded1 helicase activity on a model RNA duplex (Gao et al., 2016; Putnam et al., 2015) and mRNA recruitment of the 48S pre-initiation complex in vitro (Gupta et al., 2018). Mutation in the Ded1 human homolog protein, DBY, is linked with male infertility (Foresta et al., 2000).

Similar to the Sbp1, Npl3, and Ded1 proteins that are discussed above, many other functionally important yeast RGG motif proteins, such as Hrp1 (Gaillard & Aguilera, 2014), Hrb1 and Gbp2, are also multidomain nucleocytoplasmic shuttling proteins and associate with polysomes (Windgassen et al., 2004). It will be interesting to see whether similar mechanisms are shared among these proteins in regulating RNA fates and translation.

5.4 | FMRP

Fragile X mental retardation protein (FMRP, Figure 1), encoded by the *FMR1* gene, is an RNA-binding protein (Ashley Jr. et al., 1993) that plays important roles in the transport and translation of mRNAs in neurons (Kao et al., 2010). Epigenetic silencing of FMRP expression by excessive CGG triplet expansions within the 5′UTR of the *FMR1* gene results in an inherited intellectual disability called Fragile X syndrome (FXS; Chen & Joseph, 2015; Pieretti et al., 1991; Sutcliffe et al., 1992; Verkerk et al., 1991).

The presence of both a nuclear localization signal (NLS) and a nuclear export signal (NES) in FMRP (Figure 1) allows the protein to shuttle between the nucleus and cytoplasm (Bardoni et al., 1997; Eberhart et al., 1996; Fridell et al., 1996). FMRP contains three RNA binding motifs: two hnRNP K-homology (KH1 and KH2) motifs and one RGG motif (Ashley Jr. et al., 1993; Siomi et al., 1993; Siomi et al., 1994). The NES, not the RGG motif, plays an essential role in the protein's cytoplasmic localization (Eberhart et al., 1996).

A large number of mRNA targets bound to FMRP have been identified using high-throughput sequencing methods (Ascano Jr. et al., 2012; Darnell et al., 2011), but only a small subset of them have been biochemically verified (Menon et al., 2008; Zhang et al., 2001). G-quartets and a highly structured RNA were recognized by the RGG and KH2 domains through in vitro selection (Darnell et al., 2005, 2001) and the FMRP RGG region binds strongly to G-quartets with high affinity ($K_d = 20$ nM; Menon et al., 2008). In vivo studies show that FMRP predominantly associates with open reading frames of mRNAs belonging to synaptic transmission, neuron projection and GTPase regulator activities and stalls ribosomes on target mRNAs (Darnell et al., 2011).

The FMRP RGG region is important for MEF2-dependent synapse elimination (Pfeiffer et al., 2010). Furthermore, this region is required for association of FMRP with polysomes (Blackwell et al., 2010; Mazroui et al., 2003). The FMRP RGG is mono- and dimethylated by PRMT1 (Stetler et al., 2006), which reduces the affinity of FMRP for polysomes and a subset of its mRNA targets (Blackwell et al., 2010; Dolzhanskaya et al., 2006; Stetler et al., 2006), but promotes KH-dependent dimerization and localizes the FMRP to stress granules (Figure 4; Dolzhanskaya et al., 2006).

The relevance of translational control by FMRP to FXS was supported by a report of an FXS patient with a normal number of CGG triplet repeats, but with an Ile304Asn point mutation in the KH2 RBD (De Boulle et al., 1993; Siomi et al., 1994). The Ile304Asn mutation abolishes FMRP's association with polysomes (Feng et al., 1997), suggesting disregulation of translation could be the cause of the FXS disease. FMRP associates with both translating and stalled ribosomes (Stefani et al., 2004), and it associates with polysomes via the RGG and KH domains (Blackwell et al., 2010;

Mazroui et al., 2003). Deletion of each of the KH domains individually leads to distribution of FMRP only in monosome fractions, whereas deletion of specific arginine residues in the RGG or the RGG region results in FMRP redistributing towards the lighter polyribosome fractions (Blackwell et al., 2010; Mazroui et al., 2003).

The *FMR1* gene is ubiquitously expressed, with higher expression in the brain, thyroid gland, and reproductive tissues (Ashley Jr. et al., 1993; Siomi et al., 1993). FMRP represses translation in a tissue-specific manner (Li et al., 2001). Translation elongation repression by FMRP was supported by a cryoEM structure of an in vitro assembled 80S ribosome with truncated FMRP bound, where the KH domains bind near the ribosomal P site, and the RGG domain lies close to the A-site finger (ASF) where it could interact with the mRNA (Chen et al., 2014). However, other studies argue that FMRP binds to mRNAs via short sequences (Ascano Jr. et al., 2012), structured elements (Bechara et al., 2009) or a non-coding RNA (Zalfa et al., 2003) and represses translation at initiation (Napoli et al., 2008) or microRNA-mediated pathways (Edbauer et al., 2010; Muddashetty et al., 2011). It is also reported that FMRP enhances, rather than represses, the translation of many autism-related proteins (Greenblatt & Spradling, 2018). Clearly, the mechanisms mentioned above need not be mutually exclusive. Nevertheless, these results unambiguously indicate that FMRP plays diverse roles in transcript-specific translation.

5.5 | FUS

FUS is a nuclear DNA/RNA-binding protein belonging to the FET family (Baechtold et al., 1999; Crozat et al., 1993; Zinszner et al., 1997). Proteins in the FET family (FUS, EWS, and TAF15) share a common structural feature: an N-terminal QGSY-rich region followed by domains including RRM, zinc-finger domains and multiple RGG domains in different orders. Although it contains both a NES and a NLS, FUS primarily localizes in the nucleus (Crozat et al., 1993). It is imported into the nucleus by transportin via a proline-tyrosine nuclear localization signal (PY-NLS) in the CTD of FUS (Dormann et al., 2012; Niu et al., 2012; Yoshizawa et al., 2018). The physiological functions of FUS that have been identified thus far include transcription activation, DNA repair, alternative splicing, RNA export, and localized translation (For more details, see reviews (Efimova et al., 2017; Sama et al., 2014).

The RRM and RGG domains of FUS bind to RNAs cooperatively (Ozdilek et al., 2017). The FUS RRM binds to RNA weakly in vitro, but the interaction is enhanced with participation of either the RGG1 or RGG2 regions in the N terminus (Figure 1), as seen in the yeast protein Sbp1 (Brandariz-Nunez et al., 2018). The three RGG regions in the protein likely play different functional roles: RGG1 and RGG2 bind to the RNA as indicated by their stronger affinity for RNA compared with the C terminal RGG3 region (Ozdilek et al., 2017), and the RGG3, preceding the PY-NLS, interacts with the transportin for nuclear localization (Dormann et al., 2012; Ozdilek et al., 2017; Yoshizawa et al., 2018). Although FUS RGG3 and PY-NLS domains interact with transportin individually, the interaction is enhanced when both domains are present simultaneously (Dormann et al., 2012).

FUS is essential for localized translation at cell protrusions in the tumor-suppressor protein adenomatous polyposis coli (APC)-containing ribonucleoprotein complexes (APC-RNPs). Cells lacking FUS show reduced levels of newly synthesized proteins at the APC-RNP (Yasuda et al., 2013). On the other hand, FUS was reported to associate with stalled polysomes and represses translation in a manner that is linked with mTOR (mammalian target of rapamycin) kinase activity (Sevigny et al., 2020).

Mutations in FUS are implicated in a subset of familial amyotrophic lateral scleroses (ALS), a majority of which are clustered in the FUS C-terminal RGG3 and NLS domains (Nolan et al., 2016). The pathogenesis underlying many forms of ALS is due to mislocalization of FUS to the cytoplasm where it aggregates (Kwiatkowski Jr. et al., 2009; Vance et al., 2009), forms stress granules (Bosco et al., 2010), inhibits translation, and disrupts NMD (Kamelgarn et al., 2018; Sevigny et al., 2020). Moreover, binding of the FUS PY-NLS to the nuclear importer transportin inhibits FUS self-association and drives liquid–liquid phase separation in ALS (Yoshizawa et al., 2018). Conceivably, the mis-localization of this protein results in a loss of nuclear function and gain of toxic function in the cytoplasm.

FUS RGGs are asymmetrically dimethylated by PRMT1 or PRMT8 (Scaramuzzino et al., 2013). Arginine methylation also plays an important role in the pathogenesis of FUS-related ALS because methylation of the RGG3 domain weakens its interaction with transportin, thus impairing nuclear localization of FUS. For example, a P525L point mutation in the NLS, combined with methylation of the RGG3, resulted in a nuclear import defect of FUS (Figure 4). However, when RGG3 methylation is abolished upon PRMTI knock down, FUS-P525L re-localizes to the nucleus (Dormann et al., 2012).

Finally, repressed translation and disrupted NMD are also associated with the pathogenesis of FUS-related ALS. ALS-associated FUS mutants, such as R495X or P525L mutant proteins, repress translation of the Renilla reporter in vitro, decrease the rate of protein synthesis in vivo, and change the balance of UPF expression, which is essential to functional NMD (Kamelgarn et al., 2018). Compared with the wild type FUS, more ALS-linked FUS R521G and P525L mutants sediment with stalled polyribosomes and monosomes (Sevigny et al., 2020).

5.6 | RGG proteins in human diseases

RGG proteins play diverse roles in many cellular pathways and are frequently associated with human diseases such as cancer, muscular atrophy, and neurodegenerative disorders. Strikingly, mutations in the RGG proteins that increase their self-assembly in vitro and stress granule disassembly reduces disease state, often lead to muscular or neuro-degenerative diseases (Guo et al., 2018). Persistence of stress granules in the absence of stress could lead to high local concentrations of their components and increase the possibility of formation of amyloid fibers. Such fibrous aggregates are observed in ALS related mutations in FUS protein (Patel et al., 2015). Moreover, the RGG and QGSY regions in FUS interact with each other to drive liquid–liquid phase separation (LLPS), and aberrant LLPS is characteristic of ALS and other disorders (Wang et al., 2018). Furthermore, the accumulation of cellular granules undoubtedly disrupts nuclear-cytoplasmic transport, signal transduction, as well as ribonucleoprotein biogenesis and function, leading to disease states or even cell death (Mahboubi & Stochaj, 2017; Marcelo et al., 2021).

Furthermore, RGG proteins such as BRD4, BAZ1A, Drosha, Aven, and hnRNPs K, A1 and A2, are overexpressed in many cancer cells, but their functions in oncogenesis are yet to be determined (Chen et al., 2018; Choi et al., 2006; Jiao et al., 2020; Li et al., 2019; Peng et al., 2019; Stockley et al., 2014; Xu et al., 2017). Some of these proteins, such as hnRNPA2, can be used as biomarkers in brain (Deng et al., 2016), lung (Zhou et al., 2001) and breast cancers (Ma et al., 2020). Similarly, hnRNPA1 is also overexpressed in lung cancer (Pino et al., 2003), Burkitt lymphoma (Brockstedt et al., 1998) and leukemia (Iervolino et al., 2002). hnRNPA1 activates telomerase (LaBranche et al., 1998; Zhang et al., 2006), promotes cell cycle progression (Liu et al., 2016) and aerobic glycolysis (Clower et al., 2010). It binds to 5′UTRs and regulates the IRES-mediated translation of mRNAs involved in the cell cycle and apoptosis. Under normal physiological conditions, hnRNPA1 represses the IRES-mediated translation of Bcl-xl and XIAP mRNAs, which are functionally important for anti-apoptosis and cell survival. However, in lung cancer cells that are stimulated by basic fibroblast growth factor 2 (FGF2), activation of the kinase S6K2 site-specifically phosphorylates hnRNPA1, alleviates translation repression of these mRNAs, and promotes cancer cell survival (Roy et al., 2014). Unsurprisingly, depletion of hnRNPA1 in the cancer cells was reported to induce apoptosis (Patry et al., 2003). Similar to hnRNPA1, other RGG proteins such as nucleolin and EWS are also critical in maintaining the expression of oncogenes and tumor suppressor genes (Gonzalez & Hurley, 2010; Ishimaru et al., 2010; Li & Lee, 2000; Zhang et al., 2008). Nucleolin was shown to bind to the 3′UTR of Bcl-2, which encodes an antiapoptotic protein, and protect the Bcl-2 mRNA from deadenylation or degradation (Zhang et al., 2008). Similarly, EWS/FLI1 promotes remodeling and oncogene transcriptional activation through its RGG region (Boulay et al., 2017). The RGG region of EWS allows for its self-association and formation of high concentration hub near the DNA, thus binding to the DNA, recruiting RNA polymerase II and activating transcription (Chong, Dugast-Darzacq, et al., 2018).

5.7 | Perspectives

Thousands of proteins in the human genome contain RGG or RG repeats, and they are functionally involved in nearly every cellular pathway (Thandapani et al., 2013). More proteins containing functional RGG repeats and their biological targets are sure to be uncovered in the future. To further our understanding of this important class of proteins, four main areas of investigation are worthy of pursuing. First, studying the properties, abundance, interactions, and subcellular localization of RGG proteins in their natural environment, within the cell, will be critical. Second, structural investigations of biologically relevant molecular interactions involved in the RGG proteins will also be critical, especially of full-length RGG proteins with both structured and unstructured domains, in their free form and with their physiological partners bound. Indeed, many multidomain RGG proteins have been studied as fragments, and as important as these studies are, proteins do not function as isolated fragments or domains in the cell. Instead, multiple domains in the protein often act synergistically when carrying out their cellular functions. Therefore, to fully appreciate

their physiological functions, it is important to study entire proteins, or at least large, functionally important domain ensembles. Although characterization of structures and dynamic interactions of RGG proteins in the cell is challenging, recent technical advances in biophysical experimentation including electron cryo-microscopy offer great promise in this rewarding undertaking. Third, how do PTMs alter conformations and the molecular interactions of RGG proteins? Degrees of methylation, as well as other modifications such as phosphorylation of residues present in the RGG-domain, and their physiological relevance in vivo remain to be deciphered. Fourth, it is crucial to understand how proteins with IDRs, such as RGG repeats, contribute to the assembly and formation of higher-order cellular structure through liquid–liquid phase separation. What are the essential interactions involved in these RNP assemblies that contribute to their amorphous structure and dynamic composition? Overall, it is important to understand the mechanisms by which interactions in ordered or disordered domains, short motifs and posttranslational modification states synergistically function to control the protein functions. Further investigations are bound to provide exciting answers.

AUTHOR CONTRIBUTIONS

Mashiat Chowdhury: Investigation (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Hong Jin:** Conceptualization (lead); funding acquisition (lead); investigation (lead); supervision (lead); writing – original draft (lead); writing – review and editing (lead).

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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