The fate of eukaryotic proteins, from their synthesis to destruction, is supervised by the ubiquitin–proteasome system (UPS). The UPS is the primary pathway responsible for selective proteolysis of intracellular proteins, which is guided by covalent attachment of ubiquitin to target proteins by E1 (activating), E2 (conjugating), and E3 (ligating) enzymes in a process known as ubiquitylation. The UPS can also regulate protein synthesis by influencing multiple steps of RNA (ribonucleic acid) metabolism. Here, recent publications concerning the interplay between the UPS and different types of RNA are reviewed. This interplay mainly involves specific RNA-binding E3 ligases that link RNA-dependent processes with protein ubiquitylation. The emerging understanding of their modes of RNA binding, their RNA targets, and their molecular and cellular functions are primarily focused on. It is discussed how the UPS adapted to interact with different types of RNA and how RNA molecules influence the ubiquitin signaling components.

1. Introduction

Proteins are under constant surveillance by the protein quality control network, which tightly regulates the equilibrium between biogenesis and the timely degradation of redundant or damaged/misfolded proteins. In eukaryotes, only a small fraction of the genome is transcribed and then translated into functional proteins, yet judicious protein degradation is at the heart of many cellular processes. A key proteolytic component of the cellular protein quality control network is the ubiquitin–proteasome system (UPS).[1,2] Ubiquitylation is initiated by the conjugation of ubiquitin to the target protein. This small and highly conserved polypeptide is attached to the substrate protein via a three-step process initiated by a ubiquitin-activating enzyme (E1) and a ubiquitin-conjugation enzyme (E2). Ultimately, ubiquitin is covalently attached primarily to lysine sidechains of the substrate protein by ubiquitin-ligases (E3s). Depending on the lysine residues used for the ubiquitin–ubiquitin linkage, different types of polyubiquitin chains can be formed to serve as target or regulatory signals for the modified proteins. Proteasome complexes recognize ubiquitylated proteins and, via proteolysis, digest them into short peptides that can be further recycled.[1,2] Ubiquitylation can be antagonized by deubiquitylation enzymes (DUBs), which remove individual ubiquitin subunits or entire chains, thus influencing the fate of the ubiquitin-conjugated proteins.[3]

The functions of cells and organisms depend on the coordinated actions of two cellular systems: protein synthesis and protein degradation. The basis of protein synthesis is the generation of RNA molecules that encode the amino acid sequences of polypeptides. RNA synthesis, processing, functioning, and degradation are regulated by specific RNA-binding proteins (RBPs). Mutations in RBPs as well as aberrant RBP expression and dysfunction are correlated with disorders like cancer, autoimmune conditions, and neurological diseases.[4] Cells maintain efficient communication between RNA metabolism and ubiquitin signaling to adjust to their physiological needs. To this end, some RBPs gained ubiquitin ligase activity during evolution, thus becoming central intermediaries between the protein synthesis and degradation systems.[5] In addition to RNA-binding ubiquitin ligases (RBULs), other molecules associated with different steps of the ubiquitylation process precisely regulate RNA-dependent mechanisms in response to environmental or physiological cues. Here, we provide an overview of the evolutionary adaptations that have allowed the UPS to regulate RNA metabolism. We review recent publications that have enhanced our understanding of the interplay between the UPS and different types of RNA and RNP (ribonucleoprotein) complexes. We focus primarily on the emerging understanding of their modes of RNA binding, their RNA targets, and their molecular and cellular functions. We also discuss the interplay between noncoding RNAs and the RNA-interacting ubiquitylation machinery.

2. RNA Binding Proteins Are Involved in the UPS

2.1. Ubiquitin Signaling Relies on a Repertoire of RNA-Binding Ubiquitin Ligases and Proteases

Ubiquitin signaling mainly relies on ubiquitin ligases to oversee RNA metabolism. Therefore, we gathered a list of known E3
Table 1. List of proteins common to RBPs and UPS.

| Gene symbol | Protein name                  | E3/DUB domain | RNA-binding domain | Uniprot ID |
|-------------|-------------------------------|---------------|--------------------|------------|
| MEX3A       | RNA-binding protein MEX3A     | RING          | Two KH repeats     | A1L020     |
| MEX3B       | RNA-binding protein MEX3B     | RING          | Two KH repeats     | Q6ZN04     |
| MEX3C       | mex-3 RNA-binding family member C | RING          | Two KH repeats     | Q5USQ3     |
| MEX3D       | RNA-binding protein MEX3D isoform 2 | RING          | Two KH repeats     | Q86XN8     |
| MKRN1       | Makorin ring finger protein 1 | RING          | Four CCCH repeats  | Q5U5Q3     |
| MKRN2       | Makorin ring finger protein 2 | RING          | Four CCCH repeats  | Q86YN8     |
| MKRN3       | Makorin ring finger protein 3 | RING          | Three CCCH repeats | Q6ZN04     |
| RCH1        | Ring finger and CCCH-type domains 1 | RING          | CCCH               | Q5CTCZ     |
| RCH2        | Ring finger and CCCH-type domains 2 | RING          | CCCH               | Q5CTCZ     |
| CNOT4       | CCR4-NOT transcription complex, subunit 4 | RING          | CCCH and RRM      | Q95UH7     |
| UNK         | RING finger protein unkempt homolog | RING          | Five CCCH repeats  | Q9C080     |
| UNK1        | Unkempt family zinc finger-like | RING          | Four CCCH repeats  | Q95UH7     |
| RNF113A     | RING finger protein 113A      | RING          | CCCH               | Q5U5Q3     |
| RNF113B     | RING finger protein 113B      | RING          | CCCH               | Q86YN8     |
| ZNF598      | Zinc finger protein 598       | RING          | C2H2               | Q86UK7     |
| TRIM56      | Tripartite motif containing 56 | RING          | B-box 1 and 2      | Q9BRZ2     |
| NFX1        | Transcriptional repressor NF-X1 isoform 3 | RING          | R3H                | Q12986     |
| SCAF11      | Protein SCAF11                | RING          | Arg-Ser rich       | Q99590     |
| DZIP3       | DAZ interacting zinc finger protein 3 | RING          | Lys rich           | Q86Y13     |
| RNF17       | RING finger protein 17 isoform 2 | RING          | Four Tudor repeats | Q9BXT8     |
| ARIH2       | Ariadne RBR E3 ubiquitin protein ligase 2 | RING          | Not defined        | Q5USQ3     |
| MID1        | E3 ubiquitin-protein ligase Midline 1 | RING          | B-box 1 and 2, CCCH and SPRY | Q12986 |
| TRIM21      | Tripartite motif containing 21 | RING          | PRY/SPRY           | Q19474     |
| TRIM25      | Tripartite motif containing 25 | RING          | PRY/SPRY           | Q14258     |
| TRIM40      | Tripartite motif containing 40 | RING          | t-RNA-binding arm  | Q6P9F5     |
| TRIM71      | Tripartite motif containing 70 | RING          | NHL domain         | Q2Q1T2     |

| PRPF8       | Pre-mRNA-processing splicing factor 8 | MPN          | Not defined        | Q6P2Q9     |
| USP10       | Ubiquitin carboxyl-terminal hydrolase 10 | USP          | Not defined        | Q14694     |

*RBULs also present in RNA granules.

proteins, excluding non-catalytic E3 complex components, from the Human E3 Ubiquitin Ligases Database (https://hpcwebapps.cit.nih.gov/ESBL/Database/E3-ligases/) and compared it with lists of RBPs from two resources: RBBDB (a database of RBPs) and the “high-confidence RBPs” list (i.e., the HC-RBP list, which contains RBPs identified across three or more RBP resources studied in Ghosh et al., 2018) [6]. Via this comparison, we identified 18 proteins that appeared in both lists, revealing a repertoire of dual functional RBULs with E3 ligase and RNA-binding activities (Table 1, Figure 1). The E3 ligases hRUL138 (also known as DZIP3), RNF17, MID1, TRIM protein family members, and ARIH2, which were previously identified as RBULs [5,7] were manually added to Table 1. The need for these additions reflects the non-robustness of our strategy, which is partly attributable to the heterogeneity and unavailability of exhaustive lists of RBPs and E3 enzymes. Since the ubiquitylation level in cells is also regulated by deubiquitylating enzymes (DUBs), we also compared a list of DUBs [8] with the HC-RBP list, and we found only two proteins that appeared in both lists: PRPF8 and USP10 (Table 1).

2.2. UPS Components Adapted to Support RNA Interactions

Click chemistry-assisted RNA interactome capture (CARIC) [10] and RNA CLIP (cross-linking and immunoprecipitation)-based strategies [11–13] have dramatically expanded the catalog of RBPs with regard to their domain organization and binding-site specificity. RNA-binding domains (RBDs) are usually small stretches of tandemly repeated or non-repeated amino acid sequences that correspond to modular structures. Various RBDs allow RBPs to recognize their substrate RNAs, which can be either coding or noncoding RNAs. Known RBDs include RRMs (RNA recognition motifs), zinc (Zn)-finger motifs (CCCH), KH (K homology) domains, dsRBMs (double-stranded RNA-binding motifs), Pumilio/fem-3-binding factor (PUF) repeat domains, DEAD-box helicase domains, PAZ domains (PIWI-AGO-Zwille domains), K-rich regions, and RS domains (domains rich in alternating arginine and serine residues). [14–16] RBPs bind to their target RNAs either transiently or until they are degraded along with the RNA. [17]
Figure 1. The repertoire of RNA-binding ubiquitin ligases. Proteins from the comprehensive databases RBPDB, HC-RBP, and Human E3 ligases were analyzed using the Venn diagram to identify ubiquitin ligases, which also characterize the domains responsible for RNA binding. The analysis recognized 18 such proteins (RBULs). These RBULs are composed of either one or more RBDs and one RING domain. The RBULs are listed in the above diagram with a schematic representation of their domain composition. The domains organization of manually added RBULs (Table 1) is shown separately.

In cells, ligand RNAs exist in various structural conformation states due to multiple layers of processing (e.g., spatiotemporal expression, half-life/stability, and splicing) and modification (i.e., mRNA capping and polyadenylation and methylation). Thus, RBPs evolved two mechanisms for binding to heterogeneous RNA substrates. First, they often contain several copies of similar or different RBDs, thus increasing their ability to form stable interactions and providing sequence specificity (Figure 2). Second, RBPs exhibit structural plasticity via intrinsically disordered regions (IDRs) in their RBDs. The IDRs in RBDs not only act as biological hubs to allow interactions with multiple biomolecules, they also afford the proteins with different affinities to different substrates. It is well established that IDR-containing proteins, known as intrinsically disordered proteins (IDPs), are substrates of the proteasome system. Indeed, recent proteomic studies showed that RBPs are a frequent substrate of the proteasome. Another strong connection between the UPS and RNA metabolism stems from structural similarities in proteins involved in both pathways, as ubiquitin and the RNA-binding TGS domain share a structural likeness. The TGS domain (threonyl-tRNA synthetase [ThrRS], GTPase, and guanosine-3’,5’-bis(diphosphate) 3’-pyrophosphohydrolase [SpoT]) consists of ≈50 amino acid residues that fold into a predominantly beta-sheet structure, and it shares similarities with the alpha-L RNA-binding motif and the ubiquitin beta strap fold.

Shuffling and duplication of specific domains from an existing repertoire of domains can improve or add new functions to proteins in the cellular milieu. Furthermore, proteins that contain multiple domains display structural stability and have many advantages in their folding and functional activities. These advantages could have driven RBULs to gain their dual functionality, as most RBULs contain Zn-finger motifs, which are also present in both RBDs and the RING (Really Interesting New Gene) finger domain. Indeed, all of the RBULs that we
have listed possess a RING finger domain, which is required for their ubiquitin ligase activity, and various RNA-binding motifs (RBMs) that were acquired during their evolutionary adaptation to perform complex RNA metabolic functions (Figure 2). The RING domain is a type of Zn-finger motif that normally consists of 40–60 amino acid residues that coordinate two zinc atoms, and while they often function as a DNA recognition motif, they can also interact with RNA.[26] The TRIM (tripartite motif-containing) ubiquitin ligase family provides an example of the adaptation of RING domain-containing proteins to perform RNA-binding functions with the help of an additional RBD domain. The RBCC (RING between coiled-coil) motif in TRIM proteins mediates the ubiquitylation reaction, while their RNA-binding ability is provided by various domains, including the NCL1/H2A/LIN-41 (NHL) domain and the Sp1A kinase ryanodine receptor (SPRY) domain.[27] Interaction between an RBUL and an RNA molecule might alter the protein’s activity via allosteric modulation. For example, upon RNA binding, TRIM25 undergoes a rearrangement of its disordered L2 linker region, which has been proposed to be required for efficient TRIM25 activity.[28,29] Furthermore, RNA molecules can act as adaptors (known as ribo-bridges) to enable the recruitment of ubiquitin signaling components, including the DUBs USP10 and EL3F, to RNA granules (described in detail in Section 4).

Some RBPs evolved strategies to exploit the UPS. The TAB2, PRP21, and TAFII250 RBPs acquired ubiquitin-like (Ubl) and ubiquitin-associated (UBA) domains that allow them to participate in proteasomal degradation.[30] In summary, the UPS evolved to become a key proteolytic machine involved in the regulation of RNA-dependent processes.

3. The UPS Regulates RNA-Dependent Processes

3.1. Ubiquitin Affects mRNA Processing and Stability

The UPS can directly regulate mRNA stability; for example, MEX-3C, an RNA-binding E3 ubiquitin ligase, controls the expression level of HLA-A2, which encodes a major histocompatibility complex I receptor.[31] To do so, MEX-3C binds to the 3′-UTR of the HLA-A2 mRNA via its KH domain to induce its degradation.[31] This process depends on the RING domain of the MEX-3C protein; however, the exact mechanism of mRNA turnover is not well defined. Interestingly, this process can be antagonized by the USP7 DUB, thus revealing a multilayered regulation of mRNA stability by the UPS system.[32] How USP7 prevents mRNA degradation is not clear. Subsequent work by Cano and colleagues showed that MEX-3C also influences the rate of mRNA degradation.[32] The RNA-binding ubiquitin ligase MID1 is well known for its role in regulating the translation of several mRNAs, thus affecting processes that depend on 3-phosphoinositide-dependent protein kinase-1 (PKCδ) or an androgen receptor (AR).[37,39] MID1 has preferences for specific sequences contained in mRNA molecules. The expanded CAG repeat motif in the huntingtin (HTT) mRNA, which is translated into a polyglutamine tract in the HTT protein, is a pathological hallmark of Huntington’s disease (HD). CAG repeat regions, like that in the HTT mRNA, form double-stranded hairpin structures that recruit MID1. MID1, in collaboration with protein phosphatase 2A and 40S ribosomal S6 kinase, increases the translation of the CAG repeat expansion-containing mRNA, thus resulting in overproduction of aberrant HTT protein[41,42] (Figure 3). Similarly, MID1 influences the translation of mRNAs of other genes. For example, the GAG-rich repeats in the ataxin2 (ATXN2), ataxin3 (ATXN3), and ataxin7 (ATXN7) mRNAs are also bound by MID1, regardless of the sequences flanking the GAG repeats.[43] MID1 has become an attractive pharmacological target for the treatment of diseases associated with polyglutamine proteins, such as HD and spinocerebellar ataxias. Indeed, chemical therapeutics like Furamidine, which affects the binding of HTT mRNA to MID1, can stimulate neuroprotection.[44]

3.2. The UPS is Involved in Ribosome-Associated Quality Control (RQC)

The status of nascent polypeptide chain translation is monitored by the ribosome-associated quality-control system (RQC). When the RQC system detects defects in translation, the nascent peptide chains[45] and the associated mRNAs[46] may be directed for degradation. For example, translation of mRNAs that lack an in-frame stop codon leads to translation repression, ribosome recycling, and decay of the truncated nascent polypeptide via the RQC system. This response is initiated upon identification of aberrant mRNAs via incorrect polyadenylation in the coding region of the nascent transcript at a near-cognate poly(A) signal.[47]

Ribosomal stalling at poly(A) tracts is promoted by Makorin ring finger protein (MKRN1), a member of the evolutionarily conserved family of RNA-binding RING domain ubiquitin ligases.[48] Early studies with human HEK293 cells and mouse embryonic stem cells showed that MKRN1 interacts with multiple mRNAs and RBPs, including poly(A)-binding protein (PABP).[49,50] Recently, the role of MKRN1 in RQC was elucidated.[51] MKRN1 acts as poly(A) sensor by positioning itself at the beginning of the poly(A) tail via an interaction with PABP; however, it is not known whether PABP ubiquitylation by MKRN1 is necessary for the regulation of mRNA binding. The mRNA-bound MKRN1...
model of MID1 related neurodegeneration and neuroprotection. MID1 can support neurodegeneration by triggering PP2A proteasomal turnover. Degradation of PP2A stabilizes mTOR mediated phosphorylation of S6K kinase, leading to S6K activation and translational initiation of neurotoxic proteins (i.e., mutant huntingtin protein [HTT], amyloid precursor protein [APP], and beta-secretase enzyme [BACE1]). Neuroprotection is provided by miRNAs (miRNAs, miR-19, miR-340, miR-374, and miR-542) that suppress the expression of MID1. Other potential chemical therapeutics, including furamidine (blocks MID1-target mRNA binding) or temsirolimus (mTOR inhibitor), stimulate neuroprotection via different mechanisms. Degraded/unexpressed proteins are marked with dashed lines, and negatively regulated pathways are shown with red arrows.

is then recognized by ribosomes as a signal to stall translation. Next, MKRN1 initiates RQC by ubiquitylating the RPS10 ribosomal protein, PABP, and additional translation regulators. These functions indicate that MKRN1 participates in multiple levels of translational control.\[51\]

Zn-finger protein 598 (ZNF598), another RING domain ubiquitin ligase, is involved in RQC in mammalian cells via its ability to sense polyadenylated mRNAs.\[52–54\] ZNF598 recognizes (AAA) codon repeats in mRNAs by binding to the cognate lysine tRNAs (tRNA^[Lys](UUU)) , thus triggering ribosome stalling and subsequent degradation of the mRNA and nascent protein.\[52\] Collisions of actively translating ribosomes with blocked ribosomes can provoke endonucleolytic cleavage of stall-containing mRNAs.\[55\] ZNF598 can detect collided di-ribosome structures (mainly in polysome fractions) to initiate site-specific ubiquitylation of several ribosomal proteins, thus assisting in the resolution of poly(A)-induced stalled ribosomes.\[52–54,56,57\] It remains to be investigated whether the ubiquitin marks left by ZNF598 recruit downstream pathways that carry out mRNA cleavage and ribosome recycling. It is also unclear if ZNF98 uses the same mechanisms to sense polyadenylated mRNAs and ribosome collisions.

### 3.3. RNA Granules Are a Center of Ubiquitin Signaling

Translational inhibition due to imbalances in cellular homeostasis caused by stress conditions, proteasome inhibition, drugs, knockdown or overexpression of translation initiation factors often leads to the association of non-translated mRNAs with a set of proteins to form messenger ribonucleoprotein (mRNP) granules. mRNP complexes accumulate poly(A) mRNAs as well as RNPs and RBPs involved in mRNA transport, stability, and translation, and they function mainly as sites of mRNA triage by acting as storage centers and scaffolds. The two best-studied mRNP granules are processing bodies (P-bodies), which contain the mRNA decay machinery, and stress granules (SGs), which are characterized by the presence of translation initiation molecules.\[58–60\] Many UPS-associated proteins are found in RNA granules.\[61–65\] For instance, binding of RNA and RBPs to OTU domain-containing protein 4 (OTUD4), a deubiquitylating enzyme, is required for SG formation,\[66\] although no specific RBD has been found in OTUD4. Instead, its RNA-binding ability has been attributed to its two IDRs and its C-terminal region rich in RGG, RG, RS, and GYSY amino acids. Both of these features are common in many RBPs that lack RBDs.\[67\] MCPIP1 (monocyte chemotactic protein-induced protein 1) coordinates RNA granule organization via its DUB and RNase activities under stress conditions.\[68\] Another DUB involved in the control of SG composition is ubiquitin-specific protease 10 (USP10). USP10 localizes to SGs via an interaction with the granule-initiation protein G3BP, and depletion of USP10 reduces the rate of SG generation.\[69\] Recently, Piatnitskaia and colleagues found that USP10 is essential for the assembly of SGs that contain Tau protein, whose aggregation in neurons is a hallmark of tauopathies such as Alzheimer’s disease (AD).\[70\] Thus, USP10 can participate in the development of Tau aggregates in AD via its role in SG formation.

In addition to DUBs, ubiquitin ligases also play active roles in RNA granules. For example, members of the highly conserved Roquin and MEX3 RNA-binding E3 protein families are components of SGs and P-bodies.\[71–74\] Roquins can regulate mRNP granules via their conserved CCCH and ROQ domains, and overexpression of full-length ROQUIN or its ROQ domain alone can nucleate SG assembly.\[73\] P-bodies, unlike SGs, contain RNA degradation machinery, including mRNA decapping enzymes.
Roquins are recruited to P-bodies through interactions between their C-terminal proline-rich regions and coiled-coil regions and protein components of the RNA-decapping complexes, that is, the RNA helicase Rck (also known as DX6—DEAD-box RNA helicase 6) and the enhancer of decapping protein 4 (Edc4).\[^{[72]}\] MEX3A and MEX3B are segregated into P-bodies via their KH RBDs, wherein they colocalize with the decapping factor DCPIA and Argonauta ( Ago) proteins, which are components of the RNA-induced silencing complex.\[^{[73]}\] Interestingly, MEX3B and Ago proteins can be redistributed between P-bodies and SGs via interactions with 14-3-3 proteins (conserved adaptor molecules), suggesting that there is demand for MEX3B in different types of RNA granules.\[^{[74]}\] Another member of the MEX3 family, MEX3C, interacts with a cytoplasmic sensor of RNA viruses known as RIG-I (retinoic acid inducible gene-1) in the SGs of virally infected cells. This interaction triggers lysine-63-linked ubiquitylation of RIG-I, which then elicits antiviral immune responses.\[^{[74]}\] On the other hand, there seems to be no hard rule that RBULs are sorted to SGs upon stress induction. Unlike many cytosolic RBULs, ZNF598 does not show any change in its cytotoxic localization upon arsenite-induced stress.\[^{[72]}\]

### 3.4. Ubiquitin-Associated Proteins Affect RNA Granule Formation

Proteins that are not part of the ubiquitylation cascade (E1-E2-E3/DUB) can also affect RNA granules formation. For example, ubiquitin-associated protein 2-like (UBAP2L) is a conserved protein with an N-terminal ubiquitin-associated (UBA) domain that is likely involved in the detection of ubiquitylated substrates and the promotion of their aggregation.\[^{[76]}\] Recent studies have shown that UBAP2L has a role in SG assembly that does not require its ubiquitin-interaction ability.\[^{[77]}\] UBAP2L uses an Arg-Gly-Gly (RGG) motif to interact with SG components. Interestingly, the residue of the RGG motif can be methylated by PRMT1 (an arginine methyltransferase), and increased arginine methylation in UBAP2L affects SG assembly.\[^{[77]}\]

Liquid–liquid phase separation (LLPS) into protein-rich droplets promotes the formation of membraneless mRNP granules. Lysine is enriched in disordered regions of proteins in P-bodies and lysine-rich polypeptides phase separate into lysine/RNA-coacervates.\[^{[76]}\] Ubiquitylation of lysine residues in disordered regions would sterically interfere with the weak multivalent interactions that are prerequisite for LLPS. Consequently, ubiquitylation of disordered proteins would be a way to eliminate them from non-membrane-bound condensates for subsequent proteasomal degradation. Ubiquitin2 (UBQLN2) is a receptor and shuttle protein in the UPS that colocalizes with SGs via intrinsically disordered low-complexity domains (LCDs), which enable phase separation. By contrast, interactions with ubiquitylated substrates reverse UBQLN2 LLPS to facilitate the sorting of target proteins out of SGs toward the UPS.\[^{[84]}\] In addition to ubiquitylation, other posttranslational modifications of lysine that could also potentially affect the association of proteins with LLPS, such as methylation, sumoylation, neddylation, or acetylation, have been identified.\[^{[78,80]}\] Indeed, acetylation of lysine reverses LLPS and diminishes colocalization of tau protein with SGs.\[^{[78]}\] Nevertheless, it is not clear how the UPS shifts between supporting the ability of RNA-binding proteins to form phase-separated RNA structures and promoting the degradation of these IDPs.

Recent work revealed that SGs contain \(\approx 464\) proteins, of which \(54\)% were RBPs.\[^{[83]}\] Among the other proteins annotated in this SG protein database, a number of E3s and DUBs were detected. However, Markmiller and colleagues introduced some controversy into this story by demonstrating that the sorting of ubiquitin signaling-associated proteins into SGs is not affected by inhibition of ubiquitylation processes and that at least polyubiquitylation plays a minor role in RNA granule biogenesis.\[^{[65]}\]

On the other hand, RBPs and UPS elements constitute over half of the proteins that make up RNA granules. Thus, it can be assumed that the dynamics of these granules strictly depend on crosstalk between these two groups of proteins (Figure 4).

### 3.5. The UPS Can Destabilize rRNA

Ribosomal RNA (rRNA) metabolism, including the transcription of rRNA genes as well as pre-rRNA processing, modification, and assembly, occurs mainly in the nucleolus.\[^{[82]}\] These processes are followed by additional processing in the cytosol. Eukaryotic cytosolic ribosomes contain four rRNA molecules: the 28S (25S in yeast), 5.8S, and 5S rRNAs in the 60S ribosomal subunit and the 18S rRNA in the 40S subunit.\[^{[81]}\] E3 ligases can influence rRNA metabolism either via direct interactions with RNAs or with associated RBPs. For example, the ZNF598 ubiquitin ligase binds to the 18S rRNA via its C-terminal RBD, which is vital for the functionality of the RQC system and the no-go decay pathway (NGD), a stalled ribosome surveillance mechanism.\[^{[84]}\] ZNF598 can crosslink rRNA, tRNA, and mRNA to place itself in a privileged position on translating ribosomes. This crosslinking enables monitoring of both the mRNA coding sequence and the identity of the incoming tRNAs, leading to RQC activation upon the recognition poly(A) tails.

The UPS also monitors the status of nanostructures, such as ribosomes. For instance, formation of an E3 complex by Mms1 (methyl methane sulfonate sensitivity protein 1) and Rtt101 (regulator of Ty1 transposition protein 101, also known as Cullin-8) can trigger degradation of the 25S rRNA in defective 60S ribosomal subunits via the nonfunctional rRNA decay (NRD) pathway.\[^{[85]}\]

Under stress conditions, the ubiquitin protease E13F (eukaryotic initiation factor 3 subunit f) participates in rRNA decay via an interaction with the RBP hnRNP-K (heterogeneous nuclear ribonucleoprotein K). This interaction leads to sequestration of hnRNP-K from rRNA, which then triggers rapid rRNA degradation and attenuated translation. This molecular exchange is yet another example of how complex interconnections between translation initiation, rRNA decay, and the UPS can be governed by a single protein.

### 3.6. The UPS Is Involved in tRNA Metabolism

RNA and transfer RNA (tRNA) processing share several components, and E3 ligases involved in rRNA regulation also...
Figure 4. Ubiquitin signaling and RNA granules. The cell has evolved a protective mechanism in response to stress and sequesters essential biomolecules into RNA bodies. After a stall in translation and polysome disassembly, several RNA-RBP complexes are packaged into the membrane-less compartments. Various RBPs interact with specific E3 ligases or DUBs in the cytoplasm to recruit them to stress granules (SG) or P-bodies. This process is vital for regulating mRNP granules metabolism.

participate in tRNA metabolism. For example, the E3 ligase ZNF598 is important for the activation of the RQC system via its ability to recognize specific tRNA sequences. As discussed in Sections 3.2 and 3.5, ZNF598 crosslinks to AAA-decoding tRNA Lys (UUU) molecules to detect mRNAs with premature poly(A) tails, which then leads to RQC activation. By cooperating with different RNA types, ZNF598 can dynamically adapt RNA-related mechanisms to the protein homeostasis status in the cell.

Rsp5, the Saccharomyces cerevisiae Nedd4 homolog, is a unique E3 ligase in that it can regulate RNA-dependent processes despite two unusual features: 1) it possesses a HECT domain instead of a RING domain, and 2) it does not bind directly to nucleic acids (the modes of action of HECT and RING ubiquitin ligases have been recently reviewed in depth). Nevertheless, Rsp5 affects mRNA, rRNA, and tRNA metabolism. For instance, the role of Rsp5 in tRNA processing was evident from the nuclear accumulation of pre-tRNA molecules in rps5 mutant cells. Indeed, proteins involved in tRNA metabolism are substrates of Rsp5, including Trz1 and Lhp1, which are involved in tRNA processing, and Mtr10 and Los1, which are involved in tRNA transport. This is only one example of how the UPS uses ubiquitin ligases with different ubiquitin transfer dynamics to affect RNA metabolism, even in the absence of direct interactions with RNA.

4. The UPS Regulates Features of Noncoding RNAs

The vast majority of RNA generated in the cell lacks protein-coding sequences. Noncoding RNAs (ncRNAs) comprise a heterogeneous group of RNA species in terms of their biogenesis, biological functions, structure, and length. NcRNAs can be divided into two significant fractions according to their size: short molecules of less than 200 nucleotides (nt), for example, microRNAs (miRNAs), and longer molecules of more than 200 nt, for example, long noncoding RNAs (lncRNAs).

4.1. The UPS and miRNAs Are Functionally Linked in Some Pathways—The Status Quo

MiRNAs are a class of short, ≈20 nucleotide-long noncoding RNAs that control gene expression in diverse biological systems. MiRNA interaction sites are often located in the 3′-UTRs of their target mRNAs. Binding of a miRNA to its target mRNA induces mRNA degradation and translational repression (reviewed by O’Brien et al.). MiRNA-mediated processes are dynamically regulated by ubiquitin signaling pathways and vice versa. For example, the human miRNAs miR-19, miR-340, miR-374, and miR-542 negatively regulate the expression levels of several genes primarily associated with carcinogenesis. Interestingly, the RBUL MID1 is a common target of these four miRNAs. By binding to the 3′-UTR of MID1, these miRNAs can influence MID1 expression and, thus, the translation of several known MID1-targets, including mutant HTT (Figure 3).

Expression of mind bomb-1 (Mib1), a RING-type E3 ligase, is also regulated by various miRNAs in different cell types. For example, the brain-enriched microRNA miR-137 binds to a conserved site in the 3′-UTR of the Mib1 mRNA to regulate Mib1
expression, which is crucial for neuron maturation. Mib1 is also a target of mir10 in zebrafish and human endothelial cells, and the corresponding regulatory networks influence angiogenic processes in a Notch-dependent manner. MiRNAs can also regulate the expression levels of ubiquitin-conjugating enzymes (E2s). For instance, the decreased levels of miR-199a, miR-199b, and miR-214 in dilated cardiomyopathy (DCM) lead to stabilization of the Ubc2i and Ubc2g1 E2s. The resulting increase in the proteolytic activity of the UPS is believed to be responsible for the loss of cardiac muscle mass and cardiac failure.

In addition to being regulated by miRNAs, the UPS can also affect miRNA stability. The protein Roquin, a ubiquitously expressed RING domain-containing E3 ubiquitin ligase with RNA-binding properties, is involved in multifunctional regulation of immune homeostasis. The human genome encodes two Roquin paralogs, RC3H1 and RC3H2. RC3H1 can modulate miRNA longevity by promoting mir146a turnover in T cells. In addition, cells lacking both RC3H1 and RC3H2 show elevated levels of several other miRNAs, suggesting that Roquins influence the levels of their target miRNAs via mechanisms involving sophisticated microRNA-mediated repression. Interestingly, RNA binding by the Roquin paralog RC3H2 induces a conformational change and enhances its auto-ubiquitylation activity in an E2-dependent manner. In spite of these known processes, it is still unclear whether RNA can also modulate ubiquitin ligase specificity for protein substrates.

4.2. LncRNAs and the UPS Cooperate to Control Senescence and Neuroprotection

LncRNAs can regulate transcription by guiding specific chromatin modification complexes to target genomic loci. LncRNAs act as scaffolds for transcriptional regulators and affect the activities of transcription factors and miRNA expression levels. Due to the diverse roles of IncRNAs in transcriptional regulation, an extensive collection of literature supports the roles of IncRNAs in several pathologies, including diabetes, cancer, and neurodegeneration. Interestingly, IncRNAs can also operate as loading platforms for ubiquitin signaling components. The IncRNA Hox transcript antisense intergenic RNA (HOTAIR) acts as a scaffold to facilitate the ubiquitylation of the RBPs ataxin-1 (implicated in spinocerebellar ataxia) and snurportin-1 (a shuttling protein involved in nucleocytoplasmic transport) by the RBULs Dzip3 and Mex3b, respectively. By enabling the UPS to degrade ataxin-1 and snurportin-1, HOTAIR can counteract premature cellular senescence.

The binding of some lncRNAs to mRNAs can stimulate the translation of the latter. Ubiquitin C-terminal hydroxylase L1 (UCHL1), which is synthesized in neurons and the neuroendocrine system, is the topic of many studies due to its complicated structure and even more complex functions. UCHL1 is both a DUB and a ubiquitin chain elongating factor (a so-called E4 enzyme). Via the latter function, it extends Lys63 polyubiquitin chains on α-synuclein to prevent proteasomal degradation of α-synuclein. UCHL1 is an abundant pro-survival protein; thus, its levels must be precisely regulated to sustain neuronal protein homeostasis. Indeed, an antisense lncRNA of the Uchl1 sense mRNA to stimulate UCHL1 protein synthesis by strengthening the interaction between the protein-coding mRNA and active polysomes, thus driving its translation. Therefore, in response to neurotoxic stress, signaling pathways induce the expression of antisense Uch1l to maintain the synthesis of the neuroprotective protein UCHL1.

5. Conclusions and Outlook

Expression of multifunctional proteins is a cellular strategy to reduce energy consumption and to maintain cellular resources. The list of proteins equipped with domains enabling them to function in ubiquitin signaling and RNA-dependent processes continues to grow. This same trend is seen with RBULs, which can directly interact with nucleic acids via their RBD domains. Whether UPS proteins evolved to gain RBDs or vice versa is still debated; however, cues from other similar multidomain, multifunctional proteins offer hints for evaluating these two possibilities. Even though RBULs have significant molecular potential, their cellular functions, molecular mechanisms, binding partners, and substrates are still poorly understood. In addition, the mechanisms by which RNA binding influences the substrates selectivity and functionality of ubiquitin ligases, cooperation with E2 enzymes, and ubiquitin chain synthesis remain to be investigated. Thus, it will be beneficial to study these protein–RNA interactions structurally to decipher the mechanisms underlying their functions. Furthermore, how post-transcriptional and post-translational modifications regulate the communication between the UPS and RNA systems remains poorly understood. RBPs may operate as molecular chaperones, for example, by stabilizing IDPs. An intriguing question is whether specific RBULs function as specialized protein quality control ubiquitin ligases to maintain the solubility, stability, and functionality of aggregation prone IDPs. Future work is also necessary to shed light on the molecular mechanisms by which RBULs or RBPs that contain ubiquitin-like or ubiquitin-associated domain distinguish between supporting the formation of higher-order cellular assemblies through LLPS and promoting the degradation of these IDPs. Biomolecules, to complete their cellular functions, often communicate to act as synchronized molecular machines. The cooperation between the UPS and RNA-dependent processes is one example of such communication, and elements that induce or disrupt this crosstalk are emerging as important pharmaceutical targets.

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Conflict of Interest

The authors declare no conflict of interest.
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E3 ligase, P-bodies, ribosome-associated quality control, RNA, RNA-binding proteins, stress granules, ubiquitin-proteasome system

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