

Chapter 12

Post-Translational Modifications and RNA-Binding Proteins

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Abstract RNA-binding proteins affect cellular metabolic programs through development and in response to cellular stimuli. Though much work has been done to elucidate the roles of a handful of RNA-binding proteins and their effect on RNA metabolism, the progress of studies to understand the effects of post-translational modifications of this class of proteins is far from complete. This chapter summarizes the work that has been done to identify the consequence of post-translational modifications to some RNA-binding proteins. The effects of these modifications have been shown to increase the panoply of functions that a given RNA-binding protein can assume. We will survey the experimental methods that are used to identify the presence of several protein modifications and methods that attempt to discern the consequence of these modifications.

Keywords RNA-binding proteins • Post-translational modifications • SUMOylation • Ubiquitination • Phosphorylation

1 Introduction

RNA-binding proteins (RBPs) regulate RNAs at every stage of their existence. This includes processes that govern RNA metabolism from capping and polynucleotide extension, RNA splicing, subcellular RNA localization, cellular export, translation (initiation elongation and extension), to RNA destruction. This class of ~1200–1600 proteins has important roles in the etiology of disease and therefore advances in the understanding of these proteins hold the promise to be directly

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applicable to the treatment of human neurodegenerative diseases, cancers and developmental disorders [1–5]. It has been known for some time that mRNA splicing is coupled to signal transduction and posttranslational modifications (PTMs) [6]. A full understanding of this intertwined network of processes has been complicated by the realization that RNA-binding proteins are a diverse class of regulators which themselves undergo extensive regulation via splicing, alternative 5' and 3' ends and various post-translational modifications.

Post-translational modifications follow from various signaling pathways to cause activation of enzymes that add or remove PTM moieties (Fig. 12.1a). The set of post-translational modifications known to affect RNA-binding protein function includes at least: the reversible addition/removal of phosphate groups (PO_3) by kinases/phosphatases, of methyl groups (CH_3) by methylases/demethylases, of acetyl groups ($\text{C}_2\text{H}_3\text{O}$) by acetylases/deacetylases, of the small protein ubiquitin (~8.5 kDa protein) by ubiquitin ligases/deubiquitinating enzymes, of SUMO

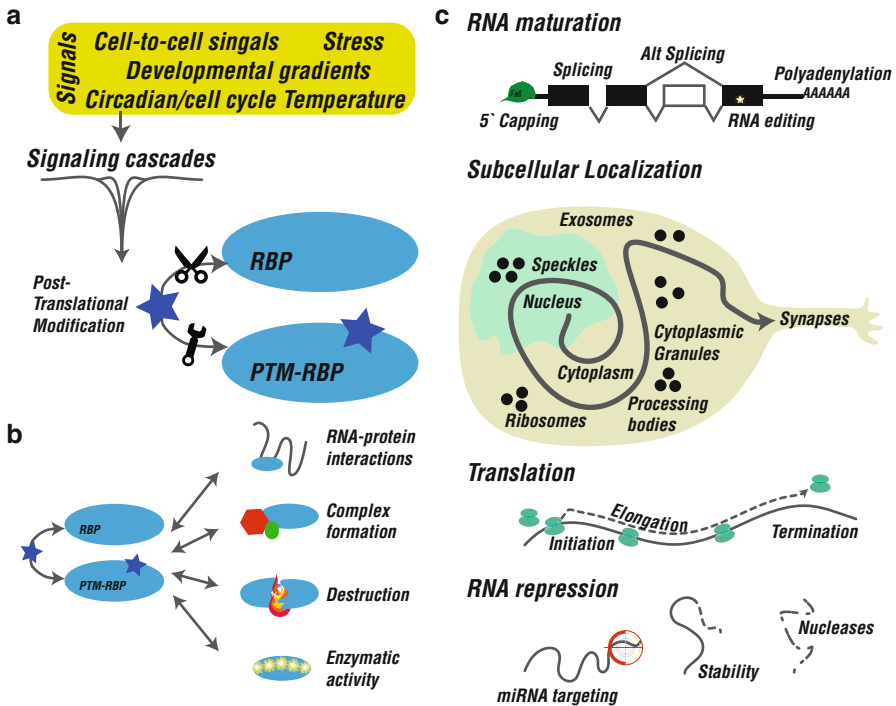


Fig. 12.1 Schematic of the effects of PTM on RNA-binding proteins. (a) Signal integration. Various signals from inside a cell and from external sources activate signaling cascades that converge on the regulators of PTM state. (b) PTM may activate or deactivate the functions of an RNA-binding protein, including altering RNA targets, protein partners, mediating protein degradation or intrinsic enzymatic activities. (c) The altered functions of RNA-binding proteins lead to overall differences in the metabolism of RNAs at every stage of their existence, from transcription through destruction

(~12 kDa proteins) by ubiquitin ligases/SUMO proteases, and of glycans (polysaccharides) by glycosyltransferases/exoglycosidases or proteolytic cleavage by proteases. Although classically ubiquitination is associated with proteasomal degradation, some studies point to other functional roles for ubiquitin conjugation including localization and regulating protein interaction partners. Further, there have been observations of functional differences in the activity of polyubiquitin chains, depending on which lysine position links the ubiquitin monomers [7].

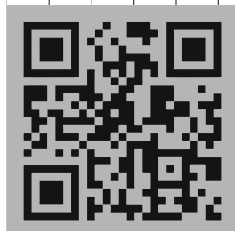
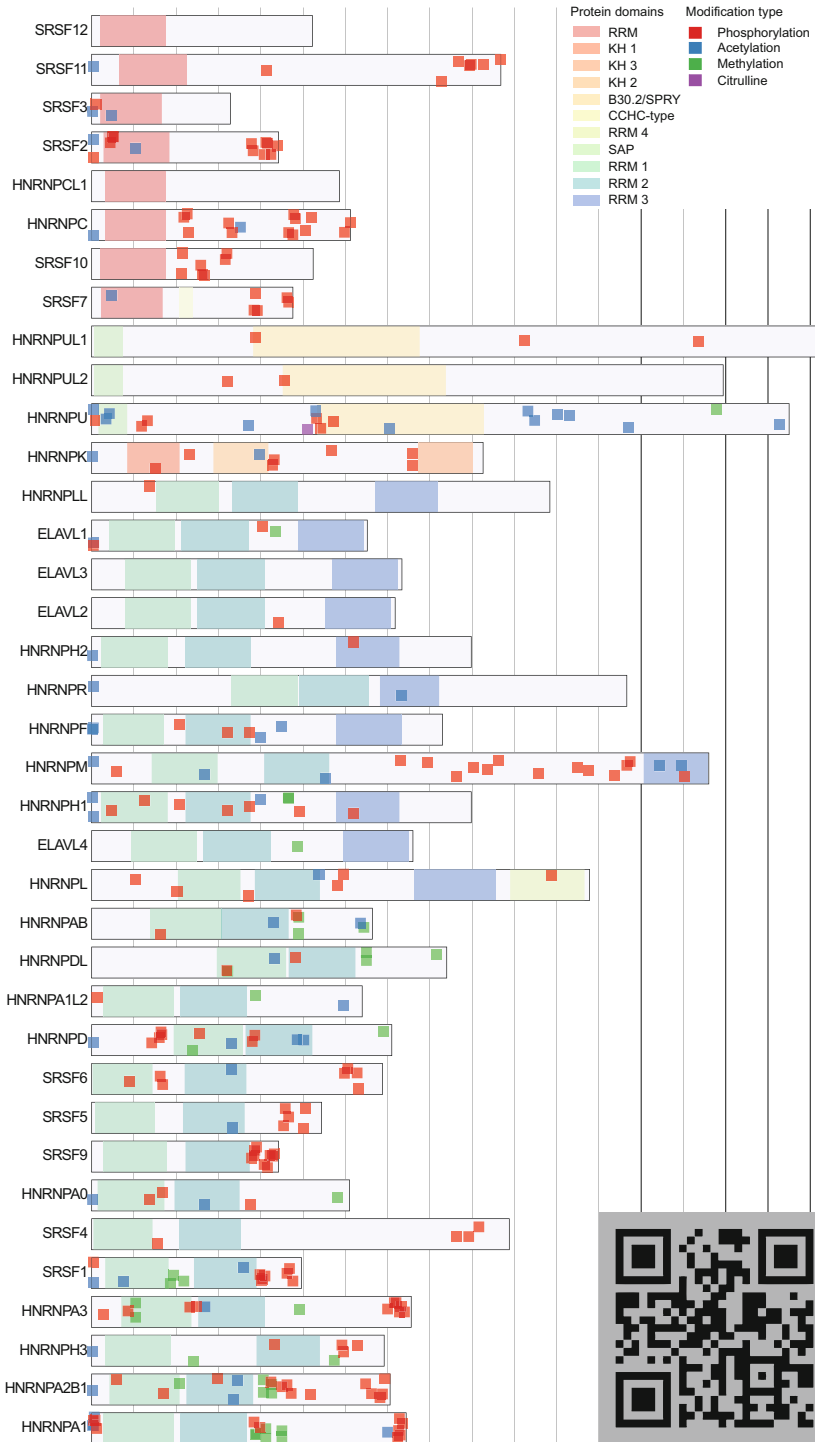
RBPs are affected by these PTMs in diverse and currently unpredictable ways. RBP subcellular localization, affinity for RNA, enzymatic activity and association with cofactors have all been shown to be directed by PTM state (Fig. 12.1b). Since these consequences on protein function on RNA fate are often indistinguishable without close inspection, the exact stage where a PTM effects change is sometimes unclear. We have organized this chapter following the life-cycle of an mRNA from transcription through splicing, then nuclear export, subcellular targeting, translation and ultimately destruction. We highlight research at each stage of the RNA life-cycle that shows the indispensability of PTMs for fidelity of RNA regulation (Fig. 12.1c). Where possible we point to studies that discuss the exact effect of PTMs and describe experiments that can reveal this information. Overall, this chapter aims to be a review of the work done to bridge the gap between proteomics and transcriptomics and answer vital questions about the diversity of ways PTMs alter RBP function.

As mass spectroscopy methodologies improve and become more accessible, protein modifications are mapped with high accuracy and with little cost [8]. Cataloguing modifications is the first step in order to understand how they participate in RBP function, and how RBP function is regulated by a certain pathway. Therefore, our intention in this chapter is far from reporting all the identified PTMs in translation control of RBPs. Instead, we will focus on some of the most studied cases hoping that they will serve as examples and predictions of what we may expect from the other members of this protein family.

There are several proteins or families of proteins discussed below that may have diverse roles that are not fully explored. As these are involved in several stages of RNA maturation, it is appropriate to first introduce them (Fig. 12.2):

HNRNPs are a diverse family of RNA-binding proteins for which post-translational modifications were discovered in the first descriptions of the proteins [9]. PTMs regulate the ability for HNRNP members to effect splicing changes and control the localization of RNA, as well as regulate translation, which will be discussed later in this chapter.

SR proteins were originally discovered for their role as splicing activators, but this view has been complicated by the nuances of effects due to PTMs [10, 11]. An abundance of literature points to the importance of PTMs in the activity of this family of proteins. Classically, SR proteins are modified by SR-protein kinases (SRPK1 and SRPK2) [12] and CDC2-like kinases (CLK1-4) [13–15]. The activity of these kinases is regulated through cell cycle, through development, and in response to cellular stresses like heat shock [10, 15, 16]. For several of the “classical” SR-proteins, including SRSF1 (aka Asf/Sf2) and SRSF2 (aka Sc35), phosphorylation induces changes in the intranuclear distribution of these phosphoproteins, causing release from nuclear speckles [17].



ELAV (aka Hu) proteins are exemplified by a well-studied member of the family, ELAVL1. ELAVL1 (aka HuR, human antigen R) has 2 N-terminal RRM domains followed by a hinge region that contains a nucleo/cytoplasmic shuttling domain (HNS), and another C-terminal RRM domain. It recognizes and binds to Adenylate-Uridylate rich elements (AREs) present in 3'UTR and/or 5'-UTR of transcripts in the nucleus and regulates their splicing, processing, nuclear export, localization, half-life and translation [18, 19]. Several features of its behavior are partially explained by PTMs. ELAVL1 has a dual effect on translation, being capable of activating translation of certain mRNAs (hypoxia-inducible factor (HIF)-1 α , p53, prothymosin- α , MKP-1, cytochrome c, heme oxygenase-1, and cationic amino acid transporter 1 [20–25]) and inhibiting others (IGFR, p27, Wnt5a e c-Myc, [25–30] for instance) with altered RNA affinity dependent upon phosphorylation. Phosphorylation on the hinge domain affects ELAVL1 localization [31], as do several phosphorylation sites on the RRM domains. There is evidence that RRM3 domain phosphorylation modulates dimerization inducing higher substrate affinity and altered protein localization.

2 PTM-Mediated Regulation of Pre-mRNA Processing

2.1 *Transcription*

The integration of signaling cascades in the control of RNA begins with RNA polymerase. Synthesis, 5' capping and 3' polyadenylation of almost all protein-coding transcripts is orchestrated by phosphorylation of the C-terminal domain (CTD) of DNA-directed RNA-polymerase 2 (POLR2A and homologs B-M) [32]. CTD hyperphosphorylation by carboxy-terminal kinases induces the recruitment of capping enzymes [33, 34] and 3' end cleavage and polyadenylation factors [35]. Phosphorylation of the CTD of POLR1* also regulates transcriptional activity of ribosomal RNAs [36]. In fact, there is an extensive body of literature devoted to the consequence of RNA-polymerase CTD phosphorylation and interested readers should read [32, 37] for a more comprehensive review of the effectors and effects of this specific PTM target. Further, acetylation by p300, indicated by a p300 dose-dependent shift in POLR2 CTD molecular weight, seems to be involved in transcription initiation or early transcription elongation of growth-factor induced genes [38]. The production of specific species of RNA that bind FUS nucleates the formation of nuclear FUS aggregates [39]. POLR2 CTD phosphorylation is reinforced by



Fig. 12.2 Large families of RBPs and their modifications. Proteins are shown as rectangles along rows. Functional domains of the protein are labelled and the proteins are sorted to group proteins with similar domains. PTMs listed in the Uniprot database are depicted as square shapes on each gene. Each vertical line is 50 amino acid residues. (Note: The QR code in the bottom-right links to an interactive version of this figure where references can also be visualized. Web link: here: <https://rawgit.com/mlovci/12365bcfbef4a32d35a/raw/f781d50b3fd96ef83d019baf9e7984374420fdc6/Figure%25202.html>)

these FUS aggregates [40]. Finally, FUS N-terminal phosphorylation by DNA protein-kinases (DNA-PK) removes FUS granules from the nucleus causing them to lose the potential to directly regulate POLR2 [41]. Thus, complex signaling and feedback control the activity of POLR2 through regulation of RBP PTM state.

Some HNRNPK PTMs serve to gate cell division checkpoints in response to DNA damage sensing. The activity of p53 tumor suppressor targets is tied to DNA damage-induced PTMs on the RNA-binding protein HNRNPK, which alter p53-HNRNPK protein-protein affinities. Methylation, phosphorylation and sumoylation of HNRNPK all regulate the p53-dependent cell cycle checkpoint [42–44]. Several signalling pathways converge to alter the function and stability of HNRNPK in response to DNA damage, including reduced expression of the E3 ligase MDM2 that targets HNRNPK to proteasomal destruction [45].

2.2 *Splicing*

Splicing is the RNA-catalyzed concatenation of exons that requires several protein scaffolds for which PTM state can control outcomes. Splicing in the nucleus is controlled by upstream signalling for DNA damage and cell cycle [46]. Indeed, it is closely tied to transcription and PTM state of histone proteins. For splicing components to mature, SMN complexes interact with U snRNAs and sm proteins to form snRNPs. This spliceosome formation occurs at Cajal bodies and requires the SMN complex. SMN components are localized, in part, by phosphorylation of the GEMIN proteins and deficiencies in this are linked to serious defects in intron recognition [47–49]. During spliceosome assembly, the targeted PTM of specific residues of snRNP must be required as both kinases and phosphatases are required for spliceosome assembly [50, 51].

S20 phosphorylation of SRSF1 initiates spliceosome assembly at intronic splice sites and is required for pre-mRNA processing fidelity [52]. This was reported to be regulated by the KIS kinase and important for bridging SRSF1 and U2AF2 in ternary SRSF1/U2AF2/RNA complexes [53]. Recent work shows with X-ray crystallography exactly the conformational shifts involved with phosphorylation of SRSF1 and reveals that only the phosphorylated version of SRSF1 can interact with U2AF65 [54]. SRSF10 (aka SRp38), is normally an unphosphorylated splicing repressor, but switches to a sequence-specific splicing activator when it is phosphorylated [17, 55, 56], presumably by inducing formation of spliceosomal complex A along with S100 [57].

2.3 *Alternative Splicing*

Alternative splicing (AS) is the regulated process of selective inclusion of specific exons into processed mRNA transcripts at specific stages of development or in response to external stimulation. Alternative splicing results from inefficient

recognition by the spliceosome or competition among 3' splice-sites for ligation to 5' splice-sites. Splicing factors are regulated through signalling cascades to either activate or repress splicing in certain environmental conditions. These include pathways that recognize extracellular signals like EGF, Wnt, insulin, cytokines and heat stress [16, 55, 58, 59].

Beside sub-nuclear localization and interactions with snRNPs, phosphorylation of SR-proteins has been shown to cause shuttling between the nucleus to the cytoplasm, usually resulting in the loss of inclusion of their splicing targets [60–62]. Proline-directed SRSF1 phosphorylation causes conformational shifts that affect enzymatic activity of the protein [63]. Lines of evidence implicating non-phosphorylation PTMs like ubiquitination and acetylation are less common but do exist, and are associated with regulation of protein turnover [64, 65]. In the case of acetylation, lysine-acetylated SRSF2 proteins by KAT5 (aka Tip60) are more likely to be subject to degradation and HDAC6-mediated deacetylation causes SRSF2 accumulation. However, KAT5-mediated PTM is accompanied by concomitant acetylation of SRPK1 and SRPK2 that causes these kinases to be excluded from the nucleus, thus the accumulated SR proteins are not actively regulating splicing in these cells [64]. Ubiquitination of SRSF1 was shown to be increased in activated T-cells, causing proteasomal destruction of the protein, but the E3 ligase that mediates this PTM is not yet known [65]. Development of small-molecule inhibitors of these SR-related PTMs has been the focus of recent research with potential applications in treatment of cancers like metastatic melanoma [66, 67].

HNRNPL S52 phosphorylation mediates signal integration via the PI3K/AKT pathway. Phosphorylated HNRNPL, but not non-phosphorylated HNRNPL out-competes HNRNPU for binding at a *cis*-element. RNA-binding assays with an antibody specific for S52-phosphorylated HNRNPL shows that when HNRNPL is phosphorylated, it associates with RNA while HNRNPU binding is diminished leading to exclusion of a pro-apoptotic caspase-9 exon; HNRNPU phosphorylation alone did not account for this change [68]. Similarly, upon neuron depolarization CAMK4 kinase activation causes phosphorylation of HNRNPL. This S513 phosphorylation increases HNRNPL affinity for CaM-kinase responsive RNA elements, out-competing assembling spliceosomal components and inhibiting exon inclusion [69]. Data obtained with a methylation-sensitive antibody indicates that PRMT1 causes constitutive methylation on HNRNPU, but the authors did not observe methylation-dependent localization shifts and could not discern a regulated function for the methylation of this protein [70].

Splicing of the stress-induced isoform of the TRA2B transcript by ELAVL1 is accomplished only when nuclear-localized ELAVL1 is phosphorylated downstream of Chk2 and p38-MAPK at positions S88 and T118 [71]. These phosphorylated residues increase ELAVL1's affinity for an intronic binding site near an exon that causes an in-frame stop-codon, in turn causing higher levels of exon inclusion and subsequent nonsense-mediated decay of the TRA2B transcript.

KHDRBS1 (aka Sam68), a member of the STAR family of RNA-binding proteins, stands out in that it has reports of multiple classes of PTMs modify its RNA regulatory activity. Phosphorylation or acetylation increases its affinity for

RNA and splicing regulatory activity as shown with point-mutants and chemical small-molecule inhibitors of phosphorylation [59, 72–75] while methylation decreases affinity for poly-(U) targets [76]. Mutational studies showed that S58, T71 and T84 phosphorylation were required for splicing activation and authors note ATP-gS, a phosphatase-resistant ATP analog, was necessary to observe this effect in *in vitro* splicing assays [59]. In addition, tyrosine phosphorylation may influence the ability of KHDRBS1 to effectively form dimers, which are required for splicing-regulatory activity [77]. SUMOylation has also been reported on KHDRBS1 but not with an RNA-regulatory effect [78].

These are just a few of the hundreds of RNA-binding protein PTMs have clear roles in regulating downstream splicing. For example, RBFOX1 (by WNK3) and RBFOX2 (by PRKCA/B) are shown to be shuttled out of the nucleus and degraded, respectively, by phosphorylation; thus, excluding these proteins from regulating their target exons [79, 80]. TRA2B has a reduced affinity for the mRNA that encodes the TRA2B protein when it is phosphorylated by CLK2 [81]. CELF1 phosphorylation downstream of Akt signaling causes changes in subcellular CELF1 distributions, affecting splicing and translational control (reviewed in detail in [82]). Hyperphosphorylation of CELF1 by PKCA/B/C was shown to be downstream of accumulation of toxic DM1 repetitive RNA in myotonic dystrophy and important for proper splicing regulation [83].

2.4 mRNA 5' G-Capping and Decapping

RNA 5' 7-methyl guanosine capping by RNA guanylyltransferase, which protects RNAs from 5' exonucleases, promotes translation and nuclear export, is tied to the phosphorylation state of RNA polymerase II CTD and this function is evolutionarily conserved to yeast [33, 35]. Decapping conversely is the first step of RNA decay and inhibits translation initiation. Decapping enzymes 1 and 2 in mammals are subject to rapid decay by ubiquitination and subsequent proteasomal degradation; thus, leading to longer RNA half-lives in general, in this case shown for a selection of targets that are subject to AU-rich element-mediated decay [84].

2.5 RNA Editing

ADAR protein levels, and consequently the extent of adenosine-to-inosine editing, are linked to the PTM state of these proteins. SUMO modification of ADAR1 at a lysine residue causes reduced editing efficacy *in vitro* and *in vivo* [85]. ADAR2 levels are decreased when phosphorylated by c-Jun kinase, resulting in reduced ADAR2-mediated A-to-I editing in pancreas [86].

3 PTM Regulation of Subcellular Localization

RBP PTMs commonly affect the ability for RBPs to move among cellular compartments. Thus, by virtue of their binding to RNA, RBPs regulate RNA localization based on their PTM state. In general, phosphorylation that affects RBP location also affects the set of bound RNAs, as may be expected since the availability of particular RNA species is not uniform across cells.

3.1 Nuclear/Cytoplasmic Shuttling

A few HNRNP proteins are sorted into the nucleus based on their PTM state. Nichols and colleagues showed with tritiated S-Adenosyl methionine then immunostaining after PRMT1 knockdown and GST-PRMT1 pull-down that HNRNPA2 arginine methylation in the RGG domain by PRMT1 is responsible for nuclear localization which is required for its regulation of alternative splicing [87]. HNRNPA1 localization is also regulated by PTM, with phosphorylation causing nuclear exclusion in a process that is activated by cellular stressors [88]. HNRNPQ has roles in splicing and mRNA stability and its localization is controlled by PRMT1-mediated methylation [89]; this may be important for controlling stability of RNA targets.

ELAVL1 is modified by an ubiquitin-like protein called NEDD8 on K313, K326 by MDM2 [90]. NEDD8 has 60 % homology with ubiquitin and its classical substrates are the cullin subunits of SCF ubiquitin E3 ligases [91]. Recently, it has been shown that MDM2 can associate with Ubc12 (the NEDD8 conjugating enzyme) and act as a NEDD8 ligase for p53 [92]. In the case of ELAVL1, neddylation promotes nuclear localization and inhibition of degradation [90].

Several kinases have been shown to be able to phosphorylate ELAVL1 and modulate its subcellular localization. For instance in the RRM domains: T118 phosphorylation by Chk2 or p38-MAPK [93, 94]; S158 phosphorylation by PRKCA and S318 phosphorylation by PRKCD [95, 96]. The hinge region (residues 186–244) is a hotspot for phosphorylation. Modifications on the hinge region affect ELAVL1 nucleocytoplasmic localization. Phosphorylation at S202 by CDK1 or CDK5, phosphorylation at S221 by PKC family members (PRKCA, PRKCD) and S242 phosphorylation by an unknown kinase all promote nuclear retention of the protein [31].

3.2 RNA Granules, P-Bodies and Nuclear Speckles

RNA-granules, processing bodies and nuclear speckles are functionally different aggregations of proteins and RNA that have modified activity and membership due to regulated changes in PTM state.

ELAVL1 phosphorylation on the hinge region outside of the HNS on Y200 by JAK3 inhibits ELAV1's localization to stress granules upon arsenite stress, leading to accelerated degradation of some of its mRNA targets (e.g. SIRT1 and VHL), but it is unclear whether mRNAs are bound to ELAVL1 during the transition to stress granules [97].

TARDBP (aka TDP-43) is acetylated at K145, K192 by CREBBP (Creb-binding protein). Based on crystal structure mapping of acetylated side-chains, the conformation of TARDBP RRM may shift and alter its ability to bind to RNA. Using glutamine to mimic acetylated lysine and forced acetylation by CREBBP, Cohen and colleagues show that acetylated lysine on TARDBP reduces RNA-binding and results in aggregation of TARDBP into cytoplasmic inclusions. When not bound to RNAs, TARDBP exits the nucleus, joins RNA granules and is phosphorylated at S410 [98], perhaps by CSK1 (casein kinase 1) [99] or TTBK1/2 (Tau tubulin kinases 1 & 2) [100]. This may represent coordinated handoff between post-translational modifiers to place TARDBP in granules. While it is possible to prevent neurodegeneration by blocking TARDBP phosphorylation at S409/S410 [101] or by preventing acetylation, it is not clear how these mechanisms interact to cause RNA granules. Further, there are other modifications that will certainly need to be considered, including ubiquitination, which likely follows aggregation and precedes proteasomal degradation [102]. These processes are of biomedical importance because phosphorylated TARDBP is a hallmark of cytoplasmic inclusions in Amyotrophic Lateral Sclerosis (ALS) [103] and mutations that are predicted to increase TARDBP phosphorylation are linked to ALS [104].

3.3 *Exosome*

Exosome loading of RNAs including microRNAs (miRNAs) and longer classes of noncoding RNA are controlled with PTMs of a few proteins. RBM7 phosphorylation downstream of the MAPK-mediated stress response sorts ncRNA in the nucleus to exosomes [105]. Exosome targeting for certain unprocessed miRNAs is similarly HNRNPA2B1 sumoylation-dependent [106]. KHSRP is an RBP phosphorylated through ATM and PI3K/ATM kinases in response to DNA damage that guides RNAs to the exosome, where they are targeted for destruction [107, 108]. Indeed exosome destruction of several RNAs is coordinated through signal integration/kinase activation of several proteins including RBM7, KHSRP, TTP and others [109].

4 PTM Regulation of Translation

Some cytoplasmic RBPs modulate protein output by either contributing to initiation, elongation or termination of translation of their mRNA substrates, thus having a large effect via downstream cellular processes coded by targeted mRNAs. Below,

we describe a few translation-regulatory RBPs, which are modified by phosphorylation, methylation, ubiquitination and oxidation.

Besides modulating subcellular localization and splicing, phosphorylation of the ELAVL1, as discussed above, PTM in ELAVL1 RRM domains can also modulate substrate affinity. For instance S38 and S100 phosphorylation by Chk2 can modulate mRNA substrate recognition. Interestingly, S100 phosphorylation seems to define the selectivity in ELAVL1 targeting. Thus, while phosphorylation induces release of Sirt1 mRNA and subsequent destabilization of Sirt1 mRNA, [93], the opposite is observed where S100 phosphorylation has been reported to increase affinity of ELAVL1 for Occludin mRNA (increasing its translation efficiency, [110]). This dual effect suggests the interesting possibility that phosphorylation may be used as a way for ELAVL1 to discriminate its different substrates and integrate dynamic signaling cues to translation decisions. It is unclear to what extent ELAVL1's promiscuity in target selection is subject to this S100 phosphorylation event or other PTMs.

At least methylation and phosphorylation modulate FMR1 protein (aka FMRP) function. Absence of FMR1 expression in neurons leads to developmental abnormalities, such as immature, thin and highly branched dendritic spines. FMR1 has two Aget domains followed by a NLS (nuclear localization signal) sequence close to its N-terminal, two KH domains (HNRNPK-homology domain) followed by a NES (nuclear export signal) signal in its middle and a RGG domain (arginine-glycine-glycine domain). The KH domain and RGG domains bind RNA. Phosphorylated FMR1 is associated with stalled polyribosome complexes. FMR1 forms a translation-inhibitory complex with the target mRNA and Cytoplasmic FMR1 Interacting Protein (CYFIP1). This complex binds translation protein EIF4E, thereby inhibiting its interaction with EIF4G. Phosphorylation is in fact necessary for FMR1 to carry out its roles in developmental timing [111]. FMR1 may also bind directly to the ribosome in polysomes to inhibit translation elongation [112]. Indeed phosphorylation status of FMR1 has also been proposed to regulate its association with translating ribosomes and stalled ribosomes. Arginine methylation of the FMR1 RGG domain by PRMT1 has been proposed to inhibit its ability to recognize target mRNAs and its assembly in translation initiation inhibitory complexes [113–115].

CPEB1 contains a PEST sequence, two conserved RRM domains and a c-terminal ZNF-domain [116]. This RBP modulates translation of target mRNAs which contain a Cytoplasmic polyadenylation element (CPE) in their 3'UTR [117]. CPEB1 binds to CPE mRNA substrates, keeping them in a translation inhibited state. When CPEB1 is phosphorylated on S174 (outside of the RRM domains) by Aurora A kinase [118–120], it recruits CPSF [119, 121] and induces polyadenylation of the mRNA, greatly inducing their expression. CPEB1 can also be phosphorylated sequentially by Cdc2 on T125 and multiple Serine residues, which recruits Plx1 that phosphorylate S191 on the PEST sequence. Once PEST is phosphorylated, the hyperphosphorylated CPEB1 is recognized by the SCFb-TrCP E3 ubiquitin ligase complex, and polyubiquitinated leading to its proteasomal degradation [122]. CPEB1 plays important roles in the development of *Xenopus* oocyte and synapse formation/long-term potentiation [123, 124].

5 PTM Regulation of RNA Stability and Destruction

5.1 *miRNA Related Repression*

miRNAs are produced through a two-stage double-stranded RNA cleavage and processing. The miRNA pathway can either affect mRNA stability or translational output through miRNA:mRNA base-pairing mediated in a RNA-protein complex called RNA-induced silencing complex (RISC). Canonical miRNAs originate from long primary mRNA transcripts, which are initially processed to miRNA precursors by the nuclear microprocessor complex in animals. The ribonuclease DROSHA and its RBP partner DGCR8 (DiGeorge syndrome critical region gene 8) are two key components of this complex (reviewed in [125]). PTM regulates this step in miRNA maturation, evidenced by co-immunoprecipitation of DROSHA and DGCR8 with components of the class I of Histone deacetylases (HDAC) [126]. In addition, the overexpression of HDAC1 in HEK293 cells results in increased affinity for primary miRNAs and higher mature miRNA availability without increasing the in corresponding primary miRNA's expression levels [126]. This was attributed to deacetylation of the DGCR8 RNA-binding domain and increased affinity to primary miRNAs [126]. Other studies have also proposed that Drosha and DGCR8 can be stabilized by phosphorylation, and it was shown that anti-MAPK/CDK substrate antibodies recognized immunopurified DGCR8 [127].

Microprocessor components are not the only proteins targeted by PTM for regulation of nuclear miRNA processing. Certain factors that regulate miRNA biogenesis are altered by PTMs. In early differentiation, LIN28 is expressed and binds to the let-7 primary transcript, but acetylation by PCAF and ubiquitination by TRIM71 causes destruction of LIN28 leading to de-repressed let-7 processing and allowing cells to progress through differentiation [128, 129]. The E3 ligase TRIM65 represses RISC assembly by targeting TNRC6 (aka GW182) for destruction [130].

After miRNA precursors are loaded into the RISC and exported from the nucleus, DICER1, an RNaseIII, is responsible for recognizing the hairpin precursor sequences and processes them to mature miRNAs. FMR1 has been shown to interact with DICER1, argonaute 2 (AGO2) and specific miRNAs. Phosphorylation holds FMR1 in association with its targets and prevents translation, perhaps by AGO2 interactions with targets. While at the same time, phosphorylation of FMRP inhibits association with DICER and reduces DICER activity [131, 132].

Argonaute proteins facilitate the interactions between the 22nt long microRNAs and target mRNAs. Several signaling pathways converge on these AGO proteins to control their activity in various cellular contexts [133, 134]. AGO protein is phosphorylated by p38-MAPK under cellular stress treatments like sodium arsenite, causing it to localize to processing bodies [135]. Certain AGO family members are preferentially subject to hydroxylation, stabilizing these proteins and potentiating the effect of miRNAs in hypoxia [134, 136].

One study suggests that phosphorylation at S499 (in the RGG domain) of FMR1 modulates translation of its target mRNA and via AGO2 [132]. Activation of mGluR

pathway leads to dephosphorylation of FMR1, followed by disassembly of its associated translation inhibitory complex and induction of PSD-95 translation/protein expression. It has been proposed that RPS6KB1 (aka S6K1) is the kinase that phosphorylates FMR1 and PP2A is the phosphatase that dephosphorylates it in the mGluR pathway [137, 138]. A separate study showed that HOXB8 mRNA is subject to the same phospho-FMR1/AGO2/miR-196a inhibitory complex [139]. This mechanism may be a way to induce gene expression of several FMR1 targets during long-term synaptic depression as depolarization leads to PP2A activation [138].

5.2 RNA Decay

QKI, a STAR-family RBP, is an essential regulator of myelination in oligodendrocytes. At early stages of development, QKI binds and stabilizes MBP mRNA. C-terminal phosphorylation by Src-Protein Tyrosine Kinases (PTK) at Y285, Y288, Y290, Y292 and Y303 decreases QKI's affinity for MBP (myelin basic protein) mRNA [140]. As src-PTK activity is reduced in early myelin development, mRNA can associate with QKI and accumulate. Indeed QKI sits downstream of several developmentally and disease-relevant pathways and understanding how PTMs affect its function will be an important goal for future studies ([140], reviewed in [141]).

ELAVL1 ubiquitination is related to the stability of its targets. ELAVL1 K48-linked ubiquitination on K182 by an unknown ubiquitin ligase promotes its proteasomal degradation [142]. However, K29-linked ubiquitination on ELAVL1 K313/K326 is reported to be a signal for protein-RNA complex disassembly. These modifications induce release of some ELAVL1 substrates (p21, MKP-1, and SIRT1 mRNAs) from ELAVL1, through recruitment of the p97-UBXD8 complex, leading to their destabilization [143]. Localization is also changed upon methylation of the hinge region by CARM1 (co-activator-associated arginine methyltransferase 1) at R217 [144]. Although the functional consequence of this modification is not completely understood, it has been shown to enhance ELAVL1's ability to regulate turnover of some of its substrate mRNAs (TNF-alpha, cyclin A, cyclin B1, c-fos, SIRT1, and p16) [145].

6 Conclusion

As the effects of PTMs are broad and unpredictable, careful follow-up on the dynamic changes in protein function are necessary. With regard to RBP function, the essential question is whether a particular PTM will affect many of the RBPs in ways we have listed above, including:

1. RNA-binding ability (i.e. QKI)
2. Protein complex formation (i.e. SRSF1/U2AF65)
3. Subcellular localization (i.e. CELF1, KDHRBS1)

- (a) Are RNAs bound during the transit?
 - (b) What mechanisms drive RBP motility?
4. Enzymatic activity of the RBP (i.e. ADAR)
 5. Initiation of RBP for destruction (i.e. LIN28)

Although the downstream consequences of these processes are varied and will be intensely studied, these are the basic features of RBP functions affected by PTMs. Answers to these simple questions for the library of RBP PTMs will be critical for accurate modeling of the effect of context and signal integration into the mRNP code. No doubt, advances in methods for probing protein structure will glean insight into the potential roles for PTMs on RBP function and provide a guide to prioritize the search for PTMs that have an impact on RNA maturation.

It should be noted again that this brief chapter is in no way a complete summary of the catalog of PTMs on RBPs. In the interest of space we had to restrict our discussions to the most well characterized examples and have left out many examples that may be relevant for basic biology or disease. These include rare post-translational modifications like nitration, which has evidence for affecting HNRNPA2B1 proteins [146], prolyl isomerization of POLR2 [147], myristoylation which affects the axonal distribution of FXR2 [148] and PARylation which can globally repress the miRNA pathway in stress [149]. Several reviews cited herein have approached the relevance of PTMs in a particular pathway, family of genes, biological process or disease. Interested readers should follow this text with a thorough examination of these and the associated primary literature. There is a great deal still unknown about the cumulative and cross-regulatory effects that each PTM on each RBP holds. If the history of DNA-binding proteins and histone modifications is any indicator, this will be an area ripe for discovery and will advance basic biology and drug development.

Acknowledgments We would like to thank members of the Massirer and Bengston labs for their critical reading of this manuscript. We also would like to acknowledge the many works not included herein that have contributed to the understanding of the role of RNA-binding protein post-translational modifications.

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