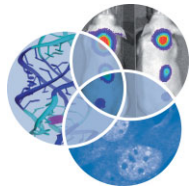
Advanced Review



Functional interactions between mRNA turnover and surveillance and the ubiquitin proteasome system

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The proteasome is a critical regulator of protein levels within the cell and is essential for maintaining homeostasis. A functional proteasome is required for effective mRNA surveillance and turnover. During transcription, the proteasome localizes to sites of DNA breaks, degrading RNA polymerase II and terminating transcription. For fully transcribed and processed messages, cytoplasmic surveillance is initiated with the pioneer round of translation. The proteasome is recruited to messages bearing premature termination codons, which trigger nonsense-mediated decay (NMD), as well as messages lacking a termination codon, which trigger nonstop decay, to degrade the aberrant protein produced from these messages. A number of proteins involved in mRNA translation are regulated in part by proteasome-mediated decay, including the initiation factors eIF4G, eIF4E, and eIF3a, and the poly(A)-binding protein (PABP) interacting protein, Paip2. eIF4E-BP (4E-BP) is differentially regulated by the proteasome: truncated to generate a protein with higher eIF4B binding or completely degraded, depending on its phosphorylation status. Finally, a functional proteasome is required for AU-rich-element (ARE)-mediated decay but the specific role the proteasome plays is unclear. There is data indicating the proteasome can bind to AREs, act as an endonuclease, and degrade ARE-binding proteins. How these events interact with the 5′-to-3′ and 3′-to-5′ decay pathways is unclear at this time; however, data is provided indicating that proteasomes colocalize with Xrn1 and the exosome RNases Rrp44 and Rrp6 in untreated HeLa cells. *WIREs RNA* 2010 1 240–252

The ubiquitin proteasome system (UPS) is involved in the regulation of almost all basic cellular processes—metabolism, development, cell cycle regulation, signal transduction, immune responses, and apoptosis.[1,2] This review focuses on the role of the UPS on mRNA metabolism, with a specific emphasis on mRNA turnover and surveillance.

UBIQUITIN PROTEASOME SYSTEM

Ubiquitin

Ubiquitin (Ub) is a highly conserved, 76 amino acid protein (Figure 1(a)) that was discovered as a macromolecular tag which could be covalently attached to proteins. Ub contains a characteristic geometry, called the Ub-fold, which consists of beta-strands that flank an alpha helical core (Figure 1(b)). By varying the extent of protein ubiquitylation, the type of poly-Ub chains, or the sites of ubiquitylation on the substrate, Ub can act as either a reversible modifier of protein function[3] or an irreversible mechanism for limiting protein levels by targeting the tagged protein for degradation by the proteasome[2] (Figure 1(c)). In the first step of ubiquitylation, an E1 ubiquitin-activating enzyme catalyzes the ATP-dependent covalent linkage of the COOH-terminus of ubiquitin to an active-site cysteine in the E1 through a thioester bond (Figure 1(d)). In the second step, ubiquitin is transferred from the E1 to an active-site cysteine on 1 of more than 20 known E2 ubiquitin-conjugating enzymes. In the final step,

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DOI: 10.1002/wrna.11

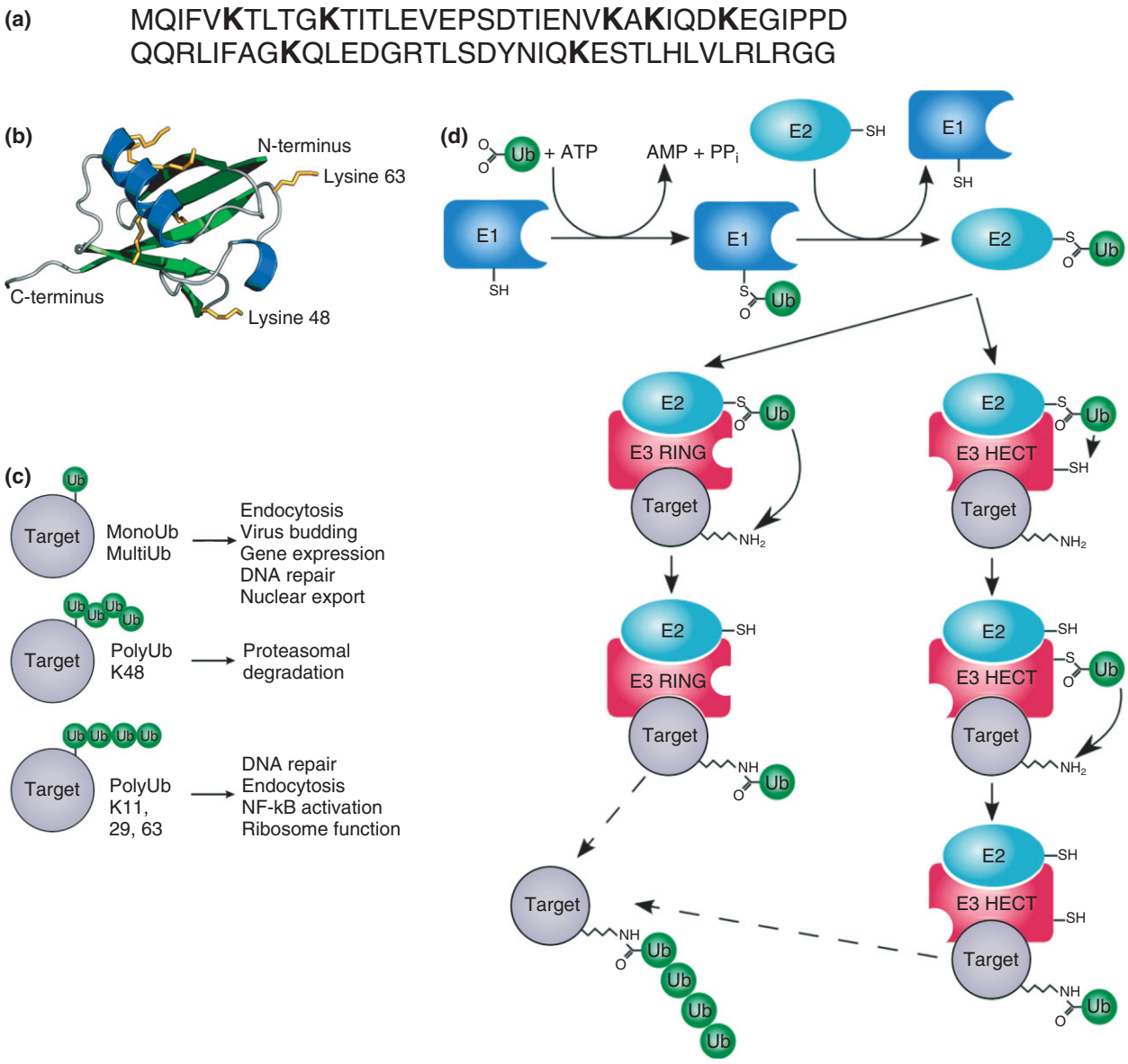

FIGURE 1 | Protein ubiquitylation pathway. (a) Primary amino acid sequence of ubiquitin. (b) Ribbon model of ubiquitin. The six lysines are shown in yellow. (c) Target proteins can be modified with single ubiquitins, multiple ubiquitins, and ubiquitin chains. Only ubiquitin chains linked through K48 target a protein for proteasomal degradation. (d) Protein ubiquitylation is a multistep process resulting in the covalent attachment of ubiquitin to a protein. Ubiquitin is attached to an E1 in an ATP-dependent process and then the ubiquitin is transferred to an E2. E3s serve as specificity determinants with each E3 interacting with one or several protein ligands. For really interesting new gene and Ub-fold E3s, ubiquitin is transferred directly from the E2 to the target protein; for homologous to E6-associated protein C-terminus E3s, the ubiquitin is first transferred to the E3 and then the target protein.

which is accomplished by the action of an E3 ubiquitin ligase,[4] ubiquitin is covalently linked by way of an isopeptide bond to a lysine in the target protein or to another ubiquitin molecule that has already been linked to the target protein. The E3 ubiquitin ligases recognize, bind, and recruit target proteins for ubiquitylation. As such, E3s form a large, diverse group of proteins, with one recent study identifying approximately 600 candidates in humans.[5] E3 ubiquitin ligases are characterized by one of several defining motifs including: HECT (homologous to E6-associated protein C-terminus), RING (really interesting new gene), or U-box (a modified RING motif without the full complement of Zn^{2+}-binding ligands) domains.[6] In the case of HECT E3 ligases, Ub is attached to the E3 and then the substrate. For

RING and U-box E3s, Ub is transferred directly from the E2 to the substrate (Figure 1(d)). Ubiquitylation is reversed by de-ubiquitylating enzymes (DUBs) that remove ubiquitin from proteins and disassemble multiubiquitin chains. The activity of DUBs provides an additional level of regulatory control, similar to the action of phosphatases, and maintain a sufficient pool of free ubiquitin molecules in the cell by removing the Ub tag from degraded proteins.[7]

Proteasome

The 26S proteasome is composed of a core catalytic complex, the 20S proteasome, capped at each end by a 19S regulatory particle[8] (Figure 2(a)). The 20S core is a large, cylindrical-shaped protease formed by 28 subunits arranged in four heptameric stacked rings, with peptidase activity localized to the inner β rings. Apart from peptidase, the 20S core is also reported to have RNase activities.[9,10] The 19S proteasome regulatory particle is subdivided into two distinct subcomplexes, the lid and base.[11] The lid resembles the COP9 signalosome.[12] The base contains six AAA-type ATPases, which function as a molecular motor unfolding and translocating the protein substrate,[11] and is important for regulating the activity of 26S proteasome complex.[13] A number of proteins contained in the 19S base have additional functions independent of the proteasome complex.

Once modified by a polyubiquitin chain of at least four K48-linked ubiquitins (Ub), a protein is able to bind either directly to intrinsic Ub receptors in the 19S complex or to adaptor proteins that link it to the 19S complex.[14] Binding of the protein to the proteasome is followed by ATPase-mediated protein unfolding, removal of the polyubiquitin chain by proteasome-associated DUBs, and translocation of the unfolded protein into the central proteolytic chamber of the 20S subunit, where it is cleaved into short peptides (Figure 2(b)).[14]

NUCLEAR mRNA TURNOVER AND SURVEILLANCE

mRNA transcription, processing, and export comprise an intimately linked series of events that are coupled and coordinated by the polymerase II (Pol-II) C-terminal domain (CTD).[15–17] Nascent pre-mRNAs are packaged into messenger ribonucleoproteins (mRNPs) concomitant with transcriptional initiation. Processing and maturation events include 5′ end capping, 3′ end cleavage and polyadenylation, and intron splicing of the pre-mRNAs. These events package the message into an export competent mRNP that is able to transit through the nuclear pore complex (NPC) into the cytoplasm.

Transcription

In eukaryotes, RNA Pol-II drives production of mRNAs, initiating gene expression (Figure 3). Transcriptional control depends on the recruitment and activity of Pol-II, which must initiate transcription at the 5′ end of a gene and successfully transcribe the complete message. mRNA surveillance begins with the initiation of transcription. In response to Pol-II stalling at a DNA lesion, Pol-II is ubiquitylated and degraded by the proteasome, thus preventing the generation of truncated messages because of DNA breaks.[18]

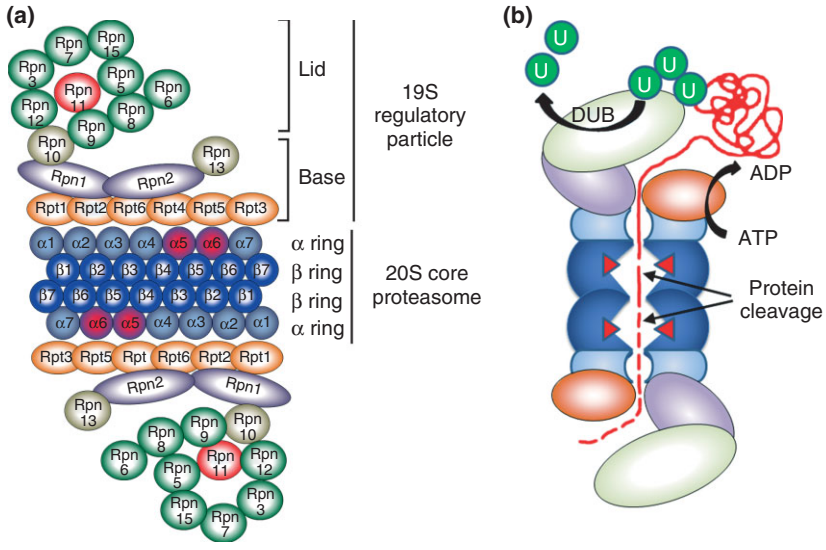

FIGURE 2 | The 26S proteasome. (a) Schematic of the protein members of the 26S proteasome. The α-ring proteins highlighted in red have RNase activity. (b) Schematic cross section of the 26S proteasome with specific functions indicated.

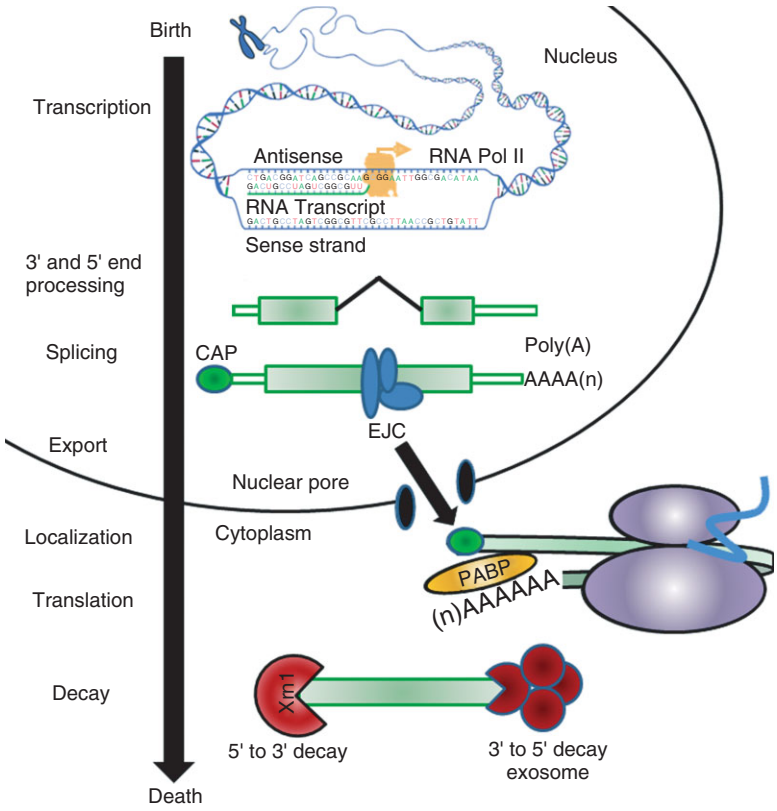

FIGURE 3 | Life cycle of mRNA. Birth of an mRNA begins with RNA-polymerase II-mediated transcription from a chromosomal gene sequence. Packaging of the message into an mRNP begins almost immediately with the initiation of transcription, with addition of the m7GpppG cap. Intron splicing from the pre-mRNA can also begin before transcription is complete and results in the deposition of the exon-junction complex (EJC). Upon transcriptional termination, the 3′ end is processed resulting in the addition of the poly(A) tail. Nuclear export of the mature message is a regulated process which in metazoans involves the EJC. Once in the cytoplasm, the message undergoes a pioneer round of translation which removes many of the proteins bound to the mRNA in the nucleus and these proteins shuttle back into the nucleus. In mammalian cells, the message is subject to several cytoplasmic surveillance mechanisms (Figure 5) during the pioneer round of translation. If the surveillance decay mechanisms are not activated, then the message is either translated into protein, stored for later translation, or degraded. Message degradation utilizes both the 5′-to-3′ Xrn1 and 3′-to-5′ exosome-mediated decay pathways.

mRNA Splicing

pre-mRNA splicing is a fundamental mechanism in metazoans, with up to 60% of pre-mRNAs undergoing alternative splicing in humans.[19] Alternative splicing allows for the generation of multiple protein products from the same gene by altering the inclusion of different exons in the mature mRNAs. Splicing events catalyze the deposition of the exon-junction complex (EJC) upstream of exon–exon unions (for review, see Ref 16). EJCs serve to mark the RNA for downstream events such as nuclear export, translation, and NMD (discussed below). Both constitutive and regulated splicing of precursor mRNAs involves members of the serine/arginine-rich (SR) protein family, and several SR proteins are regulated in part by proteasome-mediated turnover. These include SRp55,[20] SRp20,[21] human T-STAR,[22] and *Drosophila* Tra2.[23]

mRNA Export

RNA export is a regulated process that is linked with transcription via the transcription/export complex (TREX). The TREX complex is made up of the THO complex (Tho2, Hpr1, Mft1, and Thp2) as well as export factors UAP56 (Sub2 in yeast) and Aly (Yra1 in yeast) (for review see Ref 17). Hpr1 is a target of the E3 ubiquitin ligase Rsp5 and the turnover of Hpr1p is linked with active transcription.[24] Mex67 is a heterodimeric export receptor that promotes mRNP transport through the NPC by direct interactions with FG-nucleoporins.[25] Mex67 is recruited to actively transcribing genes where its UAB domain interacts with ubiquitylated Hpr1, preventing its degradation by the proteasome.[25] Thus, ubiquitin-mediated degradation does not appear to be the reason for Hpr1 ubiquitylation but rather a consequence of assembly of an export competent mRNP.

Transcripts produced in THO/sub2 mutants are subject to degradation as a result of inefficient 3′ end processing and polyadenylation.[26] The loss of polyadenylation is as a result of proteasome-mediated degradation of FIP1, a cofactor required for poly(A)-polymerase (PAP). This results in RNA decay by the nuclear exosome, utilizing the exosome RNase Rrp6, and the Trf4/Air2/Mtr4p Polyadenylation (TRAMP) complex, a multi-protein complex consisting of an RNA helicase (Mtr4), a poly(A) polymerase (PAP) (either Trf4 or Trf5) and a zinc knuckle protein (either Air1 or Air2).[26] It is important to note that both mRNA splicing and export are altered by the physiologic state of the cell, and changes in the protein components of an mRNP can alter downstream events in the cytoplasm.[27]

CYTOPLASMIC mRNA TURNOVER AND SURVEILLANCE

Once a message has been exported to the cytoplasm, the nuclear cap-binding protein CBP20–CBP80 complex initiates a pioneering round of translation that strips off EJCs and results in the replacement of CBP20–CBP80 complex by the cytoplasmic cap-binding protein eukaryotic translation initiation factor 4E (eIF4E).[28] Surveillance of the message takes place during the pioneer round of translation, which can result in rapid degradation of aberrant mRNAs; these events are discussed in detail below. The remodeled mRNP then either recruits the translation initiation complex through eIF4E, resulting in protein production, or recruits the CCR4 or PARN deadenylation complex which eventually leads to mRNA decay.[29]

Translation

Translation in eukaryotes is regulated primarily at the level of initiation, and the efficiency of translation initiation determines the rate of protein synthesis (for review, see Ref 30). Both the 5′ cap (m^7GpppN) and 3′ poly(A) tail of the mRNA play an essential role in translation initiation. The cap structure facilitates mRNA binding to the ribosome through its interaction with the eukaryotic initiation factor 4F (eIF4F). In mammals, eIF4F is a protein complex composed of three subunits: eIF4E, the cap-binding protein; eIF4A, an RNA helicase; and eIF4G which binds eIF4E and eIF4A and recruits the 40S ribosomal subunit by interacting with eIF3. The poly(A) tail of the mRNA interacts with PABP. PABP is comprised of four N-terminal RNA recognition motifs (RRM) and a proline-rich C-terminal portion. PABP and eIF4G physically interact, resulting in mRNA circularization and stimulating translation initiation. The interaction between eIF4G and eIF3 promotes 40S ribosome entry to a site near the 5′ end of the message. Subsequently, the small ribosomal subunit scans the mRNA in a 5′ to 3′ direction until an appropriate start codon has been reached. Once the ribosomal preinitiation complex is formed, the large ribosomal subunit joins and translation begins.

Initiation Factors

Proteasome-mediated degradation has been shown for three eukaryotic initiation factors: eIF4G, eIF4E, and eIF3a (Figure 4). The 20S proteasome cleaves the eIF4G subunit of eIF4F and the eIF3a subunit of eIF3, generating products with distinct functional capabilities.[31] The cleavage of either eIF4G or eIF3a selectively affects the assembly of the 48S ribosomal preinitiation complexes on different cellular and viral mRNAs *in vitro*. Inhibition of the proteasome prevents degradation of these factors, restoring assembly of the ribosomal complexes *in vitro*. Importantly, proteasome inhibition selectively alters the translation of different mRNAs *in vivo*. Given that the abundance, composition, and localization of proteasomes varies with cell cycle progression, differentiation, and environmental cues, the proportion of the cleaved to intact factors may be significantly modulated by proteasomes, thus altering the synthesis of specific proteins.

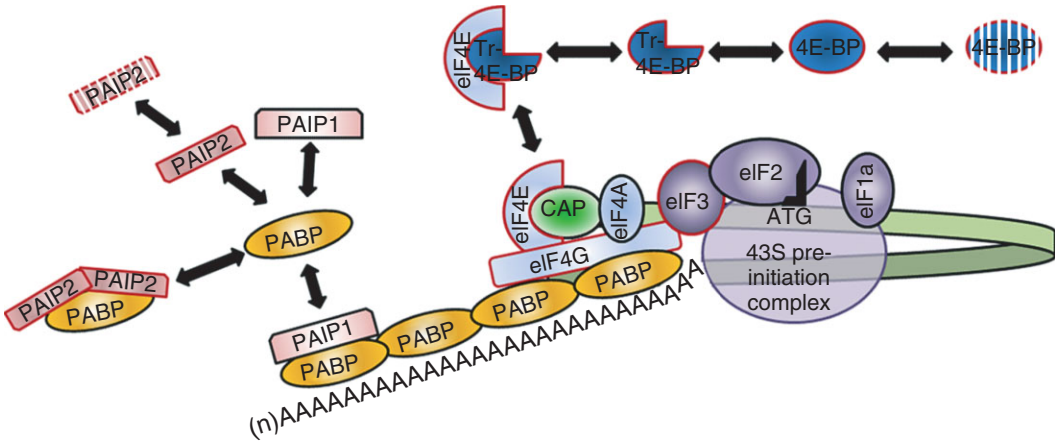

FIGURE 4 | Proteasome control of translation. The proteins outlined in red are subject to proteasome-mediated degradation or truncation. The elongation initiation factors 4E, 4G and 3 are all subject to proteasome-mediated degradation. The eIF4E-binding protein (4E-BP) is subject to two different proteasome-mediated events. In its hypophosphorylated state, 4E-BP is cleaved (Tr-4E-BP) to generate a truncated version of the protein which binds eIF4E in preference to full-length 4E-BP. In its hyperphosphorylated state, 4E-BP is completely degraded by the proteasome. PAIP2 levels are regulated by proteasome-mediated degradation. PAIP2 competes with PAIP1 for binding to poly(A)-binding protein (PABP). PAIP2 binding to PABP destabilizes the interaction of PABP with the polyadenylated tail of the message, decreasing translation efficiency and enhancing message decay.

eIF4E is ubiquitylated primarily at Lys-159 and proteasome inhibition results in an increase in eIF4E levels.[32] Ubiquitylated eIF4E retains its cap-binding ability, but the phosphorylation of eIF4E and its binding to eIF4G are reduced by ubiquitylation. Overexpression of the translational inhibitory protein 4E-BP protects eIF4E from ubiquitylation and degradation.[33] Heat shock or the exogenous expression of the carboxyl terminus of heat shock cognate protein 70-interacting protein (CHIP) increases eIF4E ubiquitylation, implicating CHIP as at least one E3 ligase responsible for eIF4E ubiquitylation.[32]

4E-BP

The proteasome is also involved in the regulation of 4E-BP. 4E-BP associates with eIF4E in competition with the initiation factor eIF4G, sequestering eIF4E in a complex that cannot participate in cap-dependent mRNA translation.[33] Interestingly, in response to p53 activation, 4E-BP1 can be partially degraded by the proteasome, generating a truncated 4E-BP1.[34] Unlike full-length 4E-BP1, which is reversibly phosphorylated at multiple sites, the truncated protein is almost completely unphosphorylated. The truncated 4E-BP is much more stable than the full-length protein and interacts with eIF4E in preference to full-length 4E-BP1. Proteasome-mediated cleavage is inhibited by the protein phosphatase inhibitor calyculin A, which prevents dephosphorylation of full-length 4E-BP1. In contrast, hyperphosphorylation of 4E-BP1 results in the complete destruction of the protein by the proteasome.[35] These data indicate that the phosphorylation state of 4E-BP1 determines two alternative fates for 4E-BP1: cleavage of the hypophosphorylated form to generate truncated 4E-BP1 or complete degradation of the hyperphosphorylated forms.

Poly(A)-binding protein

Proteasome control of translation is not exclusively localized to the 5′ cap but also includes the poly(A) tail. As noted above, the cap and poly(A) tail act synergistically to enhance translation via the interaction between eIF4G and PABP. PABP activity is modulated by two PABP-Interacting Proteins (Paips), Paip1 and Paip2.[36,37] Paip1 contains two PABP-binding sites and interacts with PABP with a 1:1 stoichiometry.[36,37] Paip1 also interacts with eIF4A, and this interaction has been shown to stimulate translation of a luciferase reporter.[38] Paip2 contains two independent PABP-binding sites[36,37] but forms a heterotrimeric complex containing one PABP molecule and two Paip2 molecules.[36] Paip2 competes with Paip1 for PABP binding and Paip2 inhibits translation both *in vitro* and *in vivo* by displacing PABP from the poly(A) tail,[37,39] abrogating mRNA circularization. Paip2 is ubiquitylated and degraded by the proteasome, providing a mechanism to control not only the level of Paip2 but also the available PABP, and consequently altering translation.[40]

Cytoplasmic mRNA Degradation

The degradation of conventional mRNAs is initiated with the onset of their translation. The current hypothesis posits that with each round of translation, the poly(A) tail is shortened, until a critical minimum level is reached. These oligo-adenylate tails cannot effectively bind PABP, and the loss of PABP disrupts the closed-loop structure of the translating mRNP.[30] At this point, two separate mechanisms can degrade the message: the 5′-to-3′ Xrn1-mediated pathway or the 3′-to-5′ exosome-mediated pathway. In the 5′-to-3′ decay pathway, the mRNP rearrangement that is brought about by poly(A) tail shortening facilitates the recruitment of the decapping enzymes Dcp1 and Dcp2 to the cap structure, resulting in message decapping.[41,42] Once decapped, the transcript is degraded by the Xrn1 5′-to-3′ exonuclease. 5′-to-3′ decay components, including Dcp1, Dcp2, and Xrn1, are concentrated in cytoplasmic granules called processing bodies (P-bodies),[43] however, P-body formation is not essential for 5′-to-3′ decay.[44] In the 3′-to-5′ decay pathway, transcripts are further deadenylated and then degraded by the exosome 3′-to-5′ exonuclease complex.[45,46] In eukaryotes, the RNase activity of the exosome is localized to two subunits, Rrp44 (Dis3) and Rrp6 (Pm/Scl-100), which are not part of the core structural exosome. Exosomes are also present in cytoplasmic granules, which do not colocalize with Dcp1 or TIA-1 containing P-bodies.[47] However, an assessment of RNase colocalization has not been performed.

ARE-Mediated Decay

The presence of an AU-rich-element (ARE), repeats of the motif AUUUA, in the 3′ UTR of a message targets the message for rapid decay via a process termed ARE-mediated decay (AMD).[29,48] Both 5′-to-3′ Xrn1-mediated decay and 3′-to-5′ exosome-mediated decay are required for efficient AMD,[49] and components of these pathways are specifically recruited to ARE-containing messages by the binding of *trans*-acting factors to the ARE[50] (Figure 5(a)). Several of these *trans*-acting RNA-binding proteins, including AUF1, TTP, HuR, and hnRNP A2, have been identified as targets for proteasome-mediated degradation.

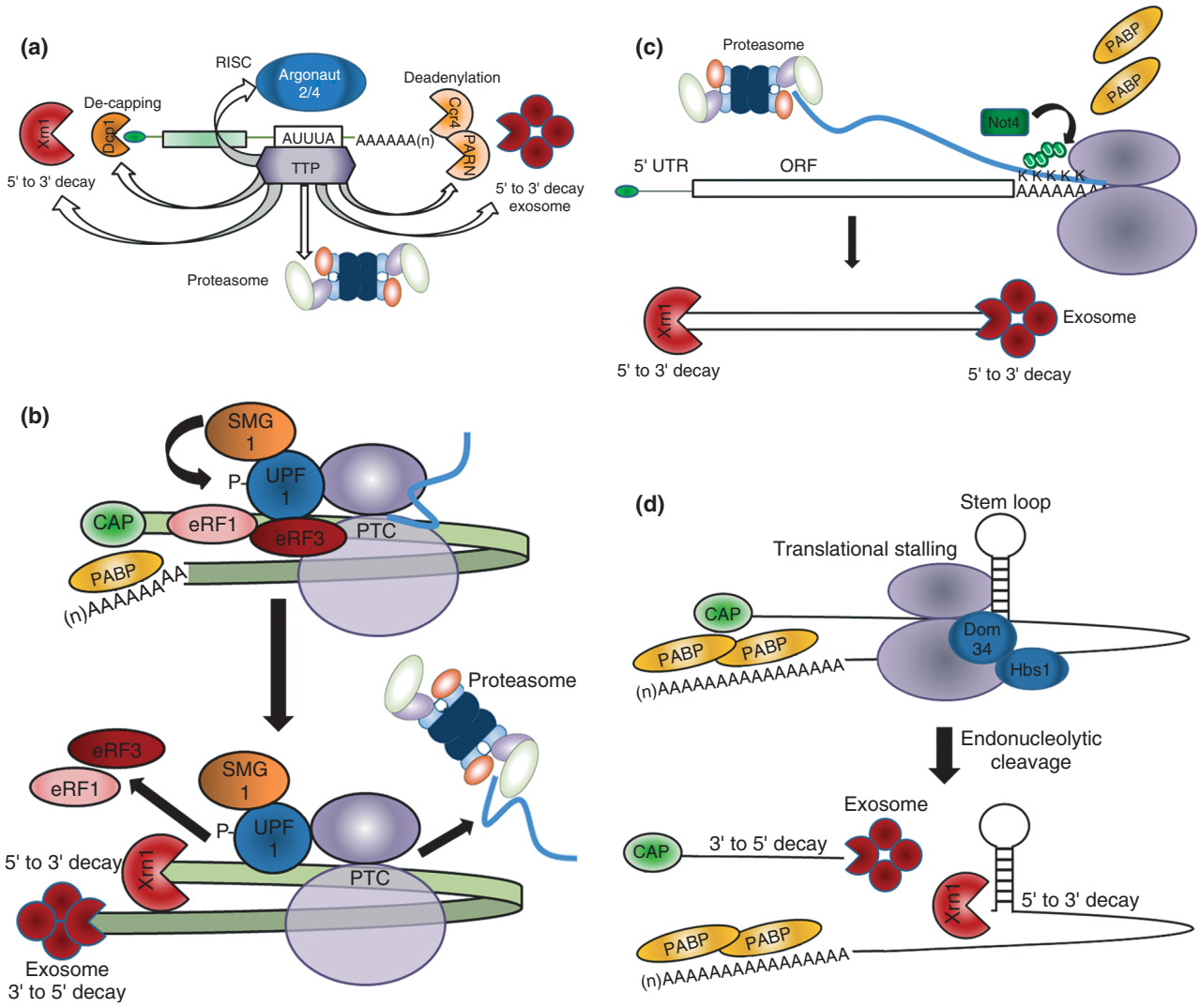

FIGURE 5 | mRNA surveillance and decay pathways. (a) AU-rich-element (ARE)-mediated decay. TTP is the best-studied decay mediating ARE-binding protein.[50] TTP has been shown to interact with components of both the PARN and CCR4 deadenylation complexes, the decapping enzyme Dcp1 and components of the 5′-to-3′ Xrn1-mediated decay pathway, the 3′-to-5′ exosome decay pathway, and the RNA induced silencing complex members Argonaut 2 and 4. TTP protein is rapidly turned over by the proteasome and, in a poorly understood mechanism, TTP turnover is linked with mRNA decay. (b) Nonsense-mediated decay (NMD). The presence of a premature termination codon in a message triggers NMD. A poor interaction between poly(A)-binding protein and Upf1 when the ribosome reaches the stop codon results in the phosphorylation of Upf1 by SMG-1 and the release of eRF1 and eRF3. Upf1 recruits the proteasome to destroy the truncated protein product as well as both the exosome and Xrn1 decay complexes to degrade the premature termination codon containing message. (c) Nonstop decay (NSD). The absence of a stop codon triggers NSD. The presence of a poly-lysine chain at the carboxyl end of the protein, resulting from translation of the poly(A) tail, results in translational arrest followed by Not4-mediated ubiquitylation of the protein. The protein is then cotranslationally degraded by the proteasome and the message subsequently degraded by both the 3′-to-5′ and 5′-to-3′ decay pathways. (d) No-Go decay. Ribosomal stalling can result in the endonucleolytic cleavage of the mRNA, release of the ribosome, and decay of the message by the exosome and Xrn1.

AUF1 is the best characterized ARE-binding protein with regard to the proteasome. Several different methodologies that alter proteasome function demonstrate the importance of proteasome-mediated AUF1 turnover in AMD. Use of a temperature sensitive E1 ubiquitin-activating enzyme, where E1 is active at the permissive temperature (30°C) but inhibited at the restrictive temperature (39.5°C), revealed that the amount of β-gal reporter mRNA containing the Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) ARE was 10–20 times higher at the restrictive temperature compared with the permissive temperature. In contrast, levels of β-gal GC-control mRNA doubled at the restrictive temperature

compared with the permissive temperature. E1 inhibition also resulted in a significant increase in the level of the AUF1 p37 isoform, but only a slight increase in p40/42 AUF1 isoforms.[51] Thus, even though there was more p37 AUF1, which generally promotes AMD, mRNA decay of the GM-CSF ARE containing reporter was inhibited. Proteasome inhibition with MG132 gave identical results demonstrating that the rapid turnover of an mRNA containing the GM-CSF ARE is associated with the ubiquitylation and degradation of at least one member of the AUF1 family.[51]

Further studies demonstrate that overexpression of the ubiquitin-specific processing protease UNP, which functions as a DUB and inhibits addition of ubiquitin to proteins, specifically blocked decay of the GM-CSF ARE-mRNA reporter. In contrast, increased expression of the DUB ubiquitin specific peptidase 8 (USP8 also known as UBPY), which increases protein ubiquitylation by recycling ubiquitin from degraded proteins, strongly and selectively accelerated ARE-mRNA decay.[52] The p37 and p40 AUF1 isoforms are ubiquitylated *in vitro*, whereas the p42 and p45 forms are not. Altering the expression of UNP and UBPY altered p37 AUF1 protein levels *in vivo*, with UNP overexpression increasing p37 AUF1 protein levels and UBPY overexpression decreasing p37 AUF1 protein levels. Neither treatment altered expression of the p45 isoform, and a differential effect on p40/p42 was difficult to resolve because of comigration of the bands.[53] Proteasome inhibition also inhibits AUF1-mediated destabilization of IL-6,[54] endothelin-1 (ET-1),[55] and DNMT1 mRNA,[56] all of which contain an ARE. In the last case, UPS-regulated changes in AUF1 protein levels during the cell cycle alter DNMT1 mRNA stability during the cell cycle. DNMT1 in turn regulates genomic DNA methylation, illustrating again the integrated involvement of the proteasome in numerous fundamental cellular processes.

Similar to AUF1, the mRNA destabilizing protein TTP is rapidly degraded by the proteasome,[57] and like AUF1, a functional proteasome is essential for TTP-mediated degradation of both reporter mRNA containing the Tumor Necrosis Factor-alpha (TNFα) 3′ UTR and endogenous TNFα.[57] Heat shock results in proteasome-mediated degradation of ARE-stabilizing protein HuR.[58] The von Hippel–Lindau (VHL) tumor suppressor pVHL functions as an E3 ubiquitin ligase regulating turnover of the Hypoxia Inducible Factor-1 (HIF-1) transcription factor. It also regulates turnover of hnRNP A2, which in turn regulates the stability and translation of the glucose transporter 1 mRNA.[59] Finally, protein levels of the polysomal ribonuclease 1 (PMR1) mRNA endonuclease are regulated in part by proteasome degradation, and proteasome degradation is inhibited by the interaction of Hsp90 with PMR1. PMR1 forms a c-Src-mediated complex with translating mRNAs, where it initiates decay by cleaving the mRNA body.[60]

Cytoplasmic mRNA Surveillance

There are at least three mRNA surveillance pathways in the cytoplasm: NMD, nonstop mediated decay, and No-Go mediated decay.

Nonsense-Mediated Decay

NMD is an mRNA surveillance mechanism that detects and degrades messages containing nonsense mutations (premature termination codons or PTCs) (Figure 5(b)). Failure to recognize and decay transcripts with PTCs results in the production of truncated proteins, and these truncated peptides potentially possess dominant negative or gain of function phenotypes. PTCs can arise as a result of germline or somatic mutations, errors in transcription, errors in posttranscriptional mRNA processing, and translational frameshifts. However, PTCs also have a specific regulatory role in some genes including mRNAs that contain upstream open reading frames (uORFs) and bicistronic mRNAs, mRNAs with abnormally long 3′-untranslated regions (3′ UTRs), as well as transcripts of pseudogenes, transposable elements, and genes that are subject to programmed rearrangements, such as immunoglobulins and T-cell receptors (for review, see Refs 61, 62).

The precise mechanism for identifying PTCs to trigger NMD is an area of active research. Although it is clear that the presence of EJCs downstream of the termination codon enhances NMD activation, EJCs alone cannot account for all NMD. The current model holds that NMD is regulated by antagonistic signals between Upf1 and PABP at translation termination (Figure 6(b)). If PABP is positioned to interact with the termination complex, then the association of Upf1 with the message is inhibited, repressing NMD. Conversely, if the interaction between PABP and the termination complex is not favorable, then Upf1 is phosphorylated and, together with SMG-1, remains in association with the termination complex, triggering NMD. The core elements in the NMD complex, referred to as the SMURF complex, appear to be SMG-1 and the translational release factors eRF1 and eRF3.[61–63]

Both the decapping-dependent 5′-to-3′ Xrn1 exoribonuclease pathway and the exosome-mediated 3′-to-5′ exoribonuclease pathway rapidly degrade mammalian mRNAs harboring PTCs[61,62]; *Drosophila melanogaster* employs a unique pathway involving

endonucleolytic cleavage near the site of the PTC.[64] Recent work has demonstrated that Upf1 stimulates degradation of not just the PTC-containing mRNA but also the proteasome-mediated destruction of the peptide derived from the aberrant message.[63] Thus Upf1 links destruction of both the PTC-containing message and the protein generated from its translation.

Nonstop Decay

Nonstop decay (NSD) is a surveillance mechanism that degrades messages lacking a termination codon (Figure 5(c)). Translation of these messages results in ribosome stalling at the 3′ end of the mRNA.[65,66] The stalled ribosome prevents further initiation and the poly-lysine chain resulting from translation of the poly(A) tail triggers ubiquitylation and cotranslational decay of the peptide by the proteasome.[67] Ubiquitylation is mediated by Not4, part of the Ccr4-Not deadenylation complex, although the other members of the complex do not appear to be required for NSD.[67] The message is then rapidly degraded via the recruitment of the exosome as well as Xrn1.

No-Go Decay

No-Go decay (NGD) is a surveillance mechanism that recognizes stalled ribosomes on a message and, in a process that involves Dom34 and Hbs1, cleaves the message near the stall site resulting in ribosome release and mRNA decay by both the exosome and Xrn1[68] (Figure 5(d)). At this time, there is no known involvement of the proteasome in NGD.

PROTEASOME RNase ACTIVITY

Over the past 20 years, a number of reports have been published demonstrating the presence of RNase activity in purified proteasomes (reviewed in Ref 10). RNase activity specifically localizes to the zeta and iota subunits of the alpha rings in the 20S proteasome core.[69] In the context of the full 26S proteasome, RNase activity is phosphorylation dependent.[70] Proteasome-mediated digestion of Tobacco Mosaic Virus (TMV) viral RNA results in a well defined pattern of RNA fragments, whereas 5S ribosomal RNA, globin mRNA, and lysyl-tRNA are not degraded.[71] In K562 cells, the specificity of the 26S proteasome's endoribonuclease activity is altered following doxorubicin treatment.[10,70] Doxorubicin did not alter proteasome-mediated degradation of the c-myc 3′ UTR, but treatment significantly inhibited degradation of the c-fos 3′ UTR compared with untreated cells; both of these 3′ UTRs contain AREs. These data indicate that the proteasome-mediated decay of mRNA is both differentially and specifically regulated.

Proteasomes copurify with RNA of heterogeneous size but most frequently in the range of 70–200 nucleotides.[72] Partial sequence analysis of these RNA fragments has not identified specific messages associated with the proteasomes, although ARE-containing fragments are enriched.[73] *In vitro* reconstitution experiments demonstrate that the 20S proteasome preferentially interacts with and degrades ARE-containing RNA oligos, including those derived from TNFα, IFNγ, and HIV-Tar.[74–76] In particular, the 45 nucleotide HIV-Tar hairpin is degraded to two bands of 6 and 39 nucleotides. Excess ARE-containing RNA oligonucleotides compete with Tar RNA for proteasome degradation, and Tar RNA is unable to interact with the proteasome if the first and last six nucleotides of the hairpin are not present.[76] Importantly, the binding site does not appear to be the poly(A) tail as the proteasome-associated RNA fragments reported so far do not have oligo(A) stretches, and proteasomes associate strongly with TMV-RNA without a poly(A) tail as well as with adenovirus mRNAs with an oligo d(T) blocked poly(A) tail.[77] These data support the findings of proteasome inhibition and mRNA stabilization seen with AUF1 and TTP.[51–53,57]

However, proteasome inhibition induces the assembly of stress granules (SGs).[78] SGs are dynamic aggregates of untranslating mRNAs in conjunction with a subset of translation initiation factors (eIF4E, eIF4G, eIF4A, eIF3, and eIF2), the 40S ribosomal subunit, and PABP (for review, see Ref 43). A number of RNA-binding proteins localize to SGs, including TIA-R, TIA-1, TTP, AUF1, and G3BP.[43] SGs form in response to problems with translation initiation; as stress responses often involve a transient inhibition of translation initiation, SGs accumulate during many stress responses.[43,78] Thus, an alternative explanation for the inhibition of AMD with proteasome inhibition is that ARE-containing messages are recruited to SGs where they are neither degraded nor translated. Supporting this hypothesis are the findings that proteasome inhibition causes disassembly of P-bodies,[78] which serve as sites of both AMD and NMD, and inhibits NMD. However, given the involvement of the proteasome in degradation of proteins generated by PTC-containing messages and the link with Upf1, it is also possible that inhibition of the proteasome disrupts an aspect of Upf1 function to prevent NMD. Contradicting the hypothesis that the induction of SGs by proteasome inhibition causes the changes in AMD is the data that AMD is inhibited by UNP overexpression and enhanced by UBPY overexpression. These findings demonstrate that a functional proteasome is required for AMD in the absence of pharmacologic proteasome

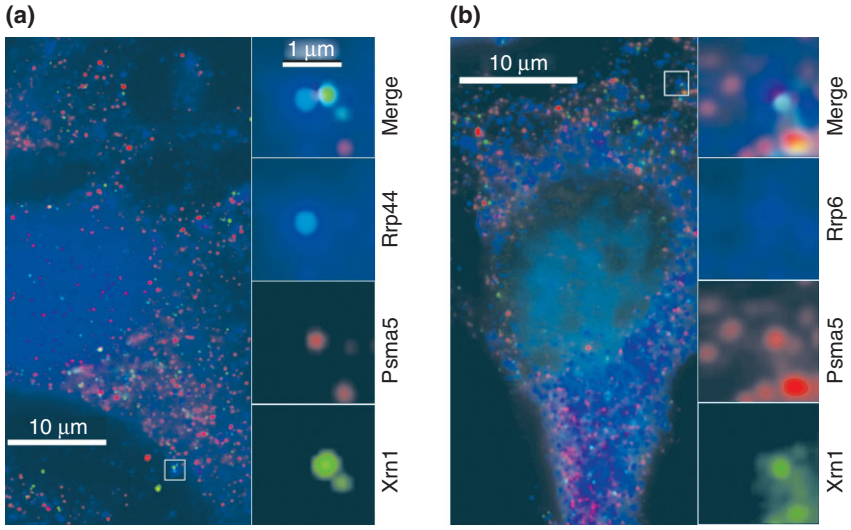

FIGURE 6 | RNase localization. HeLa cells were grown on cover slips, fixed, and stained according to the protocol in Kedersha and Anderson (2007) with the indicated antibodies. Nuclei were counter stained with Hoechst dye. For each panel, the white box in the left hand image represents the enlarged area on the right. (a) Xrn1, Psma5, and Rrp44 colocalize with each other in a subset of granules present in untreated HeLa cells. (b) Xrn1, Psma5, and Rrp6 colocalize with each other in a subset of granules present in untreated HeLa cells. An Xrn1 and Psma5 granule (yellow) is also present in the lower right of the enlarged area. Antibodies: Goat anti-Xrn1 (1:500) (Santa Cruz sc-50209); rabbit anti-Psma5 (1:500) (Abcam ab11437); mouse anti-Rrp44 (1:200) (Abcam ab68570); mouse anti-Rrp6 (1:200) (Abnova H00005394-M08). Donkey Fab fragment secondary antibodies from Jackson Research: Cy2-goat (1:200), Cy3-rabbit (1:2000), and Cy-5-mouse (1:200). Images were obtained using a Nikon Eclipse 80i with a 60× oil objective and analyzed using NIS-Elements BR 3.0 software.

inhibition.[52] Clarification of the exact role played by the proteasome in AMD will require further study, but proteasome involvement cannot be simply dismissed as an artifact of pharmacologic inhibition of the proteasome.

COLOCALIZATION OF RNases

As noted above, there are no published data examining the colocalization of the different RNases involved in mammalian mRNA decay: the 5′-to-3′ exoribonuclease Xrn1; the 3′-to-5′ exosome exoribonucleases Rrp44 (Dis3) and Rrp6 (PM/Scl-100); and the proteasome RNase subunits Psma5 (zeta) and Psma6 (iota). To address this, we performed immunocytochemistry examining the localization of Xrn1, Rrp44, and Psma5 (Figure 6(a)), as well as Xrn1, Rrp6, and Psma5 (Figure 6(b)). These data suggest that Xrn1, the exosome RNases, and the proteasome subunit Psma5 form discrete granules that are visible in untreated HeLa cells. Thus, a small proportion of the three decay components required for AMD, NMD, and NSD appear to colocalize in distinct foci in human cells. While colocalization occurs, it is also clear that a large number of the granules present do not colocalize with each other. Given that both 5′-to-3′and 3′-to-5′ RNA decay pathways, as well as the proteasome, are involved in NMD and NSD, these findings are consistent with the current understanding of these processes. What is less clear is the relationship between these proteins, their associated complexes, and ARE decay. Although both the 5′-to-3′ and 3′-to-5′ pathways are required for efficient ARE decay,[49] it has been reported that exosomes and P-bodies do not colocalize,[42] whereas P-body formation is not required for 5′-to-3′ decay.[44] It is possible that Xrn1 and the exosome RNase localize together outside of P-bodies or that P-bodies and exosome granules form distinct but interconnected structures analogous to SGs and P-bodies. It is also possible, as for any negative observation, that the lack of concordance of P-bodies and exosomes is a function of the reagents or staining methodology or physiologic status of the cells employed. These questions require additional study to resolve.

But what is the role of the proteasome in these pathways, particularly with regard to AMD? Does the proteasome act to directly decay or cleave ARE-containing messages, as indicated by the TMV-RNA data, or does the proteasome interact with the ARE while actual decay of the message is mediated by other pathways? AUF1 and TTP interact with components of both RNA decay pathways[48,50] and proteasome-mediated degradation of both proteins is required for decay of their respective mRNA ligands.[51–53,57] Perhaps the proteasome, having destroyed the instability

promoting RNA-binding protein, interacts with the ARE to ensure message decay occurs by preventing the interaction of a stabilizing protein, such as HuR.[79] Although the preponderance of the data indicates a nontrivial role for the proteasome in AMD (i.e., proteasome inhibition promotes SG formation which inhibits AMD), further study is required to elucidate the precise role of the proteasome in AMD.

CONCLUSION

Homeostasis is as much about turning off processes as about turning them on and protein degradation is an ideal mechanism to maintain homeostasis. The versatility of the proteasome to selectively degrade specific protein targets makes it ideally suited for service in most cellular processes. Although this review has focused on the role of the proteasome in mRNA surveillance and decay, the links with RNA metabolism in general are substantially larger. It is also clear that within the confines of mRNA decay and surveillance, additional proteins will be identified that are targeted for proteasome-mediated cleavage or degradation.

The proteasome is critical in the decay of protein products derived from aberrant messages. The proteasome degrades proteins derived from messages with premature termination codons as well as messages lacking stop codons; this function is essential to prevent the accumulation of dominant negative or gain of function proteins in cells. Based on these observations, it seems likely that further study of NGD will demonstrate that the protein derived during the initial translation process is degraded by the proteasome. The data in Figure 6 demonstrate colocalization of the proteasome with both the 5′-to-3′ and 3′-to-5′ RNA decay pathways, and colocalization of both the 5′-to-3′ and 3′-to-5′ RNases. Thus, decay components required for the degradation of aberrant mRNAs as well as the aberrant peptide produced from the RNA localize together within the cell. With regard to AMD, the role of the proteasome is more difficult to determine. In particular, it is unclear if the proteasome degrades ARE-containing messages *in vivo* or rather degrades ARE-binding proteins which results in, or licenses, the decay of ARE messages by the 5′-to-3′ and 3′-to-5′ pathways. Further work is required to establish the precise relationship of the proteasome, the exosome, and the 5′-to-3′ decay complex.

ACKNOWLEDGEMENT

I would like to thank Kelly Kieffer and Mathew Vincenti for their in-depth reading and comments on this manuscript. I apologize to many colleagues who generated the initial data discussed herein but whose work could not be specifically cited because of space constraints.

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