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Poly(A) RNA Binding Proteins and Polyadenosine RNA: New Members and Novel Functions

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Abstract

Poly(A) RNA binding proteins (Pabs) bind with high affinity and specificity to polyadenosine RNA. Textbook models show a nuclear Pab, PABPN1, and a cytoplasmic Pab, PABPC, where the nuclear PABPN1 modulates poly(A) tail length and the cytoplasmic PABPC stabilizes poly(A) RNA in the cytoplasm and also enhances translation. While these conventional roles are critically important, the Pab family has expanded recently both in number and in function. A number of novel roles have emerged for both PAPBPN1 and PABPC that contribute to the fine-tuning of gene expression. Furthermore, as the characterization of the nucleic acid binding properties of RNA binding proteins advances, additional proteins that show high affinity and specificity for polyadenosine RNA are being discovered. With this expansion of the Pab family, comes a concomitant increase in the potential for Pabs to modulate gene expression. Further complication comes from an expansion of the potential binding sites for Pab proteins revealed by an analysis of templated polyadenosine stretches present within the transcriptome. Thus, Pabs could influence mRNA fate and function not only by binding to non-templated poly(A) tail but also to internal stretches of adenosine. Understanding the diverse functions of Pab proteins is not only critical to understand how gene expression is regulated but also to understand the molecular basis for tissue-specific diseases that occur when Pab proteins are altered. Here we describe both conventional and recently emerged functions for PABPN1 and PABPC and then introduce and discuss three new Pab family members, ZC3H14, hnRNP-Q1, and LARP4.

Introduction

Messenger RNA (mRNA) is the intermediate molecule in the highly regulated flow of genetic information from DNA to protein. mRNA processing events, which are required throughout the life of the mRNA, are referred to as post-transcriptional processing and include the capping, splicing, cleavage and 3’end processing, export from the nucleus,

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localization, translation, and ultimate turnover of the mRNA[1]. Each of these events is tightly regulated and is mediated by a myriad of RNA binding proteins that coat the mRNA from its birth in the nucleus until its eventual degradation in the cytoplasm[1, 2]. The importance of these RNA binding proteins and the post-transcriptional processing events that they mediate is highlighted by a number of human diseases that are associated with dysregulation of their function[3, 4].

One key post-transcriptional event is the 3'end processing of an mRNA transcript, which occurs via a two-step mechanism. In the first step, the mRNA undergoes an endonucleolytic cleavage event at a designated position within the transcript. Upon cleavage, a non-templated polyadenosine "tail", or poly(A) tail, is added to the upstream cleavage product[5, 6]. The vast majority of eukaryotic mRNAs are cleaved and polyadenylated in this manner. The resulting poly(A) tails are bound by a specific family of RNA binding proteins termed poly(A) RNA binding proteins or Pabs[7]. The most conventional roles of the poly(A) tail are regulation of mRNA stability and translation[8, 9]. Two well-studied, or canonical, Pabs, poly(A) binding nuclear protein 1 (PABPN1) and cytoplasmic poly(A) binding protein (PABPC), bind to the poly(A) tail and regulate these processes[7]. PABPN1 stimulates polyadenylation and regulates poly(A) tail length in the nucleus while PABPC facilitates translation and modulates mRNA stability in the cytoplasm[10, 11]. Although these functions are critically important, Pabs perform additional functions that influence gene expression.

In recent years, a number of novel functions for the canonical Pabs have come to light[7] and will be discussed in this review. Furthermore, recent studies have also led to the identification of additional members of the Pab family, three of which are discussed here: 1) Zinc finger CCCH-type containing protein #14 (ZC3H14); 2) Heterogeneous ribonucleoprotein Q1 (hnRNP-Q1); and 3) La-related protein 4 (LARP4). These proteins expand the Pab family not only by their unique roles in regulating various aspects of post-transcriptional processing, but also by the diversity in how they recognize and bind to polyadenosine RNA. Finally, the presence of internal, or templated, polyadenosine stretches within many mRNA transcripts suggests an additional point of entry for regulation of gene expression by Pabs. Not all transcripts contain internal polyadenosine stretches and therefore may provide a clue into the specificity of Pabs for certain classes or types of RNA transcripts, thus expanding our focus on polyadenosine RNA beyond non-templated poly(A) tails to include templated internal stretches of adenosine.

In this review, we briefly summarize the well-studied, or canonical, functions of PABPN1 and PABPC and then describe the newly defined roles for these proteins that have emerged in recent years. We then describe the three novel Pab family members and discuss their roles in modulating gene expression. Finally, we present an analysis that determines the number of templated polyadenosine stretches within the human transcriptome, suggesting the potential for regulation by such a motif. By integrating the wealth of information on Pabs and polyadenosine sequences described in recent years, we gain insight into the role of this protein family in regulating gene expression and build an updated, more comprehensive model for the function of these proteins and their target sequences.

Summary of the Canonical Pabs

Conventional Pab proteins are typically defined by the presence of an RNA recognition motif (RRM) (Figure 1) combined with biochemical evidence to demonstrate specific, high affinity binding to polyadenosine RNA. A single nuclear Pab, termed PABPN1, and multiple cytoplasmic Pabs, termed PAPBC1, 3–5 and L1, have been identified in multiple organisms (Table 1). PABPC1 is ubiquitously expressed while the remaining PABPC homologs differ in their spatial and temporal expression pattern (Table 1). PABPN1 and PABPC1 are the best studied Pab proteins and are hence referred to here as the canonical Pabs. The terms PABPN1 and PABPC will be used throughout the review to refer to any ortholog of PABPN1 or PABPC1 (Table 2).

Canonical PABPN1 Functions

PABPN1 is the primary nuclear Pab and is highly conserved among eukaryotes[12]. PABPN1 is expressed ubiquitously in metazoans and PABPN1 orthologs have been analyzed in *D. melanogaster* and *C. elegans*, as well as *S. pombe*. However, to date there is no comparable PAPBN1 counterpart in *S. cerevisiae*[10] (Table 2). The PABPN1 protein consists of a number of functional domains, which are diagramed in Figure 1. The best characterized domain of PABPN1 is the RNA RRM found toward the C-terminus of the protein, which mediates high affinity polyadenosine RNA binding[13]. PABPN1 recognizes 10 to 11 nucleotides[14, 15] and detailed binding studies suggest that most of these adenosines, if not all, are bound by PABPN1 in a base-specific manner[15]. The arginine-rich region located C-terminal to the RRM also contributes to adenosine RNA recognition[16]. This domain is asymmetrically methylated; however, the methylation has no apparent effect on the affinity, specificity, or cooperativity of RNA binding[13, 16], and instead modulates interactions with the PABPN1 nuclear import receptor, transportin[17, 18]. The helical domain N-terminal to the RRM is required for stimulation of poly(A) polymerase (PAP). Of note, the stretch of ten alanines located immediately adjacent to the initiating methionine is expanded to anywhere from 12–17 alanines in the late-onset human disease, oculopharyngeal muscular dystrophy (OPMD), which is characterized by a progressive weakening of a specific group of muscles in the head as well as limb muscles in some instances of the disease[12]. How this modest alanine expansion leads to this tissue-specific disease is unknown.

The role of PABPN1 in polyadenylation has been extensively studied largely through elegant biochemical studies[19–21]. These conventional functions of PABPN1 are schematized in Figure 2. As stated in the Introduction, 3’-end processing of pre-mRNA transcripts occurs via a two-part process consisting of cleavage and subsequent polyadenylation. These processing events are mediated by a large, heterooligomeric protein complex that is comprised in part of PAP and the cleavage and polyadenylation specificity factor (CPSF)[6]. Upon cleavage of the pre-mRNA by CPSF-73, PAP begins to add adenosine residues to the upstream cleavage product in a slow, or distributive, manner. Binding of PABPN1 to the first 11 adenosines stimulates PAP activity and triggers processive polyadenylation[19, 20]. Evidence that PABPN1 increases the affinity of PAP for RNA, thus leading to processive polyadenylation by a tethering mechanism is provided through analysis of RNA binding mutants of PABPN1 that are unable to stimulate processive polyadenylation[21]. In addition,

amino acid changes in the helical domain of PABPN1, which do not affect the affinity of PABPN1 for RNA, but do abrogate binding to PAP, also eliminate the ability PABPN1-mediated stimulation of processive polyadenylation[20]. In the "molecular ruler" model, PABPN1 binds the initial 11–14 adenosines, and then coats the growing poly(A) tail to form 21 nm spherical particles that enable the RNA to fold back and maintain an interaction between CPSF and PAP[21, 22]. When a length of 200–300 adenosines is reached, this complex is disrupted and the phase of processive polyadenylation is complete (Figure 2)[21]. This model is supported by experiments using electron and scanning force microscopy with oligo(A) RNA and purified PABPN1 protein[22]. These studies using primarily purified proteins have provided the field with significant insight into the role of PABPN1 in regulating polyadenylation; however, further studies to assess the role of PABPN1 in the context of other RNA binding proteins as well as other functions that have emerged for this protein are required to build a complete model of polyadenylation.

Proper 3'end processing and polyadenylation of transcripts is required for efficient mRNA export from the nucleus to the cytoplasm[6]. The finding that PABPN1 can shuttle between the nucleus and the cytoplasm has led to the suggestion that PABPN1 could contribute to poly(A) RNA export from the nucleus[23] (Figure 2). Consistent with this suggestion, nuclear accumulation of poly(A) RNA is observed in cells expressing the viral NS1A protein, which binds to and inhibits the nuclear-cytoplasmic shuttling of PABPN1[24]. Furthermore, primary muscle cells depleted of PABPN1 also display nuclear accumulation of bulk poly(A) RNA[25]. Altered steps in the polyadenylation process could cause this nuclear poly(A) RNA retention, as proper polyadenylation is required for efficient mRNA export from the nucleus[6, 26, 27]. However, two independent studies using templated poly(A) tracts present at the 3'-ends of reporter transcripts show that these artificial substrates are exported,[28, 29] suggesting that the presence of a tail alone may be sufficient for RNA export and therefore raising the question of how the steps of polyadenylation are coordinated with the generation of an export-competent mRNP complex. These studies suggest that PABPN1 could regulate mRNA export from the nucleus, but further analyses are required to understand whether PABPN1 plays a direct role in modulating this critical step in gene expression or whether effects on upstream events such as polyadenylation impair subsequent mRNA export.

Altogether, these studies underscore the critically important role of PABPN1 in stimulating and regulating the polyadenylation of nascent mRNA transcripts. Whether PABPN1 plays a direct role in modulating mRNA export to the nucleus remains unclear but the question highlights the complexity of understanding the direct molecular functions of proteins involved in the highly interconnected steps of post-transcriptional processing.

Canonical PABPC Functions

The major cytoplasmic poly(A) binding protein, PABPC, is ubiquitously expressed and found in all eukaryotes (Table 2). As shown in Figure 1, PABPC contains four tandem RNA recognition motifs (RRMs), an unstructured linker region and a C-terminal helical domain with five α-helices[10, 11]. PABPC contacts polyadenosine RNA through the RRMs and requires a minimum of 12 nucleotides for high affinity binding[30]. Together RRMs 1 and 2 mediate oligo(A) binding while other pairwise combinations or single RRMs are not

sufficient for binding[31]. However, binding of all four RRMs is required to account for the ~25 nucleotide footprint of PABPC[30, 32]. In addition, the N-terminal RRMs and the C-terminal α-helical domain serve as protein-protein interaction domains to mediate the various functions of PABPC. The RRMs facilitate binding to eukaryotic initiation factor 4G (eIF4G)[33], the α-helices facilitate binding to eukaryotic release factor 3 (eRF3)[34] and promote PABPC dimerization[35] and both of these domains are involved in binding Poly(A) binding protein Interacting Proteins (PAIPs)[36, 37] and GW182, an RNA induced silencing complex (RISC) protein[38]. Thus, PABPC serves as a platform to coordinate multiple players that modulate gene expression.

The role of PABPC in regulating mRNA translation has been extensively studied[10, 11] (Figure 2). Studies with *S. cerevisiae* reveal that cytoplasmic mRNAs form a closed loop structure to promote 40S ribosomal subunit recruitment[39]. PABPC is required to assume this structure, which facilitates efficient translation[33, 40, 41]. The cap-binding complex, eIF4F, enables formation of the closed loop structure with the eIF4G subunit linking the 5' cap-bound eIF4E subunit to poly(A) tail-bound PABPC[41, 42]. Further evidence suggests that PABPC could play a role in 60S ribosomal subunit joining[8, 43, 44] and promote ribosome recycling by interacting with eukaryotic release factor 3 (eRF3)[45]. In summary, PABPC mediates a number of interactions between the translationally poised mRNA and ribosome that underlie the role in ensuring efficient translation.

Similar to the role in translation, the function of PABPC in modulating mRNA decay is well established[10, 11] (Figure 2). mRNA decay is a multi-step process that is typically initiated by poly(A) tail shortening to a length of 10–15 nucleotides[46]. Deadenylation is followed by decapping with the Dcp1–Dcp2 complex and digestion by the 5'- to 3'-exoribonuclease Xrn1[46]. PABPC inhibits the deadenylase, which blocks the initial step of mRNA decay[47, 48]. PABPC also interacts with other RNA binding proteins, including the novel Pab hnRNP-Q1 (discussed below), to inhibit the decay of specific mRNA transcripts by blocking deadenylase or endonuclease activity[49–51]. Therefore, PABPC affects both global and transcript-specific mRNA decay.

The well-studied roles of the canonical Pabs, PABPN1 and PABPC, in modulating polyadenylation, translation, and mRNA decay demonstrate the importance of these proteins in post-transcriptional processing. However, recent studies reveal diverse, novel functions of these canonical Pabs[7], expanding our understanding of how these key proteins regulate gene expression and pointing toward a new model that integrates the novel and canonical functions of PABPN1 and PABPC.

Novel Functions of the Canonical Pabs

As discussed in the previous section, PABPN1 and PABPC play well-defined roles in polyadenylation, poly(A) tail length regulation, mRNA stability, and translation[10, 11]. However, in recent years a number of studies have unveiled multiple novel functions[7] (Figure 3) for these two proteins in the post-transcriptional regulation of mRNAs which, can influence both the temporal and spatial control of gene expression.

Novel PABPN1 Functions

Alternative polyadenylation—Recent work has uncovered a role for PABPN1 in influencing alternative cleavage and polyadenylation site selection (Figure 3A). This role for PABPN1 was initially uncovered in an unbiased genome-wide screen for modulators of alternative polyadenylation (APA) using an APA reporter[52]. This study found that siRNA-mediated depletion of PABPN1 results in 3'UTR shortening and preferential usage of proximal poly(A) sites (PASs). An alternative approach also uncovered a role for PABPN1 in APA in muscle, the tissue affected in OPMD patients[12]. This analysis employed 3'-end RNA-Seq analysis on transgenic mouse tissues that express mutant alanine-expanded PAPBPN1 (Ala17) to interrogate the impact of mutant PABPN1 on RNA[53]. Consistent with the study directed at defining modulators of APA[52], this analysis revealed a major shift toward proximal PAS utilization in tissues expressing mutant PABPN1. Thus, two distinct approaches reveal a critical role for PABPN1 in modulating PAS site selection. The consequences of this shift to proximal cleavage sites include a decrease in repression by 3'UTR binding factors such as miRNAs. Additional experiments in both of these studies led both groups to invoke a model in which alanine-expanded PABPN1 sequesters endogenous PABPN1 in nuclear aggregates, which are commonly observed in OPMD patient cells[12], resulting in a decrease of functional PABPN1 in the nucleus[54]. A decrease in functional PABPN1 in OPMD patient muscles could lead to shifts in APA that could contribute to patient phenotypes. These independent approaches strongly support a role for PABPN1 in APA; however, the mechanism by which PABPN1 modulates APA is unclear, as evidenced by distinct mechanistic models for how PABPN1 modulates APA selection proposed in the two studies. These differences demonstrate that further biochemical analyses are required to elucidate the precise mechanism by which PABPN1 influences APA.

lncRNAs—A role for PABPN1 in the regulation of long noncoding RNAs (lncRNAs), which are defined as non-coding RNAs that are greater than 200 nucleotides in length[55], has recently been reported[56] (Figure 3A). This function for PABPN1 emerged from an RNA-Seq analysis of the transcriptome of HeLa cells depleted of PABPN1 by siRNA. Interestingly, the majority of transcripts from protein-coding genes were unaffected by knockdown of PABPN1, which was somewhat surprising given the well-defined biochemical role of PABPN1 in 3'end processing and polyadenylation[12]. However, a subset of lncRNAs showed an increase in steady-state levels upon knockdown of PABPN1. A model lncRNA derived from a snoRNA host gene was used to demonstrate that PABPN1 promotes lncRNA turnover through an oligoadenylation-dependent mechanism. Further analysis revealed that this class of PABPN1-sensitive lncRNAs is targeted by specific subunits of the human Trf4-Air2-Mtr4 Polyadenylation (TRAMP) complex, an exosome co-factor[57], in cooperation with the exosome, suggesting that PABPN1 regulates lncRNA expression, in a manner similar to that described for PABPN1 in polyadenylation-dependent RNA decay in fission yeast[58].

Nuclear RNA Quality Control—A role for PABPN1 in promoting the decay of mRNAs subject to nuclear RNA quality control as well as polyadenylated nuclear noncoding RNAs (ncRNAs) has also recently been reported[59] (Figure 3A). A nuclear RNA decay pathway that includes PABPN1, two isoforms of PAP (PAPα and PAPγ), and the catalytically active

exonuclease subunits of the RNA exosome (RRP6 and DIS3) was defined in this work. In this pathway, PABPN1 and the PAP enzymes produce hyperadenylated RNA products that are subsequently targeted and degraded by the exosome. Experiments using cordycepin, which causes premature termination of polyadenylation[60], and PAP-stimulating mutants of PABPN1 demonstrate that the extension of the poly(A) tail is necessary for decay as cordycepin treatment led to the accumulation of more stable transcripts with shorter poly(A) tails. Transcripts that undergo efficient splicing and export are not impacted by this pathway, invoking a model in which PABPN1, together with PAPα and PAPγ, plays a role in nuclear RNA quality control and promotes exosome-mediated decay of nuclear polyadenylated transcripts that are delayed in processing.

Pioneer round of translation—The pioneer round of translation of an mRNA is a key quality control checkpoint that selects for correctly processed and translation-competent mRNAs[61]. Pioneer translation initiation complexes differ from steady-state translation initiation complexes by the complement and stoichiometry of RNA-binding proteins and associated complexes[61]. However, how the process of translation facilitates the remodeling of the pioneer translation initiation complexes into steady-state translation initiation complexes is not yet known. PABPN1, which localizes to the nucleus at steady-state, can shuttle to the cytoplasm and associate with nascent mRNA transcripts that have undergone the pioneer round of translation[62]. However, in polysome profile analyses, PABPN1 is associated with fewer ribosomes than PABPC[63], suggesting that PABPN1 may be replaced by PABPC on translating mRNAs. Immunoprecipitation of PABPN1 and PABPC from HeLa cell lysates expressing iron-responsive mRNA reporters revealed a significant decrease in the amount of reporter mRNA that was enriched upon PABPN1 pulldown following the stimulation of translation[64]. Together, these data support a model in which the process of translation either stimulates the removal of PABPN1 and/or enhances binding of PABPC, ultimately resulting in the replacement of PABPN1 by PABPC on translating mRNAs (Figure 3A).

These recent studies greatly expand our understanding of the function of PABPN1, extending beyond the regulation of polyadenylation in the nucleus to diverse roles in mRNA processing events. These newly discovered roles for PABPN1 as a key factor in influencing poly(A) site selection, regulating lncRNA expression, and coordinating a novel nuclear RNA decay pathway paint a new picture of PABPN1 as a multifunctional player in the highly regulated post-transcriptional control of gene expression.

Novel PABPC Functions

miRNA-mediated translational repression, deadenylation and decay—In an extension of the canonical role for PABPC in regulating mRNA translation and stability, PABPC and the poly(A) tail have been implicated in facilitating miRNA-mediated translational repression, deadenylation and decay[7, 38] (Figure 3B). Recent work supports a model where PABPC promotes the association of the RNA induced silencing complex (RISC), specifically Argonaute 1 (AGO1), with target mRNAs[65] and the interaction of the RISC component GW182 with both PABPC and deadenylase complexes is required for efficient silencing[66]. PABPC is released from the target mRNA prior to deadenylation and

the recruitment of the deadenylase complexes to the miRNA target by GW182 is required for PABPC release to occur[67]. Subsequently, eIF4E and eIF4G are released from the mRNA in a decapping- and deadenylation-dependent manner, respectively[67], and GW182 is released from the mRNA after deadenylation suggesting that it is only required to initiate silencing[68]. Taken together, these data suggest that binding of the RNA induced silencing complex (RISC), which includes AGO1 and GW182, leads to translational repression, deadenylation and decay of the miRNA target due to removal of PABPC and consequently the disruption of the closed loop structure which inhibits translation and allows deadenylase access to the poly(A) tail (Figure 3B). However, PABPC does not appear to play a role in miRNA-mediated repression in *D. melanogaster* S2 cells or *D. rerio* embryos, which suggests that this mechanism may not be conserved in all organisms and/or experimental systems[69, 70].

Nonsense mediated decay—PABPC plays a role in identifying premature termination codons in the nonsense mediated decay (NMD) pathway[7] (Figure 3B). NMD is an mRNA surveillance pathway that leads to the degradation of mRNAs that contain premature termination/stop codons (PTCs)[71]. This process involves an interaction between the central NMD protein Upf1, an ATPase and RNA helicase, and the eukaryotic release factors eRF1 and eRF3, which bind to ribosomes that are positioned over stop codons[71]. Only recently have studies begun to unravel how cells distinguish PTCs from normal stop codons. The conventional model for how the NMD machinery distinguishes between these two centers around the position of the exon junction complex (EJC), which marks exon-exon junctions that result from splicing[71]. This model posits that stop codons upstream of splice junctions and hence, deposited EJCs, are recognized as premature[71]. However, this mechanism does not explain NMD of transcripts not subject to splicing or with a PTC located after the terminal splice site.

Recent results suggest that PABPC may also contribute to the identification of PTCs and consequently NMD[7] (Figure 3B). PTCs located in close proximity to the 3'UTR evade NMD more efficiently than those more distal[72]. Studies implicating PABPC in PTC recognition show that PABPC inhibits NMD at a distal PTC when it is tethered immediately downstream[73]. Thus, PABPC marks the end of the mRNA transcript and that the translational machinery distinguishes a normal stop codon from a PTC by the presence or absence of PABPC, respectively. However, PTCs located proximal to the AUG start codon can also evade NMD, which may be due to the continued binding of PABPC to the 43S ribosomal complex throughout the AUG scanning process[74]. NMD evasion by tethered PABPC is dependent on the C-terminus of PABPC[74], which contains the binding site for eRF3[10, 11]. PABPC and Upf1 compete for binding to eRF3[75], which leads to a model in which a PABPC-eRF3 interaction suppresses NMD and a Upf1-eRF3 interaction facilitates NMD (Figure 3B). However, this mechanism remains controversial[76] and how PABPC and the EJC coordinate NMD remains to be elucidated.

Long interspersed nuclear elements (LINE) retrotransposition—Long interspersed nuclear elements (L1 or LINEs) are 500–8,000 base pair, non-long terminal repeat (non-LTR) retrotransposons that are very abundant in humans, accounting for ~17%

of the genome[77]. Shortening the poly(A) tail of the L1 RNA or depletion of PABPC significantly reduced L1 retrotransposition[78]. Furthermore, PABPC is a component of the L1 ribonucleoprotein (RNP)[78], which is an RNA-protein complex that is required for retrotransposition[79]. Depletion of PABPC leads to reduced accumulation of the L1 RNP protein, Orf1, in the nucleus[78]. These results suggest that PABPC affects retrotransposition by facilitating nuclear accumulation of the L1 RNP (Figure 3B). PABPC also interacts with templated A-rich sequences in short interspersed nuclear elements (SINEs)[80], which are 100–300 base pair, non-LTR retrotransposons that account for ~11% of the human genome[77], but evidence supporting a role for PABPC in regulating the retrotransposition of SINEs is lacking[81–83]. The mechanisms underlying these contributions of PABPC to regulation of transposition have not yet been defined.

mRNA localization—mRNAs are localized in many cell types as a mechanism to spatially regulate gene expression and this localization is particularly key in developmental processes[84]. A role for PABPC in mRNA localization has been proposed based on studies in *D. melanogaster* embryos (Figure 3B), where PABPC co-immunoprecipitates, in an RNA-dependent manner[85], with the Egl/Bic-D transport machinery, which functions as an adaptor to link mRNA cargoes to dynein motors[84]. Of interest, PABPC also co-localizes with *osk* mRNA and the localization of *osk* mRNA to the posterior compartment of the embryo is disrupted in PABPC mutants[85]. PABPC mutants also show reduced *osk* mRNA levels suggesting that the localization defect detected may be due to altered *osk* mRNA stability and/or localization[85]. Furthermore, the *osk* mRNA sequences required for this regulation have been mapped to an A-rich region in the 3'UTR, which may be bound by PABPC[85]. PABPC is also part of a complex of proteins that associates with RNA fragments containing the minimal localization signal of the endogenous, localized mRNA *bicoid*, further supporting the idea that PABPC could influence mRNA localization through the formation of a multiprotein complex[86] (Figure 3B). However, additional work is needed to dissect whether PABPBC plays direct or indirect roles in mRNA localization.

Translation and local translation—PABPC regulates the translation of some specific mRNAs through mechanisms that appear to be independent of the poly(A) tail (Figure 3B). For example, PABPC binds to A-rich sequences in the 5'-UTR of the *PABPC* mRNA transcript and consequently represses its own translation *in vitro* and *in vivo*[87–89]. PABPC also binds to an ~50 nucleotide A-rich sequence in the 3'-UTR of Y-box binding protein 1 (YB-1) mRNA to relieve translational inhibition by bound YB-1 protein[90], which normally functions as a transcription factor[91]. These studies demonstrate that PABPC can regulate translation by binding to templated adenosines within the mRNA transcript. In addition, PABPC binds to BC200 and BC1[80], noncoding RNAs in *H. sapiens* and *M. musculus*[92, 93], respectively, which inhibit translation by blocking the helicase activity of eIF4A[81–83]. PABPC blocks BC200 and BC1-mediated translation inhibition both *in vitro* and *in vivo*[94]. These noncoding RNAs contain over 40 nucleotides of adenosine-rich sequences, and nine consecutive adenosine residues were shown to be the minimal sequence necessary for binding by PABPC RRM domains[95]. In addition, BC200 and BC1 are localized to the dendrites of neurons[96, 97] where they were reported to interact with FMRP[98], a local translation regulator, and hnRNP-Q1[99]. These results suggest that PABPC may also play a

role in regulating translation locally in neuronal processes. In support of this hypothesis, PABPC binds to Vasopressin mRNA through sequences in the end of the coding region and/or the 3'UTR[100]. These regions of the mRNA are also required for localization of the mRNA to dendrites where Vasopressin mRNA is locally translated[100, 101]. In summary, PABPC regulates translation through a variety of mechanisms and has also been implicated in mRNA transport, which suggests a role for PABPC in regulating translation locally within specific subcellular compartments (Figure 3B).

These recent findings demonstrate that PABPC plays important roles in virtually all cytoplasmic post-transcriptional regulatory events, including mRNA transport, translation and decay (Figure 3B). PABPC regulates multiple aspects of these processes, either directly or indirectly, indicating that PABPC plays a key role in coordinating the post-transcriptional control of gene expression. Future studies of interest include analyzing how the multiple functions of PABPN1 and PABPC are coordinated to influence gene expression, as well as how novel Pabs such as ZC3H14, hnRNP-Q1, and LARP4 fit into the picture of post-transcriptional processing via binding to polyadenosine RNA.

New Pab Family Members

The ongoing discovery and characterization of RNA binding proteins has led to the expansion of many classes of RNA binding proteins, including the Pab protein family. Here we present three novel members of the Pab family: ZC3H14, hnRNP-Q1 and LARP4 (Figure 1). These proteins are defined as Pabs based on *in vitro* binding studies that demonstrate specific, high affinity binding to polyadenosine RNA. ZC3H14, hnRNP-Q1, and LARP4 play diverse roles in the post-transcriptional control of gene expression, which are schematized in Figure 4.

ZC3H14

Zinc finger CCCH-type containing protein #14 (ZC3H14) is an evolutionarily conserved nuclear Pab that binds to polyadenosine RNA via tandem CCCH zinc fingers[102]. Although most Pab proteins bind polyadenosine RNA via RRM motifs, biochemical studies provide evidence that the zinc fingers in ZC3H14 confer specific binding to polyadenosine RNA[102]. Interest in this class of zinc finger Pabs has been sparked by recent studies showing that a mutation of *ZC3H14* that results in loss of this ubiquitously expressed protein leads to an autosomal recessive form of nonsyndromic intellectual disability[103]. The brain-specific phenotype in these patients suggests that the function of ZC3H14 is particularly critical in the highly specialized cells found in the brain.

Putative orthologs of ZC3H14, which share a common domain structure (Figure 1), have been studied in a variety of model organisms (Table 2), including *S. cerevisiae*[104] (Nab2), *D. melanogaster*[103] (dNab2), *C. elegans*[105] (SUT-2), and *M. musculus*[106] (ZC3H14/MSUT2). The N-terminus of ZC3H14 adopts a proline tryptophan isoleucine (PWI)-like fold, which mediates interactions with nuclear pore-associated proteins and facilitates proper mRNA export from the nucleus[107]. A classical nuclear localization signal (cNLS) is thought to target ZC3H14 to the nucleus[108]. The tandem CCCH zinc fingers located at the C-terminus of the protein are responsible for high affinity polyadenosine RNA

recognition[102, 104]. Extensive binding studies demonstrate that in *S. cerevisiae*, only three out of the seven total zinc fingers are necessary for high affinity polyadenosine RNA recognition. The additional zinc fingers may bind other nucleic acid sequences or even other proteins[102, 109].

Recent structural analyses of both *S. cerevisiae* and *C. thermophilum* ZC3H14 reveal that three zinc fingers interact to form an RNA binding module[110, 111]. The crystal structure of zinc fingers 3–5 of *C. thermophilum* Nab2 in complex with an A_8 RNA oligo revealed that these zinc fingers form the binding unit that interacts with polyadenosine RNA with base-specific interactions on multiple adenosine residues[111]. These three zinc fingers share the greatest homology with hZC3H14 zinc fingers 1–3 and *S. cerevisiae* Nab2 zinc fingers 5–7, which is consistent with previous studies implicating the final three zinc fingers of Nab2 in high affinity polyadenosine RNA binding[102] as well as poly(A) tail length control[112, 113]. Further analysis of the crystal structure revealed that although multiple adenosine-specific interactions are present, the spacing of the individual zinc fingers within the binding unit could result in Nab2 also binding to degenerate, or A-rich sequences instead of solely to poly(A) tracts. This information opens the possibility that this family of proteins could bind to poly(A) tails as well as A-rich sequences, and presents a model in which ZC3H14 could interact with a diverse spectrum of RNA transcripts.

Although little is known about the molecular function of human ZC3H14, studies in model organisms provide insight into potential roles in post-transcriptional processing. The majority of these studies have been carried out in *S. cerevisiae* analyzing Nab2. The Nab2 protein localizes to the nucleus at steady-state, but shuttles between the nucleus and cytoplasm[114]. The ZC3H14 protein also localizes to the nucleus at steady-state, more specifically to nuclear speckles, which are sites of RNA processing[108, 115]. Nab2 associates with poly(A) mRNA transcripts prior to export, likely modulating polyadenylation and facilitating the generation of export-competent mRNPs[116]. *S. cerevisiae* cells expressing N-terminal deletion mutants of Nab2 display nuclear accumulation of poly(A) RNA as well as extended poly(A) tails[114, 117]. However, the existence of Nab2 RNA binding mutants that confer defects in poly(A) tail length with no nuclear accumulation of poly(A) RNA highlights the critical role of the zinc finger domain in poly(A) tail length regulation[118]. Additional insight into a potential function for ZC3H14 has been obtained from a *D. melanogaster* loss-of-function model of *ZC3H14*, *dNab2* null flies[103]. Studies in this model demonstrate that dNab2 has specificity for polyadenosine RNA *in vitro* and that RNA isolated from *dNab2* null flies displays extended poly(A) tails. However, no defect in bulk poly(A) mRNA export has been detected[103], lending support to a primary role for ZC3H14 in control of poly(A) tail length. Consistent with this model, siRNA-mediated depletion of ZC3H14 from cultured mammalian cells causes extended poly(A) tails with no apparent impact on poly(A) RNA localization[119].

With respect to the role of zinc finger Pabs in the brain suggested by the phenotype of *ZC3H14* patients, behavioral defects observed in *dNab2* null flies can be rescued by transgenic expression of either *Drosophila* Nab2 or human ZC3H14 specifically in neurons[119]. This result demonstrates the critical role of Nab2/ZC3H14 in ensuring proper neuronal function and also provides experimental evidence for the functional conservation

of this protein family through evolution. Rescue of neuronal phenotypes is accompanied by rescue of the molecular phenotype of extended poly(A) tails also observed in *dNab2* mutant flies[103, 119]. Together, these data suggest that the role of ZC3H14 in poly(A) tail length control may underlie its function in development and disease. Two simple models for how ZC3H14 could limit poly(A) tail length include: 1) a limiting model where ZC3H14 limits the activity of poly(A) polymerase; or 2) a model in which ZC3H14 recruits or triggers a ribonuclease to trim the poly(A) tail[118] (Figure 4).

An independent line of experimentation provides a further link between ZC3H14 and neuronal function. A screen for suppressors of the neurotoxicity of aggregated tau protein in *C. elegans* identified mutations in the Suppressor of Tauopathy 2 (*SUT-2*) gene, which encodes the nematode ortholog of ZC3H14[105]. Loss of function mutations in *SUT-2* suppressed the uncoordinated phenotype, tau aggregation, and the observed neurodegenerative changes in this worm model, suggesting that SUT-2, when present, enhances tau-induced neurotoxicity[105]. Further analysis of the human protein ZC3H14/mammalian SUT-2 (MSUT2) demonstrate reduced MSUT2 staining in the hippocampus, a region of the brain affected by tau pathology[120], in patients with Alzheimer's disease[106]. The authors suggest that decreased MSUT2 staining in patient neurons may reflect a population of neurons that are spared from tau pathology subsequent neurodegeneration[106].

The existence of multiple loss-of-function models of ZC3H14 with neuronal phenotypes strongly suggests a critical role for ZC3H14 in neurons. However, ZC3H14 is ubiquitously expressed, so the protein likely cooperates with PABPN1 to achieve precise control of polyadenylation. Whether ZC3H14, like the canonical Pabs, has additional, perhaps neuron-specific functions is not yet known.

hnRNPQ1

Heterogeneous ribonucleoprotein Q1 (hnRNP-Q1; also termed Nsap1,[121] Syncrip[122] and hnRNP-Q2[123]) is the cytoplasmic isoform of the mRNA binding protein hnRNP-Q[124] (*Mus musculus*: NP_062770.1; *Homo Sapiens*: NP_001153149.1). Alternative splicing of the gene also leads to two nuclear isoforms of hnRNP-Q, termed hnRNP-Q2 and hnRNP-Q3[124], but the function of these proteins is less well-characterized as compared to hnRNP-Q1. hnRNP-Q1 is ubiquitously expressed[122, 125, 126] and evolutionarily conserved (Table 2). The domain structure of hnRNP-Q1 consists of an N-terminal acidic domain, three RRMs, a putative nuclear localization sequence and a C-terminal RGG box[124]. hnRNP-Q2 and hnRNP-Q3 share the same domains but have an additional NLS and a different C-terminus that results from alternative splicing[124]. Both the acidic domain and the RGG box are involved in protein-protein interactions[124, 127] and the RRM domains are presumed to be required for binding RNA. However, experimental studies to define specific domains required for RNA binding have not yet been performed.

hnRNP-Q1 has been implicated in a variety of post-transcriptional regulatory processes including mRNA splicing[124, 128–130], editing[127, 131, 132], transport[133–137], translation[99, 126, 138–145], and degradation[51, 140, 146–149]. In addition, hnRNP-Q1 was recently identified as a novel polyadenosine RNA binding protein[123], consistent with previous studies that revealed binding to oligo(A) RNA[122]. Data demonstrating that hnRNP-

Q1 binds specifically to polyadenosine RNA with high affinity include *in vitro* binding assays coupled with competition experiments. The *in vitro* binding studies demonstrate that neither poly(C) nor poly(G) RNA can compete with poly(A) RNA for binding to hnRNP-Q1[122, 123].

Recent work suggests that hnRNP-Q1 competes with PABPC for binding to poly(A) tails and inhibits translation[123] (Figure 4). In this model, hnRNP-Q1 represses translation by inhibiting eIF4F complex recruitment to the mRNA and consequently 48S and 80S initiation complex formation and ultimately cap-dependent translation[123]. This mechanism is consistent with previous studies that have identified hnRNP-Q1 as a translational repressor[126, 144]. Furthermore, hnRNP-Q1-mediated translational repression depends on both the poly(A) tail and PABPC. The fold change of hnRNP-Q1-mediated translational inhibition increases with the length of the poly(A) tail[123]. Additionally, when PABPC is sequestered by the repressor poly(A) binding protein interacting protein 2 (Paip2), hnRNP-Q1 does not affect translation[123]. Depletion of hnRNP-Q via shRNA sequences targeting all isoforms of hnRNPQ leads to increased global translation as detected by [^{35}S]methionine/cysteine incorporation[123]. However, previous microarray data identified only ~2,250 mRNAs enriched in hnRNP-Q1 immunoprecipitation pellets which corresponds to ~10% of the mRNAs interrogated. These results suggest that hnRNP-Q1 does not bind to all mRNA targets and that there is some specificity involved in this mechanism[134]. In fact, two consensus sequences for hnRNP-Q1 binding were identified from the microarray analysis: AYAAYY and UAUYRR (Y = C/U and R = A/G)[134]. Furthermore, hnRNP-Q1 binds to the 3'-UTR of RhoA mRNA, which does not contain A-rich sequences[126]. Therefore, hnRNP-Q1 may bind to both A-rich and non-A-rich sequences within an mRNA transcript and specifically repress the translation of a subset of mRNAs unlike the other Pab family members.

These results demonstrate that hnRNP-Q1 represses translation by competing with PABPC for binding to poly(A) tails (Figure 4). However, the mechanism that defines the interplay between hnRNP-Q1 and PABPC and consequently whether translation is enhanced or repressed has yet to be identified. Similarly, whether hnRNP-Q1 affects the translation of all mRNAs is unknown as is the possible contribution of the nuclear isoforms of hnRNP-Q, which contain the same RNA binding domains as hnRNP-Q1.

LARP4

La-related protein 4, or LARP4, is one member of the superfamily of La-related RNA binding proteins[150] that are conserved across eukaryotic evolution[151, 152] (Table 2). The La family of proteins use a La motif (LaM) as well as an RRM to recognize terminal UUU-3'OHs on small, nascent RNA transcripts to protect the bound RNAs from 3' exonucleases, such as the RNA exosome[153]. LARPs also contain the LaM-RRM or LaM-RRM-like domain arrangement and have varying specificities for different classes of RNAs[150]. Although the overall domain structure of LARP4 (Figure 1) is similar to other family members, LARP4 harbors critical divergence from La as well as other LARP family members in its LaM. This change is in two or three key residues involved in UUU-3'OH recognition, suggesting that LARP4 may have unique RNA targets and functions. These

critical amino acid changes in LARP4 suggest that it is the most diverged member of the La family[152].

A recent study demonstrates that LARP4 is a *bona fide* cytoplasmic poly(A) RNA binding protein that also binds to PABPC using a variant PABP-interacting Motif (PAM2) to influence global translation as well as the stability of target mRNA transcripts[154]. The N-terminal domain of LARP4, which contains both the LaM and the RRM, has high affinity for a 20-mer of adenosine RNA, but has low affinity for U20 and essentially no affinity for C20 or G20. Although La binds U10 with high affinity, LARP4 does not bind to A10 with high affinity, but requires a longer stretch of adenosine. Thus, LARP4 binds with high affinity to polyadenosine RNA with a length requirement of more than 10 adenosines[154]. Polysome profile analysis revealed that LARP4 associates with 40S ribosomes as well as translating polyribosomes[154]. Subsequent studies uncovered an interaction between LARP4 and the 40S ribosomal-associated protein, RACK1, which is a scaffold protein that recruits activated protein kinase C to the ribosome to stimulate translation[154, 155]. This interaction may partially explain the association between LARP4 and 40S ribosomal subunits.

In addition to interactions with the 40S ribosome, LARP4 may also directly interact with PABPC. LARP4 contains a conserved N-terminal domain with a PAM2 motif. The PAM2 motif in LARP4 harbors a tryptophan in place of a typically invariant phenylalanine and is consequently designated a PAM2w motif. Indeed, LARP4 and PABPC can be co-immunoprecipitated and the interaction is RNase-resistant, suggesting a protein-protein interaction. Analysis of a series of LARP4 deletion mutants reveals that the PAM2w domain as well as a region C-terminal to the RRM is required for PABPC interaction. Analysis of these LARP4 deletion mutants by polysome profiling reveals a diminished association with polysomes, suggesting that the interaction with PABPC is necessary for LARP4 to maintain association with polysomes. Further studies also implicate the RNA binding motifs in mediating association with 40S components and polysomes. LARP4 RNA binding mutants that are not associated with 40S components also show decreased interaction with PABPC, suggesting that RNA binding by LARP4 stabilizes the LARP4-PABPC interaction. The affinity of LARP4 for polyadenosine RNA suggests that LARP4 and PABPC may associate on the poly(A) tails of mRNAs. In fact, further analyses provide evidence that PABPC and LARP4 interact with one another only in the presence of polyadenosine RNA[154].

The primary function ascribed to La proteins is modulation of RNA stability[150]. Stability assays reveal that LARP4 stabilizes a luciferase reporter as well as several endogenous mRNAs analyzed. However, the stability of some mRNA transcripts, including the non-polyadenylated histone H2A mRNA[156] and GAPDH mRNA[154], was not affected by LARP4 overexpression. Thus, LARP4 appears to stabilize some, but not all, mRNAs. Results of this study suggest that LARP4 associates with translating polyadenylated mRNAs and that LARP4, via interaction with PABPC, may be one member of a network of proteins that compete for interaction with PABPC. Finally, because overexpression of LARP4 stabilizes some mRNAs, LARP4 may also compete with degradation machinery such as the Pan2/Pan3 and/or the Ccr4-Not-Caf1-Tob2 deadenylation complexes[157] to modulate transcript stability. LARP4 clearly plays an important role in influencing gene expression and further analysis is required to not only identify the precise function of LARP4 in modulating

translation and stability, but also to understand how LARP4 fits in with the other Pab family members.

These studies not only highlight the diverse functions of the novel Pabs, ZC3H14, hnRNP-Q1 and LARP4, but they also underscore the importance of Pabs in influencing gene expression. Further comprehensive study of Pab family members should reveal whether they interact with or coordinate with other Pab family members to regulate post-transcriptional processes. The ongoing discovery and characterization of novel RNA binding proteins suggests there could be additional Pab family members that we have yet to discover, which would then need to be integrated into an overall picture of Pab protein function.

Novel Polyadenosine Stretches

As discussed extensively in this review, the canonical function of Pab proteins is to bind the poly(A) tails of mRNA transcripts and carry out diverse functions that are essential for proper gene expression. However, these non-templated polyadenosine tracts are not the only stretches of polyadenosine found in the transcriptome. For instance, a handful of studies have described polyadenosine stretches ranging from 9–26 adenosines within mRNA transcripts[158–160], but little work has been done to determine the function of these sequences. Given the length of these internal stretches and the typical Pab footprint of ~11–12 A's[15, 30], Pabs may bind to these internal sequences as well as to poly(A) tails. In fact, there is precedence for this mode of regulation. As discussed earlier, human PABPC binds to A-rich sequences in the 5'UTR of *PABPC* mRNA and the 3'UTR of *YB-1* mRNA to modulate the translation of these transcripts[87–90]. Furthermore, binding of *S. cerevisiae* Nab2 to a stretch of 26 adenosines in the 3'UTR of *NAB2* mRNA mediates a negative feedback response in which recruitment of the exosome drives subsequent degradation of the *NAB2* transcript[161]. The human ortholog of Nab2, ZC3H14, also contains a polyadenosine stretch (A_{15}) within the 3' UTR and preliminary studies suggest that ZC3H14 can bind to its own transcript (unpublished data). Together these data suggest that internal polyadenosine sequences could play an important role in regulating gene expression.

To gain insight into the prevalence of internal polyadenosine stretches within the human transcriptome, we analyzed all annotated human transcripts from the UCSC HG19 genome annotation for the presence of polyadenosine stretches. PABPN1 and PABPC require a stretch of 11–12 adenosines for high affinity binding[15, 30], so we extracted stretches containing at least 12 consecutive adenosines, filtered the data for overlapping regions, and mapped the resulting stretches to their locations within transcripts. As a proof of principle, we verified the presence of mRNA transcripts that are known to contain ≥12 nucleotide (nt) adenosine stretches (Dystrophin[160] and Vitamin D Receptor[158]) within our final dataset. Our results indicate that there are 139,334 instances of ≥12 nt adenosine stretches in the human transcriptome, many of which occur in multiple differentially spliced isoforms of the same gene (Figure 5A). As expected, the overwhelming majority of adenosine stretches occur in introns (136,655 instances). Interestingly, there are only 2,464 adenosine stretches that occur exclusively in exons (i.e. in the mature transcript)--1.8% of total instances. Of those, roughly 82% occur exclusively in the UTRs of the gene in which it resides (the majority of which occur in the 3' UTR) (Figure 5B), suggesting that the aforementioned examples of

regulatory mechanisms that depend upon templated polyadenosine stretches in UTRs could be more prevalent than previously appreciated. Remarkably, less than 0.02% of templated polyadenosine instances occur in the coding region of mRNA transcripts, with the remainder found in non-coding RNAs (Figure 5C). These preliminary results reveal that many mRNAs contain internal polyadenosine stretches and therefore have the potential to be bound and modulated by poly(A) binding proteins not only via their non-templated poly(A) tails, but also through these templated internal sequences. The vast enrichment of these stretches in the 3'UTRs of mature transcripts supports the well-establish function of the 3'UTR in post-transcriptional processing[162] and suggests a potential additional point of regulation via 3'UTRs. In the future, it will be interesting to test the function of these internal polyadenosine sequences as well as investigate whether these sequences are enriched in certain types or classes of RNA transcripts.

Future Directions and Remaining Questions

As described here, our understanding of the functions and members of the Pab protein family has increased in recent years. The studies presented in this review highlight the diversity found within the Pab family, as represented not only by the diverse roles of Pabs in modulating gene expression, but also by novel Pab family members that bind to RNA via unconventional polyadenosine RNA binding modules. The function of Pab proteins likely depends on the cellular context, specific regulatory elements, and the complement of RNA binding proteins present. Highlighting the importance of context for Pab protein function are the tissue-specific diseases that result from mutation of *PABPN1* or *ZC3H14*. Both PABPN1 and ZC3H14 play important roles in RNA processing, which are likely critical in numerous cell types, so why defects in PABPN1 cause muscle-specific pathology and loss of ZC3H14 causes defects specifically in brain function is not at all obvious. Clearly to understand these tissue-specific diseases, studies will not only need to assess contributions of these proteins within the biological context of the affected tissues but also to define the complete molecular functions of the Pabs that underlie these contributions. Indeed, there are likely additional roles that have yet to be discovered for members of the Pab protein family raising several key questions for contemplation.

The first question is how the diverse functions of the multiple Pab proteins are coordinated throughout the intimately linked steps of post-transcriptional processing. Given that all Pab proteins bind to polyadenosine, they should all technically compete for the same target sequences. Presumably there is remodeling and/or replacement of the complement of Pabs associated with a poly(A) RNA as that RNA moves through processing within the nucleus to ultimately fulfill its cytoplasmic destiny. The question of whether all polyadenylated RNAs are bound at some point in their life by the full complement of Pabs or whether Pabs show preferential binding to specific transcripts or subsets of transcripts will also need to be addressed in order to understand how overall Pab family function is coordinated. Very little is known about how Pab proteins could be exchanged for one another at different stages of poly(A) RNA biogenesis and/or function. One possible mode of Pab regulation is association with protein binding partners. For example, PABPC is regulated by binding to a number of PAb-Interacting Proteins (PAIPs), including LARP4. Given this precedent, there are likely additional partners that interact with and regulate other Pab family members. Such

interactions could contribute to selectivity for certain transcripts in cases where these Pab binding partners themselves are sequence-specific RNA binding proteins. Clearly, further studies are needed to define molecular interactions with both target RNAs and protein partners to fully understand how members of the Pab family of proteins cooperate to regulate gene expression.

A second important and related question is whether all adenosine sequences are created equal. For instance, the non-templated poly(A) tails present at the 3' end of mRNAs represent just one type of polyadenosine found in the human transcriptome. Our analysis of internal, templated polyadenosine stretches in RNAs reveals clear enrichment of these sequences within the introns and 3'UTRs of mRNAs. These sequences could be bound by Pabs to confer regulation. Only in a few cases have the function of such templated A stretches been analyzed but those studies do confirm the potential for their functional importance. With regard to the question of whether all polyadenosine stretches are equivalent, recent work revealed that methylation of adenosine residues (m^6A) on circadian clock transcripts contributes to their processing efficiency and plays a role in maintaining proper circadian rhythm[163]. The observation that post-transcriptional modification of adenosine residues modulates mRNA processing presents the possibility that adenosine residues on poly(A) tails or within internal polyadenosine stretches could be methylated to create distinct motifs that could be differentially recognized by Pab proteins. Future analyses should address the function of internal polyadenosine sequences and also explore whether modification of adenine within Pab target sequences influences Pab recognition and/or regulation.

In summary, the family of Pab proteins is expanding in both number and diversity of function. In fact, there are likely additional Pab family members that we have yet to define as such or to discover. While insight into the molecular roles of the individual Pab proteins is growing, developing a comprehensive model for how the functions of this protein family are coordinated to modulate gene expression remains the goal for the future.

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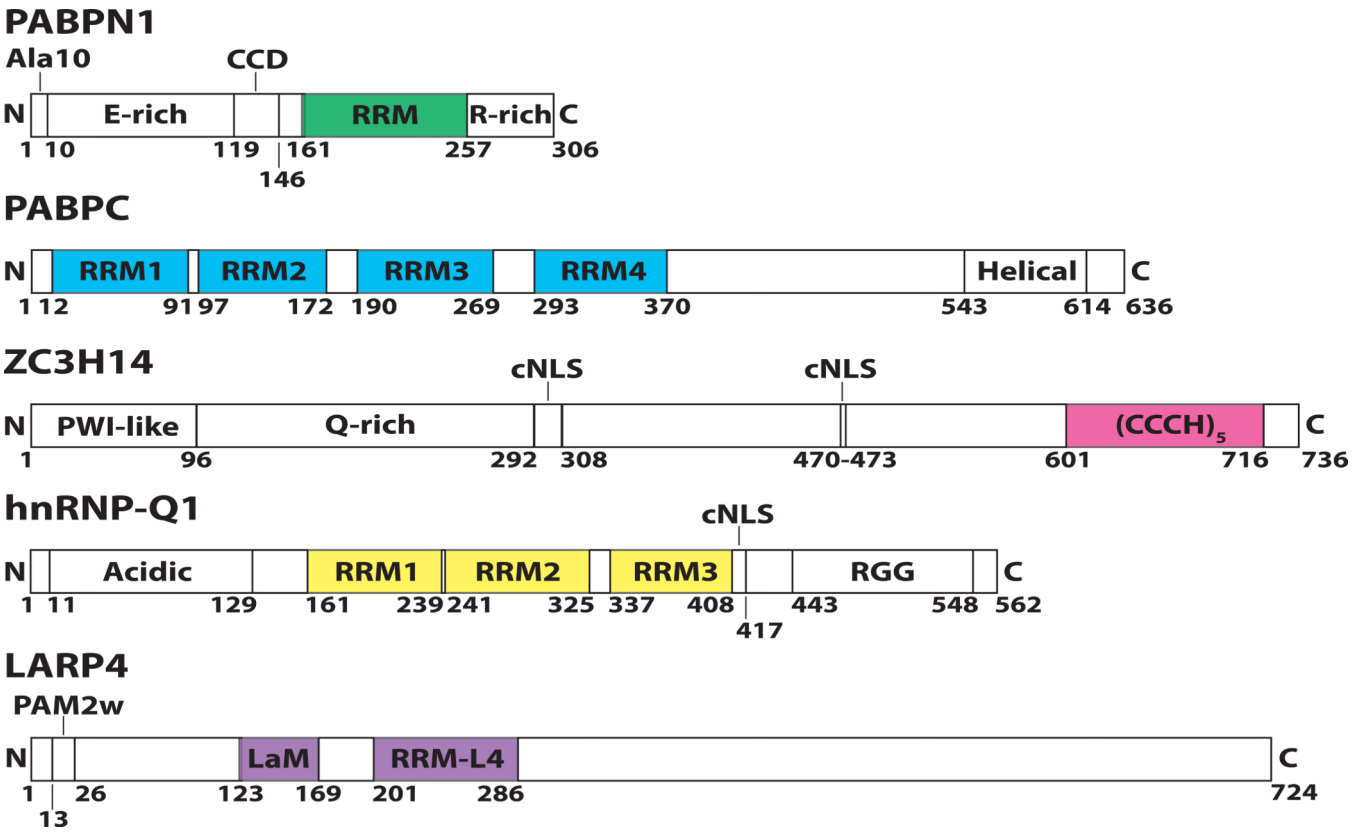

Figure 1. Pab protein domain structures and family members

The domain structures for the five Pab family members discussed in this review (PABPN1, PABPC, ZC3H14, hnRNP-Q1, and LARP4) are schematized here. RNA binding modules are highlighted in colors that correspond with each of these proteins in subsequent figures. PABPN1 is a nuclear Pab that contains a stretch of 10 alanines (Ala10) that are expanded in OPMD, a glutamic acid-rich domain (E-rich), a coiled-coil domain (CCD), an RNA recognition motif (RRM) that is responsible for high affinity polyadenosine RNA binding, as well as an arginine-rich (R-rich) domain at the C-terminus. PABPC is a cytoplasmic Pab that interacts with polyadenosine RNA via RRMs (RRM1-4) and contains a C-terminal Helical domain. ZC3H14 is a novel nuclear Pab that interacts with polyadenosine RNA via tandem CysCysCysHis (CCCH) zinc fingers and also contains an N-terminal Proline Tryptophan Isoleucine-like (PWI-like) fold that mediates interactions with the nuclear pore, a glutamine-rich (Q-rich) domain of unknown function, and two putative classical nuclear localization signals (cNLS). hnRNP-Q1 is a novel cytoplasmic Pab that is presumed to bind polyadenosine RNA via RRMs (RRM1-3) and contains an Acidic N-terminal domain as well as an Arginine Glycine Glycine (RGG) domain, both of which mediate protein-protein interactions. A putative weak cNLS is also present in hnRNP-Q1. LARP4 is a novel cytoplasmic Pab that interacts with polyadenosine RNA via a La Motif (LaM) in conjunction with an RRM-like 4 domain (RRM-L4). LARP4 interacts with PABPC via a poly(A) binding protein interacting motif (PAM2) domain that contains an atypical tryptophan in the consensus sequence (PAM2w).

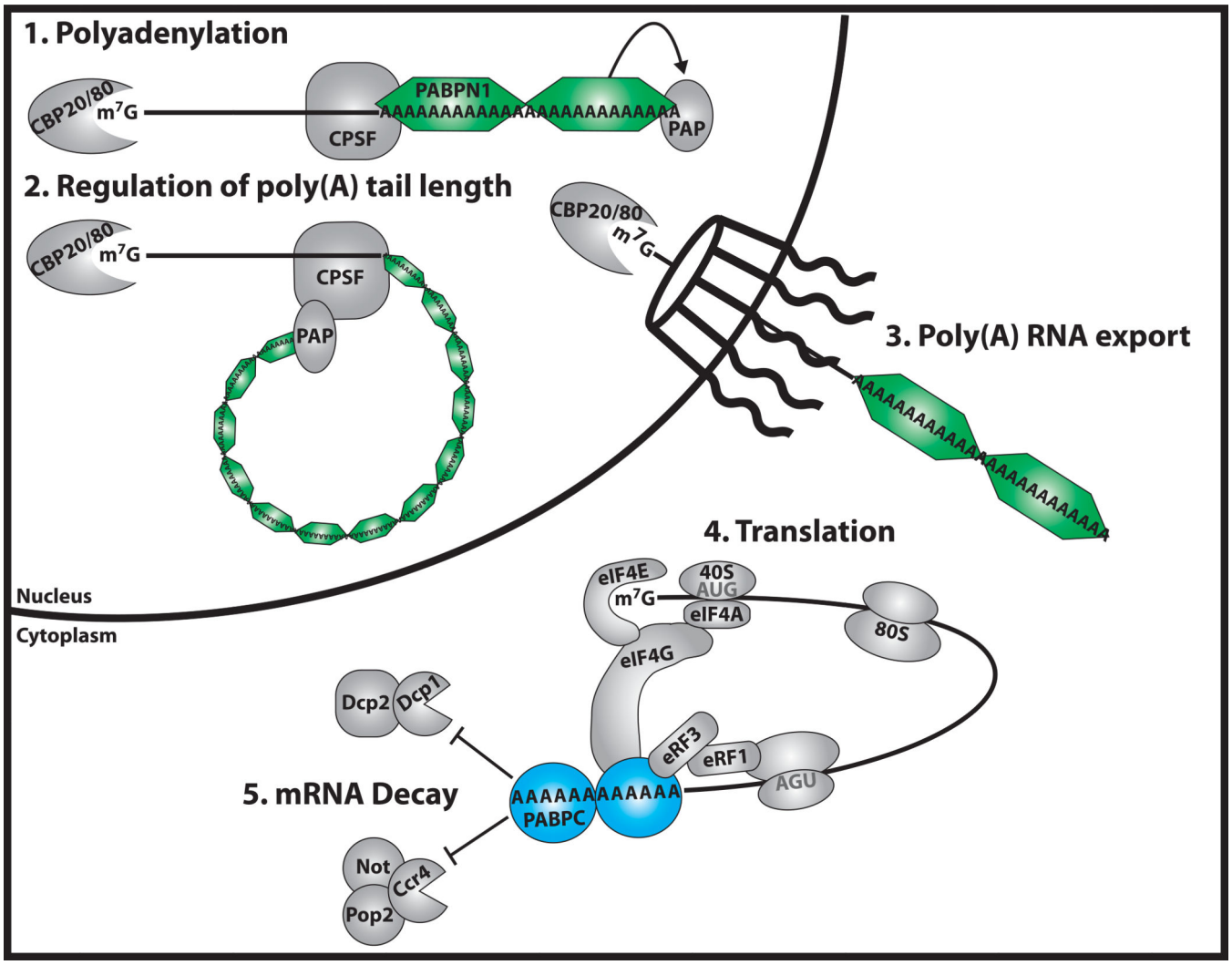

Figure 2. Model for the Canonical Functions of PABPN1 and PABPC

The role of **PABPN1 (green hexagon)** in modulating 3'end processing of mRNA transcripts is well established and consists of the following molecular functions: **1. Polyadenylation:** PABPN1 interacts with poly(A) polymerase to stimulate processive polyadenylation; **2. Regulation of poly(A) tail length**: PABPN1 interacts with the cleavage and polyadenylation specificity factor (CPSF) to modulate and ensure proper poly(A) tail length; and **3. Poly(A) RNA export:** Although whether the role is direct or indirect is unknown, defects in PABPN1 function can lead to nuclear accumulation of poly(A) RNA. This observation together with the fact that PABPN1 shuttles between the nucleus and the cytoplasm have led to the suggestion that PABPN1 function is required for efficient poly(A) RNA export from the nucleus. **PABPC (blue circle)** plays a well-defined role in modulating gene expression including: **4. Translation:** PABPC binds to eukaryotic translation initiation factor 4G (eIF4G), which bridges interactions between the 5'- and 3'-ends of the mRNA and facilitates efficient translation initiation and **5. mRNA decay:** PABPC binds to eukaryotic release factor 3 (eRF3), which facilitates ribosome recycling and thus inhibits mRNA decay by protecting the poly(A) tail from decapping enzymes (Dcp1 and Dcp2) as well as

exonucleases such as the Ccr4-Pop2-Not complex. The following factors are also incorporated into the model shown: Cap binding proteins 20 and 80 (CBP20/80); 7 methylguanosine cap (m^7G); eukaryotic translation initiation factor 4E (eIF4E); eukaryotic small ribosome (40S); eukaryotic translation initiation factor 4A (eIF4A); eukaryotic ribosome (80S); and eukaryotic release factor 1 (eRF1).

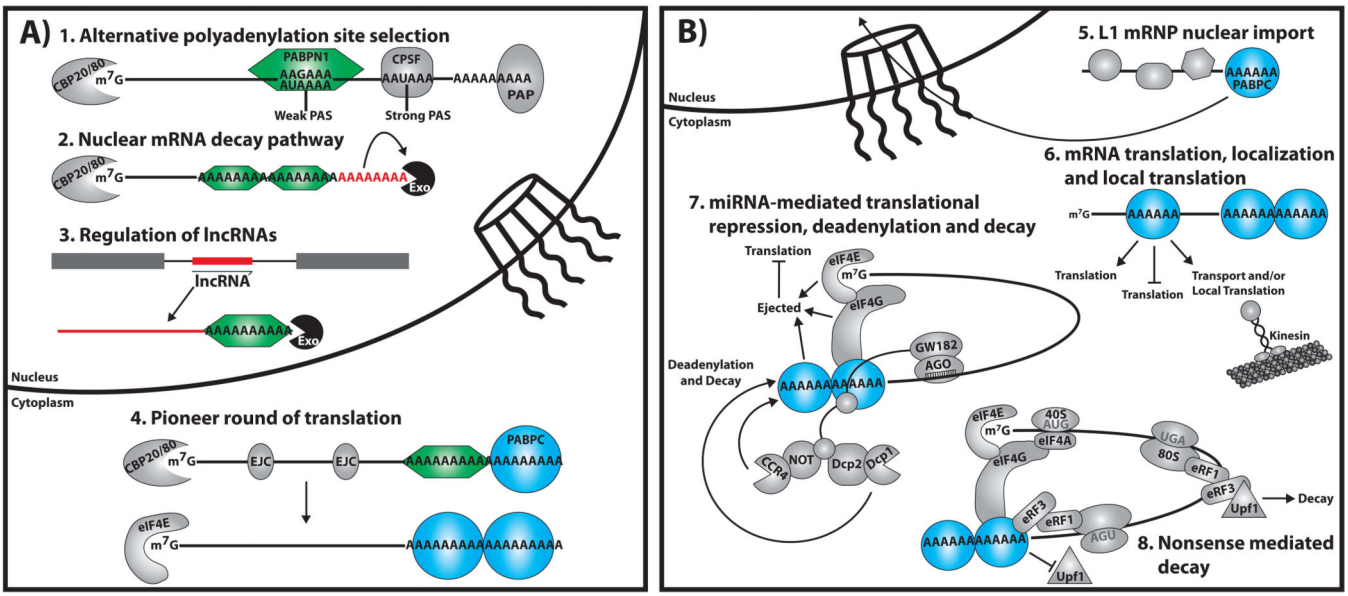

Figure 3. Model for Novel Functions of PABPN1 and PABPC

A) Recent studies reveal that **PABPN1 (green hexagon)** plays diverse roles in modulating gene expression that extend beyond those shown in Figure 2 including impacting: **1. Alternative polyadenylation site selection:** Loss of PABPN1 function leads to preferential use of proximal PolyAdenylation Sites (PAS), which are often weak sites relative to the commonly used distal sites; **2. Nuclear mRNA decay pathway:** PABPN1 coordinates with other RNA processing factors (not shown) to generate hyperadenylated mRNAs (indicated by red As) that target the transcript for degradation via a novel nuclear mRNA decay pathway; **3. Regulation of lncRNA:** PABPN1 also promotes the turnover, via the RNA exosome (Exo), of a specific subset of long noncoding RNAs (lncRNAs) in an oligoadenylation-dependent manner; and **4. Pioneer round of translation:** PABPN1 remains bound to mRNAs as they exit the nucleus and enter the pioneer round of translation. PABPN1 likely cooperates with the exon junction complex (EJC) and PABPC and is replaced by PABPC following the pioneer round of translation. **B)** Recent work reveals that **PABPC (blue circle)** is required for a number of functions beyond control of translation and regulation of mRNA decay illustrated in Figure 2. These functions include: **5. L1 mRNP nuclear import:** Although the mechanism is unknown, PABPC is required for the nuclear accumulation of the LINE1 Ribonucleoprotein (L1 RNP); **6. mRNA translation, localization, and local translation:** PABPC can modulate the translation and may modulate the localization and local translation of specific mRNA transcripts by binding to internal A-rich sequences; **7. miRNA-mediated translational repression, deadenylation, and decay:** PABPC promotes RNA-induced silencing complex (RISC) recruitment by binding to the RISC protein, GW182, which leads to the removal of PABPC, disruption of the closed loop structure and deadenylation by recruited deadenylase complexes; and **8. Nonsense mediated decay:** PABPC inhibits recruitment of the nonsense mediated decay protein, Upf1, by interacting with eukaryotic release factor 3 (eRF3). The following factors are also incorporated into the model shown: Cap binding proteins 20 and 80 (CBP20/80); 7 methylguanosine cap (m^7G); cleavage and polyadenylation specificity factor (CPSF);

Poly(A) polymerase (PAP); eukaryotic translation initiation factor 4E (eIF4E); eukaryotic small ribosome (40S); eukaryotic translation initiation factor 4A (eIF4A); eukaryotic ribosome (80S); and eukaryotic release factor 1 (eRF1).

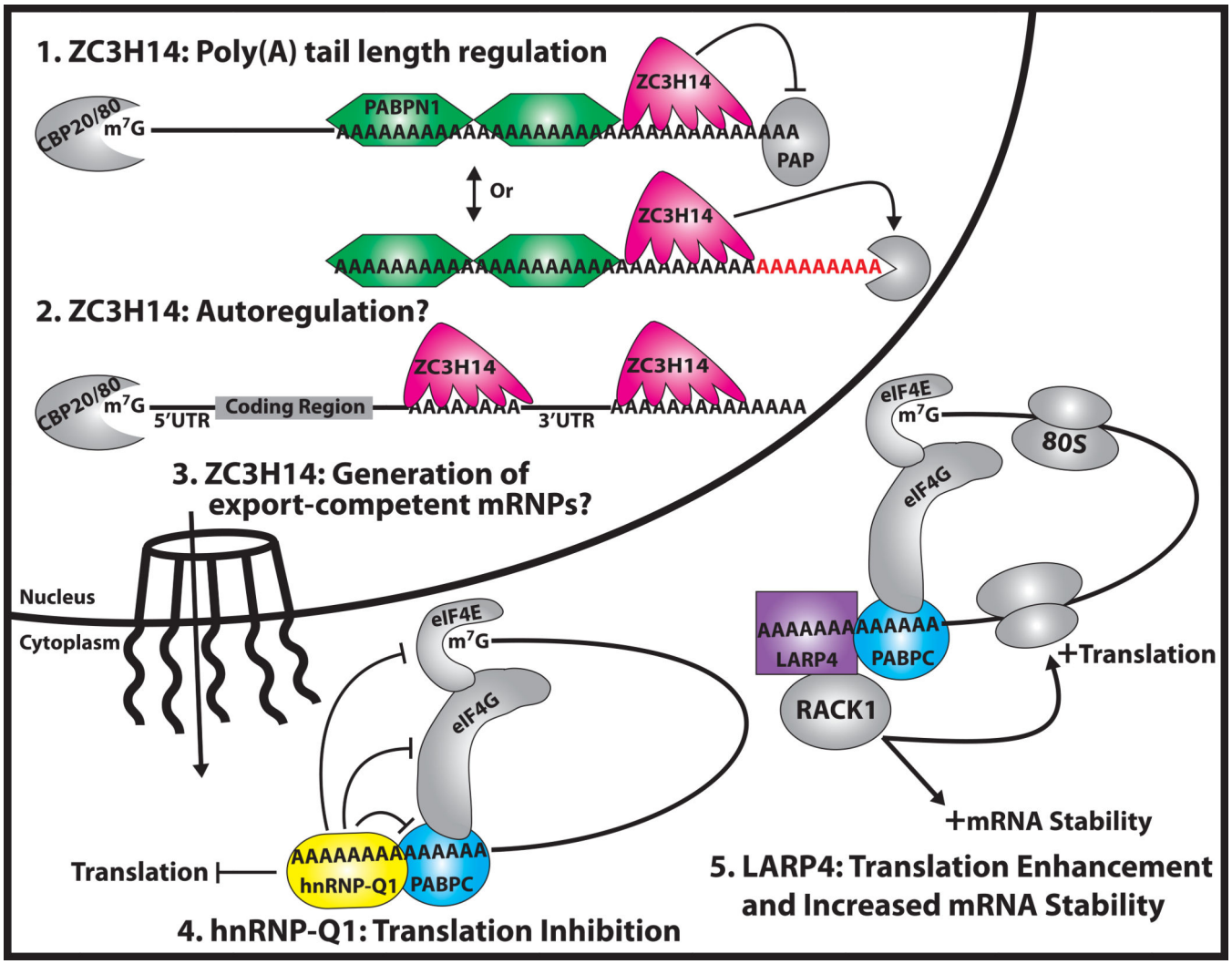

Figure 4. Model for the Function of Novel Pab Family Members

The functions proposed for the new members of the Pab family described here (ZC3H14, hnRNP-Q1, and LARP4) are illustrated. The novel nuclear zinc finger Pab, **ZC3H14 (pink five-fingered shape)** plays a role in **1. poly(A) tail length regulation:** ZC3H14 could limit poly(A) tail length either by inhibiting Poly(A) Polymerase (PAP) or by recruiting a 3'–5' exonuclease (grey Pac-man); **2. Autoregulation:** Like its *S. cerevisiae* counterpart, Nab2, ZC3H14 may bind to and autoregulate its own mRNA transcript via an A15 stretch present in the 3'UTR; and **3. Generation of export-competent mRNPs:** ZC3H14 could play a direct role in the generation of properly packaged mRNPs that are poised for export but most data to support this function comes from studies of S. cerevisiae Nab2. Alternatively, proper polyadenylation could be required to assemble export-competent mRNPs and the role for Nab2/ZC3H14 could be indirect. The novel cytoplasmic Pab, **hnRNP-Q1 (yellow ellipse)**, plays a role in **4. Translation inhibition:** hnRNP-Q1 competes with PABPC for binding to poly(A) tails and consequently preventing the formation of the translation initiation complex. The other novel cytoplasmic Pab, **LARP4 (purple rectangle)**, is implicated in **5. Translation enhancement and increased mRNA stability:** LARP4 interacts with PABPC

as well as the ribosome-associated protein, RACK1, to positively modulate mRNA translation and decay. The following factors are also incorporated into the model shown: Cap binding proteins 20 and 80 (CBP20/80); 7 methylguanosine cap (m^7G); eukaryotic translation initiation factor 4E (eIF4E); eukaryotic translation initiation factor 4G (eIF4G); and eukaryotic ribosome (80S).

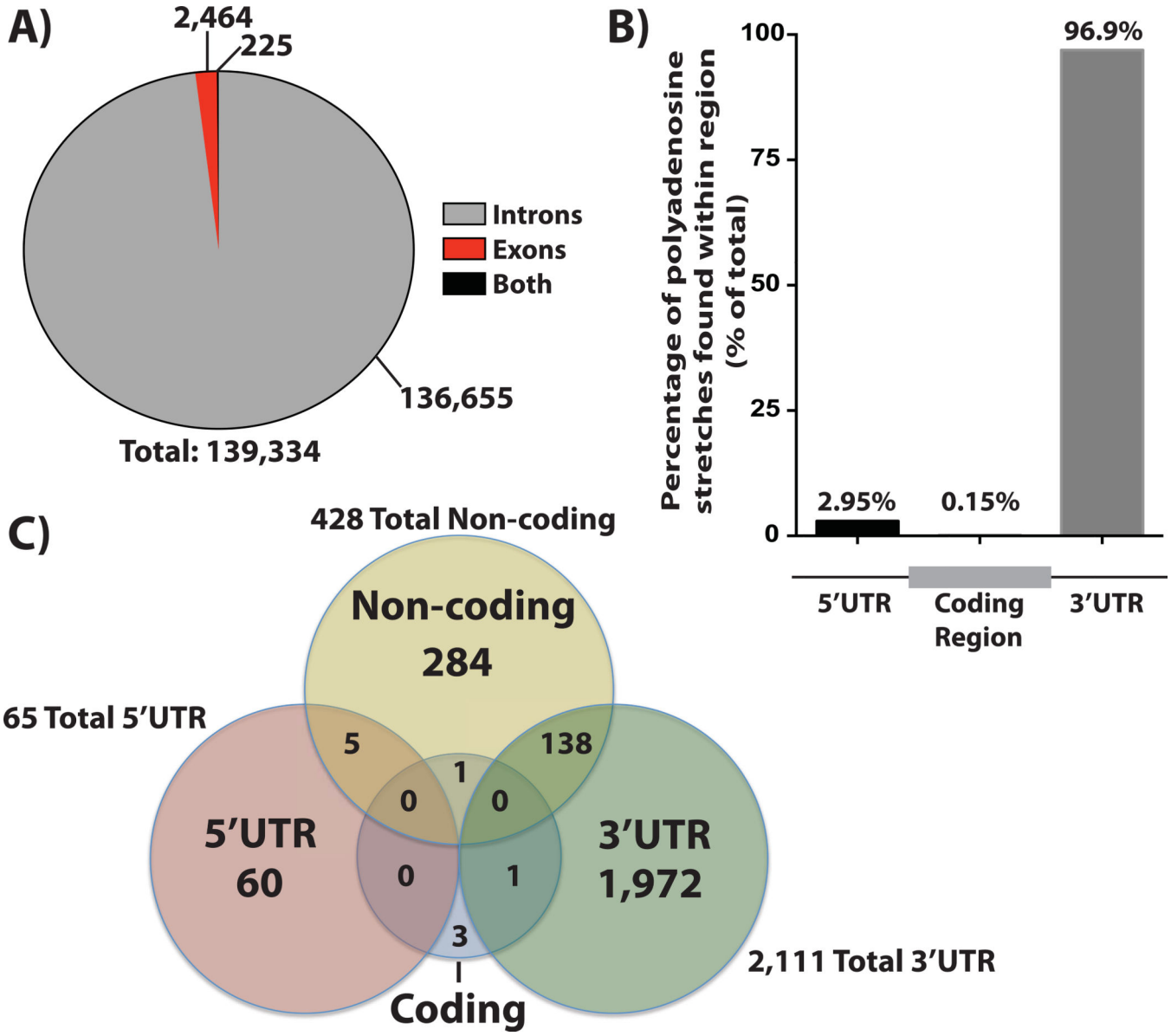

Figure 5. Prevalence and Location of Templated, Internal Polyadenosine Stretches Within the Human Transcriptome

A) Analysis of the human transcriptome for the frequency and enrichment of internal polyadenosine stretches containing at least 12 consecutive adenosines reveals that the vast majority (136,655 out of 139,334) of these sequences are located in the introns of mRNAs whereas a much smaller fraction are located in exonic regions (2,464 out of 139,334), which includes 5' and 3'UTR regions. A much smaller number of these internal adenosine stretches are found in sequences that can be either introns or exons as a result of alternative splice variants. **B)** Internal polyadenosine stretches found in exonic sequences are almost exclusively located in untranslated regions. Further analysis of the ≥12nt polyadenosine sequences that occur in mature mRNA transcripts reveals that almost all (99.9%) of these instances are located in the UTRs, with an extremely large percentage present in the 3'UTRs (96.9%) of mRNA transcripts. **C)** Non-coding RNAs also include templated stretches of

polyadenosine. A number (428) of noncoding RNAs (yellow circle) contain templated polyadenosine sequences, suggesting that Pabs could modulate non-coding RNAs through this templated binding site. Consistent with a model of 3' UTR regulation, a large number of the internal polyadenosine stretches identified in ncRNAs (138) are transcribed from regions that correspond to the 3'UTRs of other/host genes (yellow and green overlap).

Table 1

PABPC Family Members

PABPC homologs present in *H. sapiens* are listed. The mRNA expression pattern of each homolog, as described by the UCSC Genome Browser, is listed. Known protein functions and percent protein similarity to PABPC1 are indicated.

Gene Name	Expression	Known Functions	Similarity
PABPC1[164]	Ubiquitous	mRNA Translation, mRNA Decay, miRNA-mediated Repression, NMD, L1 Retrotransposition, mRNA Localization, Local Translation	--------
PABPC3[165]	Testes-specific	Spermatid mRNA Translation	64%
PABPC4[165]	T Cells & Other Tissues	Erythroid Differentiation, Upregulated upon T Cell Activation, PABPC1 Compensation	67%
PABPC5[166]	Fetal Brain & Other Adult Tissues	PABPC1 Compensation	7%
PABPC1L[167]	Ovaries, Testes & Other Adult Tissues	Oocyte mRNA Translation	59%

Table 2

Poly(A) Binding Protein Family Members

The protein names of Pab family members discussed are shown. The names of the corresponding proteins in a number of common model organisms are provided. Proteins in parentheses have not been extensively studied in the organisms indicated. A dashed line indicates no known/obvious/described ortholog present in that organism.

		Poly(A) Binding Proteins				
Species	***H. sapiens***	**PABPN1**	**PABPC1**	**ZC3H14**	**hnRNP-Q1**	**LARP4**
	M. musculus	Pabpn1	Pabpc1	Zc3h14	Syncrip	(Larp4a)
	X. laevis	pabpn1	pabpc1a/b	(zc3h14)	(syncrip)	(larp4a/b)
	D. rerio	(pabpn1)	pabpc1a/b	(zc3h14)	(syncrip)	(larp4a/b)
	D. melanogaster	Pabp2	PABP	dNab2	Syncrip	(Larp4)
	C. elegans	PABP-2	PAB-2	SUT-2	HRP-2	(LARP-5)
	S. pombe	pab2	pabp	nab2	-----	-----
	S. cerevisiae	-----	Pab1	Nab2	-----	-----