RNA BIOLOGY
2021, VOL. 18, NO. 2, 259–274
https://doi.org/10.1080/15476286.2020.1868753

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REVIEW

LARP1 and LARP4: up close with PABP for mRNA 3′ poly(A) protection and stabilization

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ABSTRACT

La-related proteins (LARPs) share a La motif (LaM) followed by an RNA recognition motif (RRM). Together these are termed the La-module that, in the prototypical nuclear La protein and LARP7, mediates binding to the UUU-3′OH termination motif of nascent RNA polymerase III transcripts. We briefly review La and LARP7 activities for RNA 3′ end binding and protection from exonucleases before moving to the more recently uncovered poly(A)-related activities of LARP1 and LARP4. Two features shared by LARP1 and LARP4 are direct binding to poly(A) and to the cytoplasmic poly(A)-binding protein (PABP, also known as PABPC1). LARP1, LARP4 and other proteins involved in mRNA translation, deadenylation, and decay, contain PAM2 motifs with variable affinities for the MLLE domain of PABP. We discuss a model in which these PABP-interacting activities contribute to poly(A) pruning of active mRNPs. Evidence that the SARS-CoV-2 RNA virus targets PABP, LARP1, LARP 4 and LARP 4B to control mRNP activity is also briefly reviewed. Recent data suggests that LARP4 opposes deadenylation by stabilizing PABP on mRNA poly(A) tails. Other data suggest that LARP1 can protect mRNA from deadenylation. This is dependent on a PAM2 motif with unique characteristics present in its La-module. Thus, while nuclear La and LARP7 stabilize small RNAs with 3′ oligo(U) from decay, LARP1 and LARP4 bind and protect mRNA 3′ poly(A) tails from deadenylases through close contact with PABP.

Abbreviations: 5′TOP: 5′ terminal oligopyrimidine, LaM: La motif, LARP: La-related protein, LARP1: La-related protein 1, MLLE: mademoiselle, NTR: N-terminal region, PABP: cytoplasmic poly(A)-binding protein (PABPC1), Pol III: RNA polymerase III, PAM2: PABP-interacting motif 2, PB: processing body, RRM: RNA recognition motif, SG: stress granule.

ARTICLE HISTORY
Received 22 August 2020
Revised 6 December 2020
Accepted 17 December 2020

KEYWORDS
Deadenylation; poly(A) phasing; pabp; pam2; mlle; ccr4

Introduction

The family of La-related proteins (LARPs) share a 'La-module' consisting of two tandem RNA-binding domains: a La motif (LaM) and an RNA recognition motif (RRM) [1]. Although RRM domains are present in hundreds of genes in vertebrates, the LaM is rare and the La-module is found only in the five subfamilies of LARP proteins: 1, 3, 4, 6 and 7. In humans, there are seven LARP genes: LARP1, 1B, 3, 4, 4B, 6 and 7 (1B and 4B arose by relatively recent gene duplications of 1 and 4) [1,2]. Among many potential isoforms, one major mRNA species represents each LARP examined in the cells tested [3].

Diagrams of the seven LARPs are shown in Fig. 1. The ancestral LARP is the nuclear La protein (aka LARP3) that binds the oligo(U) 3′ends of precursor-tRNAs and other nascent RNA polymerase III (Pol III) transcripts and protects these from digestion by 3′ exonucleases [4–6] (**reviewed in** [7,8]). LARP7 is nuclear and a closely related La paralog that binds and protects a subset of RNAs with 3′ oligo(U) [9–11]. LARPs 1, 4 and 6 are highly divergent and reside in the cytoplasm. Evidence of LARP4 and LARP1 in mRNA 3′ poly(A) tail metabolism became apparent more recently [12,13]. While LARP1 has been well characterized as an mRNA translational repressor [14–18] whereas LARP4 (and 4B) promotes mRNA translation [12,19], recent data suggest that the La-module related activities of these LARPs may be more similar than was initially apparent. LARPs 1 and 4 both exhibit 3′ poly(A) tail length protection-mRNA stabilization associated with their La-modules and dependent on interactions with cytoplasmic poly(A)-binding protein (PABP, aka PABPC1). For both, PABP-binding is mediated by the PABP-interacting motif-2 (PAM2) adjacent to the La-module.

PAM2 motifs bind to a conserved binding site on the MLLE domain of PABP [20]. A summary of proteins containing PAM2 motifs is presented in Table 1. The PAM2 motif of LARP4 is unique with a tryptophan at position-10 that contributes to both poly(A) RNA and PABP binding [12,21]. The PAM2 within the La-module of LARP1 identified by Fonsesca et al. [14]. was recently characterized and the importance of an N-terminal residue for high affinity binding to PABP demonstrated [22]. Consideration of LARPs 1 and 4 as factors that bind and protect mRNA 3′ poly(A), dependent on PABP

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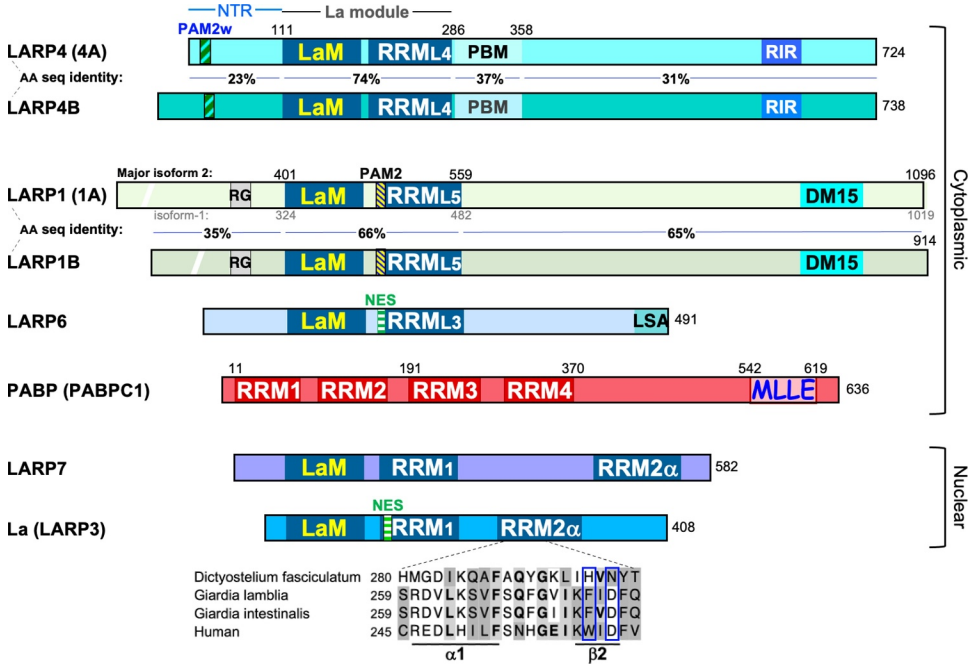

Figure 1. Linear schematic features of the human proteins relevant to this review. LARPs 4 and 4B are distinct gene products, as are LARPs 1 and 1B. For each of these pairs, the % amino acid (AA) sequence identity of different subregions is indicated. For LARP1 the numbering is shown for isoform 2, a dominantly expressed form [3] (the isoform 1 numbering is also indicated; isoforms −1 and −2 differ in their N-terminal regions only and are identical beginning with aa 68 of the former). The PAM2 sequence FSQLLNCPEFVP [22] begins towards the end of the interdomain linker between the La motif (LaM) and predicted RRM of LARP1 and continues through the predicted β1 strand of the RRM [1]. This PAM2 is extended on its N-terminus by a key residue, F419 relative to that previously noted [14]. Curiously, this PAM2 resides in a very similar relative position as a nuclear export sequence (NES) in the β1 strand of the LARP6 RRM [23–25]. A functionally mapped NES in the α1 helix of the La protein La-module RRM is also shown [45]. It should be noted that LARP6b and c homologs in plants have functional PAM2 sequences positioned similarly to LARPs 4 and 4B, whereas human LARP6 lacks a PAM2 [26]. An excerpt of a multiple sequence alignment is shown under the human La schematic RRM2α (the C-terminal RRM has also been referred to as RRM2 and xRRM, see text). These aligned sequences were obtained as the only full length La protein homologs in the single-cell organisms indicated; demarcation of the secondary structure elements corresponding to α1 and β2 under the sequence were from Jacks et al [68].; the vertical rectangles indicate conservation of RNP3 residues (F/Y/W/H)-x-(D/Q/E/N) [71,73]. Abbreviations are as follows. NTR: N-terminal region, LaM: La motif, RRM: RNA recognition motif, PBM: PABP binding motif, RIR: RACK1 interacting region, PAM2: PABP interacting motif-2, RG: conserved region consisting of eight arginine-glycine repeats [2], DM15: 5′TOP motif binding region, NES: nuclear export sequence, MLLE: mademoiselle domain.

albeit by different mechanisms [22], is the objective of this review.

Working on RNA 3′ ends is a tradition in the LARP family

Although La and LARP7 differ from LARP1 and 4, an overview of their activities and functions is fitting. The La-modules of LARP7 and La are relatively conserved as compared to the other LARPs (**reviewed in** [33]). A unique feature of La and LARP7 is the presence of a second RRM domain, designated RRM2α, which will be described in the next section. The prototypic La-module recognizes UUU-3′OH in a sequence- and length-dependent manner [5,6] and protects the RNA from 3′ exonucleolytic digestion [34–-34–36] (**reviewed in** [33,37]). Many precursor-tRNAs (and pre-mRNAs) are degraded in wild-type cells by a nuclear surveillance system that detects transcriptional and other errors [38]. La is known to protect pre-tRNAs from the degradative activities of 3′-directed nuclear surveillance while also serving as a chaperone that can assist structurally challenged pre-tRNAs in their correct folding [35,39–42] (**reviewed in** [33,37]). As a molecular chaperone [43] with nuclear retention activity [44–46], La also prolongs the time window of protection for maturation events that stabilize local folds for formation of the correct tRNA structure.

RNA association with La is transient and its dissociation can be critical to the RNA maturation pathways for tRNAs, the spliceosomal U6 snRNA, and 7SK RNA (related to P-TEFb [9–11], below) [43,47,48] (**reviewed in** [49,50]). Pre-U6 snRNA undergoes multiple enzymatic 3′ end processing activities and involving La protein before acquiring its mature form as a core component of the spliceosome [51–53] (**reviewed in** [54]). Unlike nascent pre-tRNAs and pre-U6, the nascent 7SK RNA does not undergo 3′ processing but is likely transferred as UUU-3′OH from La to LARP7 (**see** [49]). Also, unlike pre-tRNAs, the 5′ ends of 7SK, U6 and some other Pol III transcripts are not removed by processing but are capped with a monomethyl moiety on the gamma (γ) phosphate (ppp-CH_3), which decreases the affinity for La [49,54,55] and presumably facilitates movement along their maturation pathways [49,54].

The La-module of LARP7 binds UUU-3′OH while its RRM2α stably binds a hairpin specific to 7SK RNA. This enables LARP7 to function in controlling P-TEFb (positive transcription elongation factor-b) [9–11] which activates elongation of Pol II that is paused near the transcription start site and regulates a large number of mRNA as well as snRNA and snoRNA

Table 1. PAM2 proteins, alignment of their PAM2 sequences, and their properties.

Protein	PAM2 sequences[1]	Affinity for PABP MLLE (K_d, μM)[2]	Structure (PDB)	Another PABP-binding region	Properties/Function	SG[3] or PB[4]	Refs
position:	1 3 7 *	**3.8**			Translation inhibition of 5′TOP mRNAs and stabilization, poly(A) length protection	SG	[14,22]
LARP1	FSQLLNCPE**F**VP						
LARP1B	FSQLIDCPE**F**VP						[22]
eRF3 C	FV**P**NVH**A**AE**F**VP	**3.1**[2]	3KUJ		Translation termination		[99,113]
NFX1	FK**F**NTD**A**AE**F**IP				Regulates telomerase levels		[27]
PAIP1	SK**L**SVN**A**PE**F**YP	**1.4**	3NTW	yes, PAM1	Translation enhancement		[28,29]
PAIP2	SN**L**NPN**A**KE**F**VP	**0.2**	1JGN/3KUS/ 3KUT	yes, PAM1	Translation inhibition	SG	[99,113]
PAIP2B	SN**L**NPD**A**KE**F**IP			yes, PAM1	Translation inhibition	SG	[30]
USP10	ST**L**NPQ**A**PE**F**IL	**26**			Ubiquitin specific peptidase-10, protein stability	SG	[144]
Tob2 265	SQ**L**SPN**A**KE**F**VY				Associates with CCR4-CAF1-NOT1 deadenylation complex		[138,144]
Tob1 265	SA**L**SPN**A**KE**F**IF						[98]
ATXN-2	ST**L**NPN**A**KE**F**NP	**0.7**	3KTR		Translation regulation, deficiency associated with spinocerebellar ataxia	SG	[106,144]
LARP4B	SE**L**NPN**A**EV**W**GA		3PTH	yes, PBM	mRNA stabilization, translation	SG	[37,100,178]
LARP4	TG**L**NPN**A**KV**W**QE	**22**	3PKN	yes, PBM	mRNA stabilization, 3′ poly(A) length protection, opposes deadenylation	SG	[12]
TTC3/TPRD	LQ**L**NPA**A**RE**F**KP	**5.4**			Neurodegenerative down syndrome		[144]
eRF3 N	RK**L**NVN**A**KP**F**VP	**3.9**[2]	3KUI		Translation termination		[99,113]
Tob2 125	SS**F**NPD**A**QV**F**VP	**16**			Associates with CCR4-CAF1-NOT1 deadenylation complex	PB	[138,144]
Tob1 125	NS**F**NPE**A**QV**F**MP						[98]
PAN3	QT**P**NPT**A**SE**F**IP	**40**			Deadenylase subunit	PB	[87,145]
TNRC6A (GW182)	.....WPPE**F**RPGEPWKGY			yes	Deadenylation, miRISC assembly		
TNRC6B	.....WPPE**F**QPGVPWKGI			yes			
TNRC6C	.....WPPE**F**HPGVPWKGL	**6**	2X04/3KTP	yes	Deadenylation, miRISC assembly	PB	[31,32,106]

[1]The PAM2 consensus positions are indicated by numbers above the aligned sequences except position-10 which is indicated by an asterisk. Position-10 is the most important for high affinity binding to the MLLE of PABP [113] **(reviewed in** [91])(see text). Position-3 typically has leucine (L), is sometimes phenylalanine (F) or proline (P), but in LARP1 and 1B is glutamine (Q). Leucine-3 was found to be the second most important for high affinity binding to the MLLE of PABP [113]. Position-10 is always F except in LARP4 and 4B in which it is tryptophan (W).

[2]The two overlapping PAM2s of eRF3, -C and -N, together provide a K_d of 1.0 μM.

[3] Stress granule (SG).

[4] Processing body (PB).

transcripts [56] (**see** [49]). Cellular levels of P-TEFb activity are negatively regulated by binding to 7SK RNA [57]. LARP7 is a stably bound subunit of 7SK RNP which serves as a reservoir of P-TEFb activity, the latter of which can dissociate and bind on cue [9–11]. LARP7 levels determine 7SK RNA levels.

LARP7 also associates albeit transiently with metazoan U6 snRNA and thereby directs its 2′-*O*-methylation by a sequence-specific subfamily of box C/D snoRNPs [58,59] (**reviewed in** [49]). In this capacity, LARP7 binds U6 via its La-module and simultaneously binds the guide-snoRNA via its RRM2α. This allows it to function as a strand-annealing chaperone by assisting the complementary base pairing of the U6 and snoRNA [58].

La and LARP7 are involved in RNA 3′ end metabolism and may have co-existed in an ancient nucleated organism [60]. Other LARPs diverged within and beyond their La-modules [1,2]. LARPs 1 and 4 are highly divergent, yet their La-modules appear to exhibit surprisingly similar activities in RNA 3′ poly(A) tail metabolism [22].

La-module evolution

The milestone analysis of LARPs revealed that while their LaMs were highly conserved, they were segregated into five families on the basis of their RRM domains [1]. The RRM is considered an RNA-binding platform with 'extreme structural versatility,' comprised of a core of secondary structure elements with variations and extensions [61]. More than 500 high-resolution RRM structures have been resolved, ~20% of which include a bound RNA [62]. Several atypical RRMs subtypes with distinctive features have evolved [63]. Different RRMs can exhibit unique RNA binding specificities, e.g., individual members of the LARP7 family (below).

Sequence alignments and predicted secondary structure elements of the LARPs [1], benefited from high-resolution RRM structures including unexpected features of La protein bound to UUU-3′OH [6,64–66]. The RRMs in La and LARP7 La-modules were considered classic, whereas those in LARPs 6, 4 and 1 were variably divergent, termed RRM-L3, -L4, and -L5, respectively [1]. Further analysis suggested that the LaM and RRM coevolved with the various LARPs [1]. High-resolution structures for LARPs 4, 6, and 7 later emerged and while not all secondary structure elements aligned with or matched the initial predictions, the models generally fit. A suitable example is that despite strong evolutionary conservation of the La-modules of La and LARP7, a structure of the latter revealed only three β strands in its RRM1 [67] instead of the canonical four as in La (**see** [33]).

Phylogenetics indicate a La gene duplication in an ancestral eukaryote and that some alleles had one RRM and others had two. The C-terminal RRMs of LARP7 and La were named RRM2 [68–70] but have since been referred to as RRM2α [8,33] because of the most prominent feature of this atypical RRM, its α3 helical extension (Fig. 1). Two types of genuine La protein and two types of LARP7 exist in representatives of ancient eukaryotes, each with and without an RRM2 [1,2]. This suggests that an intragenic RRM duplication accompanied a gene duplication and raises the possibility that the two RRMs could have exchanged places during La-module evolution. Yeast La proteins lack RRM2 [8] and their La-module RRMs were noted to exhibit characteristics of RRM2α [45,71].

Certain atypical features of RRM2α appear to have differentially evolved in lineages of La and LARP7 homologs, so much so that it is referred to as xRRM in the latter [71,72]. RRM2α is unique to LARPs, found only in La and LARP7 homologs which include the telomerase RNA-associated p43 and p65 in ciliates [73–75] and Pof8 in *S. pombe* [60,72,76]. Because ciliates contain huge numbers of linear minichromosomes with telomeres, they require high levels of telomerase RNA which is synthesized by Pol III [77,78], the polymerase specialized for high output of short transcripts [79]. The La-module of p65 binds UUU-3′OH and its xRRM binds to a hairpin on the Tel-RNA with high affinity and sequence-specificity [80].

The α3 extension of a classic RRM was discovered as an integral element that lies across the RRM β-sheet surface of the human La RRM2 [68]. Multiple additional features of this atypical RRM were also noted: i) a five stranded β-sheet, unprecedented at the time, ii) lack of aromatic residues at conserved positions in the β3 and β1 strands of the RNP1 and RNP2 motifs, and iii) enrichment of acidic residues in the strands [68]. Structures of p65 and LARP7 bound to their different sequence-specific RNAs revealed these features along with a key RNP3 motif on β2 and a conserved arginine on β3 that now appear distinctive of xRRM [71,73,74,80]. RNP3 is a short sequence motif, often including an acidic residue, on the β2 strand that participates with α3 in RNA binding [71].

Distinctive among the RRM2α domains is that the α3 helices of LARP7 and p65 RRMs are partially unstructured in the absence of RNA but become extended upon binding their sequence-specific ligands, and mutations that prevent this decrease their high affinity RNA binding (**reviewed in** [72]). Differences in the amino acid composition of α3 in p65 and LARP7 appear to contribute to their RNA-binding sequence specificity [72]. Thus, differential α3 features contribute to high affinity and recognition-specificity for their different cognate RNAs, whereas Pof8 and La protein appear to be towards the other end of the α3 helix length spectrum [72]. Consistent with previous perspectives [33], the RRM2α domains exhibit distinctive and interesting features in their various host proteins.

Some atypical features of La RRM2α may be discerned by sequence alignment and structure prediction. Sequence conservation of RNP3 on β2 of La RRM2α extends from human to flies (**R.M., unpublished, see** [72,74]). Sequence evidence of α3 in the La RRM2α of *Giardia, Dictyostelium* and other deep rooted eukaryotes was reported [8,33]. Evidence of an RNP3 motif is also found in La proteins of some *Giardia* and *Dictyostelium* species (Fig. 1).

Features of RRM2α of La and LARP7 were recently examined, including the nature of the β strand-α3 interactions [72]. Notably, the La RRM2α, which bears a relatively short α3 (in absence of RNA) and lacks a β3-arginine that may otherwise contribute to RNA binding, is consistent with its quite low affinity for RNA and reported broad substrate recognition ([72] **and refs therein**). Although La requires its RRM2α for binding to hepatitis C virus domain IV RNA, no sequence-

specificity could be found [81]. Also, despite its evolutionary conservation and apparent correlation of the presence of an RRM2α in La protein and the essentiality of that La protein for viability in the species examined (**see** [8]) no sequence-specific RNA ligand of any La RRM2α has been identified.

Additional evidence that the RRM1 of the prototypic La-module and the RRM2α share evolutionary roots is the very unusual asymmetric function of their β-sheets. The β2 strand is the major component of the β-sheet for RNA binding by RRM1 [6,64] and by the RRM2α/xRRM [73,80]. Neither RRM1 of prototypic La and LARP7 nor the RRM2α /xRRM of LARP7 and p65 interact with their RNA ligands through their β-sheet. Instead, they principally use the β2 strand to interact with RNA [6,71,73]. In RRM2α, the α3 helix lies across much of the β-sheet surface, forcing RNA interactions towards the edge defined by the β2 strand and its RNP3 amino acids [80].

The idea that the two RRMs could have exchanged during early LARP evolution is consistent with the observation that RRMs L3-L5 are deficient in RNP1 and RNP2 conserved residues [1]. The LARP4 La-module RRM lacks RNP1 and RNP2 conserved residues and binds RNA with low affinity [21]. Although LARP6 La-module RRM contains RNP1 and RNP2 residues, they appear blocked from RNA binding by contacts with a nearby α-helical element, somewhat reminiscent of RNP1 and RNP2 position interactions with α3 in xRRM [72].

While La and LARP7 are highly related and appear to have evolved in parallel, they provide different types of function. La interacts transiently with its RNA ligands and is not part of a stable RNP, whereas LARP7 homologs bind stably to RNAs and other proteins as part of stable RNPs. LARPs 1 and 4 appear much more divergent, yet exhibit similar type activity, each via stable binding to PABP involved in mRNA 3′ poly(A) related functions. We review PABP next, the central factor and integrator of mRNA translation, deadenylation and decay activities.

PABP helps form and stabilize translation complexes

PABP is a multifaceted factor that promotes efficient translation by facilitating multiple steps in the process [82],[83],[84], ([85]'and refs therein). It contributes to stable assembly of the pre-initiation complex comprised of cap-dependent translation initiation factors. It is also centrally involved with various factors in the stabilization and decay of mRNAs as well as translation termination [86]. In the latter case, interaction with translation termination factor, eRF3, was linked to mRNA stability through PABP [87].

PABP binds poly(A) with its four RRMs in an ordered manner; multiple PABP molecules oligomerize on poly(A) in a length-dependent manner with a footprint of ~27 nucleotides [88–90]. PABP-PABP interactions stabilize its binding to poly-(A) [88]. Following the four RRM domains, PABP contains a mademoiselle (MLLE) domain (aka PABC) to which various PAM2-containing proteins can dock [91] (Figs. 1 & 2).

An example of an intricate PABP pre-initiation complex network follows. Eukaryotic initiation factor eIF4G interacts via the backside of the β-sheet RNA-binding surface of PABP RRM2 [92,93] while the β-sheet is bound to the mRNA poly-(A) tail. In the pre-initiation complex, eIF4G is bound to eIF4E, the 5′ m^7Gppp-cap binding protein (**reviewed in** [94]) a central regulatory point factor in health and disease [95–97]. Thus, PABP bridges the 5′-end and the 3′-poly(A) of an mRNA. Note that because this PABP-eIF4G interaction

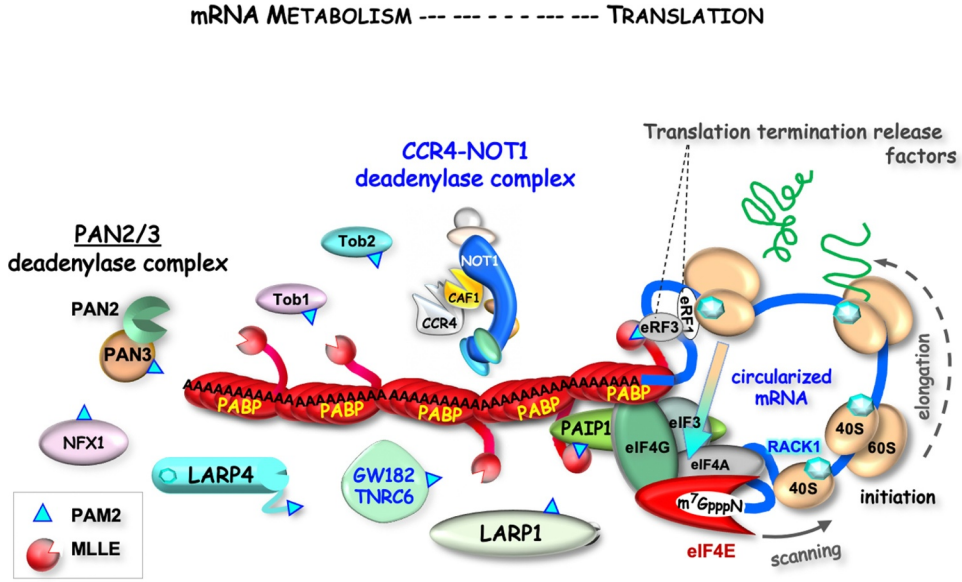

Figure 2. Schematized cartoon model of closed-loop mRNP-poly(A)-oligomerized-PABP. The 3′ end metabolism and translation activities of a closed-loop mRNP with a poly(A) tail of ~150 nucleotides bound by five PABP molecules is depicted. The 40S and 60S ribosome subunits and other factors are not to scale. Several of the known factors with a PAM2 motif, the latter depicted as small blue triangle, are shown. The MLLE domain tethered to RRM4 of PABP which binds to the PAM2 peptides is shown, as well as factors associated with the circularized mRNA according to the closed-loop model of high efficiency translation [87,91,107,108,115,190,192]. The figure depicts a fixed poly(A) length although as discussed, as deadenylation occurs a limiting number of MLLE-PABP binding sites may focus competing PAM2 proteins, for example for mRNA pruning [111]. Note that PAM2 proteins known to reside proximal to the translating mRNA, e.g., eRF3 tend to exhibit relative high affinity for MLLE (Table 1), whereas PAN2/3 and Tob2, known to work more distally have lower affinity.

does not involve the MLLE, the latter is free to interact with PAM2-proteins. Other proteins increase the potential for interconnecting contacts in 'closed-loop' mRNA-ribonucleoproteins (mRNPs) which are recognized as efficiently translated (Fig. 2, right side). Moreover, the multiple PABPs on the poly(A) tail each with a MLLE domain is a potential PAM2–protein interaction site (Fig. 2, left).

PABP may integrate PAM2-associated activities via its MLLE domain

While PABP interacts with different proteins via its RRMs, it binds a larger number via its highly conserved MLLE which has a binding surface for a peptide motif known as PAM2 (Table 1)(**see** [86])' [91]. Alignment of >150 sequences representing multiple proteins revealed 12 amino acids comprising a PAM2 consensus [20,98]. Of the ~20 distinct PAM2-proteins, several are involved in mRNA processing and/or translation, including the deadenylase complex PAN2/PAN3 and TOB1/2 proteins that regulate the CCR4-NOT deadenylase complex (Table 1) [87,99]. Notably, PABP, LARPs 1, 4, 4B [12,100–102] and others also localize to stress granules (SGs) or processing bodies (PBs) (Table 1), in which mRNPs are isolated in a dormant state [103]. PBs contain deadenylated mRNPs, the translation repression and deadenylation protein 4E-T, PAN2/PAN3, CCR4-NOT-Tob1/2, GW182/TNRC6, and notably lack PABP [103–105].

Most PAM2 peptides interact with PABP in a similar way in which the conserved hydrophobic residues at positions 3 and 10 of their PAM2 sequence bind in two hydrophobic pockets separated on the surface of the MLLE [91]. However, the PAM2 or related peptides of some proteins, e.g., eRF3-C (the second of overlapping PAM2s in eRF3, see footnote 2 in Table 1) and GW182 bind MLLE in atypical ways [99,106]. As is reflected in the PAM2 sequence alignment in Table 1, the GW182 binding peptide is homologous only to the C-terminal part of the PAM2 consensus, and structures have shown that it interacts asymmetrically with MLLE relative to other PAM2 peptides. It engages only one of the hydrophobic pockets, leaving the other vacant, and uses its additional downstream residues that have no counterpart in other PAM2-MLLE interactions to bind an extended surface [106].

Binding affinities for PAM2 peptides and the MLLE span a 200-fold range in K_d, from 0.2 to 40 μM [91] (Table 1). Elegant studies provided evidence of competition among three PAM2 proteins *in vivo* with functional outcomes. This led to a model of competition for PABP between translation and mRNA deadenylation factors involving the translation termination release factor eRF3, CCR4-NOT and Pan2/Pan3 in human cells [87]. eRF3 contains overlapping PAM2 sequences that contribute to high affinity MLLE binding [99,107,108]. Further studies of eRF3 led to a model of stepwise assembly of a translation termination complex that is directed in part by PABP [109]. It should be expected that other PAM2 proteins also compete for the MLLE domain of PABP (**also see** [99]).

As noted above, PABP oligomerization on poly(A) can provide multiple docking sites for PAM2 proteins. This suggests potential for poly(A) length-dependent concentration of PABP-interacting proteins around mRNPs (Fig. 2), for example, in cell peripheral regions [110]. Such length-dependence might be relevant to an mRNP pruning model in which short poly(A) tails might limit less competitive PABP-interacting activities and concentrate others for efficient translation and/or protection of translational functions [111]. LARP4 contains a variant designated PAM2w that binds MLLE similarly to most PAM2 proteins but as will be reviewed below also contributes to poly(A) binding [12]. The PAM2 sequence of LARP1 is unique in multiple features and binds with relative high affinity (Table 1) [22]. The PAM2s of LARPs 1 and 1B differ at one position which is highly conserved in each of their lineages [22]. Curiously, the translation repressive proteins PAIPs 2 and 2B also differ in aspartic acid versus asparagine at position-6 (Table 1). The PAM2w sequences of LARPs 4 and 4B differ by 50% which are conserved in their lineages [2].

PAM2w in the LARP4 N-terminal region participates in and directs binding to poly(A) or PABP

LARP4 and LARP4B have a unique variant PAM2 sequence near their N-termini [37] known as PAM2w [12] which contains tryptophan in position-10 [12,100], where all other PAM2 sequences contain phenylalanine [98,112]. Phenylalanine-10 is considered the most important of the PAM2 amino acids for MLLE interaction based on binding affinities of systematic point mutated peptides (position-3 was the second most important), and because of severe binding deficiencies upon mutation of position-10 in various proteins [113] (**reviewed in** [91]). Nonetheless, the LARP4 and 4B PAM2w peptides were found to interact with the same residues on MLLE as other PAM2s, including tryptophan at position-10 [12,100]. PAM2s also reside near the N-termini of LARPs 6B and 6 C of plants[104], [26] similar to LARP4 but contain phenylalanine at position-10 [12,100].

The principal determinant of poly(A) binding by LARP4 is the NTR (amino acids 1–111), of which PAM2w occupies positions 13–24, while the La-module contributes a minor role and this is mostly limited to interactions with the RRM [21]. Multiple analytical approaches indicate that the NTR contains disordered regions and transient helical and β-strand structural elements that were disrupted by PAM2w mutations, suggesting that such structure in the NTR is a required for poly(A) binding [21]. The NTR exists in an equilibrium state that supports rapid sampling of closed-open conformations with regard to interacting with the nearby La-module [21]. Most relevant is that maximal poly(A) binding to the NTR was dependent on W22 in position-10 of the PAM2w. A W22F substitution reduced poly(A) binding affinity more than 20-fold but did not significantly reduce binding to MLLE [21]. Thus, the LARP4-PAM2w can direct NTR binding to either the MLLE or poly(A) suggesting intricacy of LARP4, poly(A) and PABP dynamics [21]. That PAM2w uses Trp to engage MLLE similar to Phe in other PAM2s but in the context of LARP4 NTR which can uniquely bind poly(A) and with 10-fold higher affinity than for MLLE [21], suggests that

this PAM2w is a determinant specialized for mRNA-poly(A)-PABP related function.

A unique PAM2 emerged in LARP1

PABP binding to LARP1 was attributed to a PAM2-like sequence that was identified by visual scrutiny [14]. However, at only eleven residues, lacking the invariant alanine at position-7 [91], and requiring a gap to align the critical L3 and F10 positions, the proposed sequence SQL-LNCPEFVP was very unusual [14]. Nonetheless, mutagenesis of the phenylalanine that was aligned with the PAM2 consensus position-10 decreased the amount of PABP that coimmunoprecipitated with LARP1 [14]. While this is important, this mutation does not exclude that this unusual sequence might represent an atypical/asymmetrical PAM2 such as the GW182 peptide [106] (Table 1).

A recent study characterized the PAM2 of LARP1 and identified an N-terminal Phe missing in the original identification [22]. The study showed the affinity of a LARP1-derived peptide was comparable to other PAM2 peptides. Point mutations of key residues in the LARP1 PAM2 demonstrated its importance in cellular assays of mRNA 3′ poly(A) tail protection and stabilization by full length LARP1 [22]. It was shown that a PAM2 mutation that impaired coimmunoprecipitation of cellular PABP with the LARP1 La-module that also impaired mRNA poly(A) protection-stabilization, did not affect oligo(A) binding by the recombinant, purified La-module [22].

As discussed above, LARPs 1 and 4 independently bind poly-(A) and PABP. Another shared characteristic is association with the ribosome protein mRNAs, which are abundant, efficiently translated and tightly regulated [16,33,114]. As noted previously, LARP1 is a repressor whereas LARP4 promotes mRNA translation. We next discuss features relevant to these LARPs and translation before moving to deadenylation and decay.

Highly expressed and translated mRNAs have short poly(A) tails and other key features

Although *de novo* recruitment of 40S ribosomes to the start codon is considered a measure of translation efficiency [94], post-termination re-initiation in a closed-loop complex may also contribute to high efficiency [115] (Fig. 2). The closed-loop model involves the critical factors, m^7Gppp-cap-eIF4E-eIF4G-PABP-poly(A) [116] although analysis in yeast emphasizes these are not exclusive to closed-loops [85]. LARPs 1 and 4 bind the principal factor PABP and involve interactions at both ends

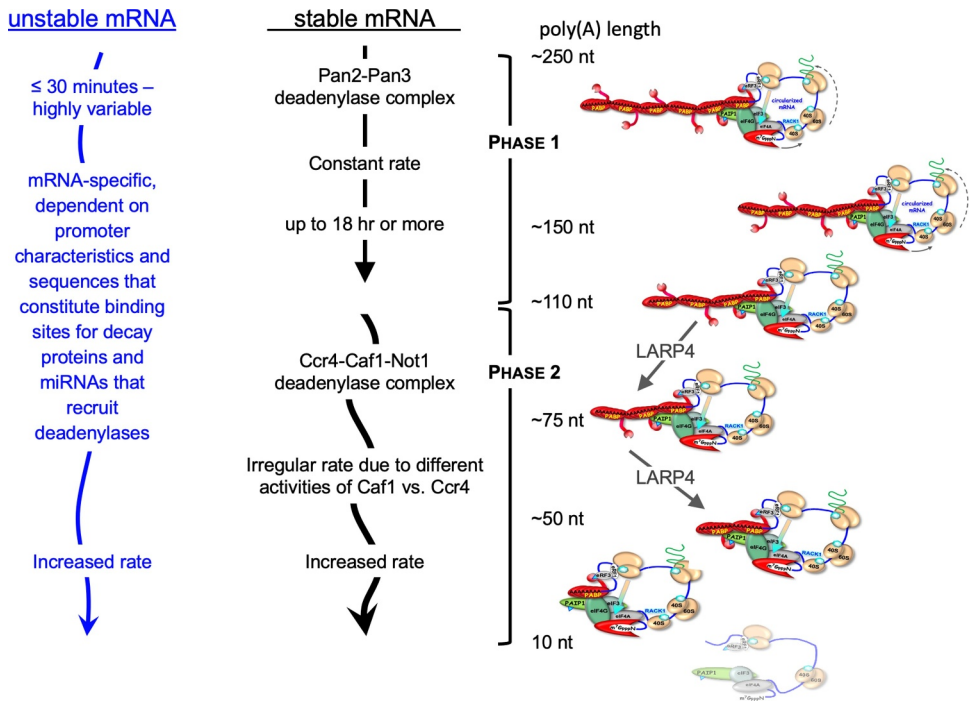

Figure 3. Schematic of model deadenylation pathways in mammalian cells. Model pathways for unstable mRNAs (left) and stable mRNAs (middle) derived from studies of reporter constructs and certain cellular mRNAs (see text). Classic stable mRNAs which have a paucity of destabilizing elements, follow a default pathway with biphasic deadenylation kinetics. The PAN2/3 initiates and carries out the first phase on nascent poly(A) tails of ~250 nucleotides with regular or constant rate depicted by straight downward arrow. After trimming to poly(A) lengths of 150–110 residues the mRNAs become preferred substrates of the CCR4-CAF1-NOT1 deadenylase complex CCR4-CNOT [.125,129] whose rate is irregular as depicted by the curved arrow. Unstable mRNAs, e.g., with AREs and/or miRNA binding sites in their 3′ UTR actively recruit deadenylases. Many mRNAs bear sequence elements or are engaged by other means that lead to active recruitment of the PAN2/3 and/or CCR4-NOT deadenylase complexes **(see** [134] **and refs therein)**. The CCR4 and CAF1 deadenylase subunits of the CCR4-CNOT complex exhibit different types of deadenylase activity; CCR4 is active on poly(A) that is bound by PABP whereas CAF1 is active only on unbound substrate. Such differences as well as loss of cooperative interactions between PABP monomers and progressive instability on poly(A) contribute to the associated irregular and accelerating rates of deadenylation [88,129,143]. mRNA decay and translation rates are also coupled via codon content relative to tRNA activity [143]. Right: Schematized mRNP-PABP complexes with different poly(A) lengths. Diagonal green arrows depict poly(A) lengths representing those found by SM-PAT-seq profiling to be altered in cells in which LARePA was present or absent [149]. Two effects were observed; poly(A) length phasing of ribosomal protein-mRNAs suggested apparent impediment of conversion of mRNPs with presumed four PABPs to three PABPs in the presence of LARP4. Time-course profiling revealed apparent impediment of conversion of mRNPs with three PABPs to two PABPs in the presence of LARP4[149]

(including via the receptor for activated C kinase 1, RACK1 [12,19] below).

Poly(A) tail length is correlated with translation efficiency in early embryos, but not thereafter [117]. The poly(A) tails of the abundant ribosomal protein mRNAs are relatively short at steady state. The short poly(A) tails of such mRNPs are referred to as being maintained in a pruned state as pruning activities may be protective of the efficient translation and stability of these mRNPs [111]. Notable features of highly expressed, translated and closed-loop mRNPs include short ORFs and short poly(A) tails of 30–70 nucleotides [111,115,117]. mRNAs in this class often have short 5′UTRs which facilitate efficient *de novo* initiation [16]. Thus, a combination of features distinguish the ribosomal protein mRNAs: stability, short poly(A), high translation efficiency, favourable codon use, and also short 3′UTRs, the vast majority of which (human) are ≤100 nucleotides [118] **(see** [119] **for UTR lengths)**, likely significant as 3′UTR length is associated with destabilizing elements that recruit deadenylases [120–122].

It is envisaged that mRNAs recruit abundant PABP in a poly(A) length-dependent manner (Figs. 2 & 3). As reviewed above, evidence exists of functional competition for PABP by PAM2 proteins [87,108] **(see** [123]). These observations prompt a model in which deadenylation-mediated pruning may be protective of translational function [111] as poly(A) shortening would tend to focus PAM2-associated activities to those with high affinity for PABP at the closed-loop, excluding those with low affinity (Table 1).

Model pathways of poly(A) deadenylation and mRNA decay

In their excellent review, Chen and Shyu note that poly(A) tails of ~250 nucleotides in mammalian cells (or 70–90 in yeast) are shortened by a 'default' pathway as soon as mRNAs enter the cytoplasm [124]. mRNAs with short UTRs contain few if any elements that recruit deadenylases and therefore maintain high stability [124].

Deadenylation is the initiating and rate-limiting step in mRNA decay and is regulated. Deadenylation occurs prior to decay of the mRNA body [124]. Features of biphasic deadenylation kinetics and mRNA decay are schematized in Fig. 3 which shows that the model pathways for stable and unstable mRNAs differ in the mechanisms of recruitment and relative timing of the deadenylases used [120,125]. According to current data and models, most mRNA decay becomes associated with deadenylation only when the poly(A) tails are short [126–128].

In classic work, Yamashita et al. showed a biphasic deadenylation pattern for stable β-globin mRNA [125]. Deadenylation in phase one is slow and synchronous, i.e., with fairly constant rate, but without mRNA decay [125] **(see** [124]). Using a reporter mRNA system, the PAN2-PAN3 deadenylase complex was attributed with the first phase of deadenylation, which is the trimming of long poly(A) tails down to ~150 nucleotides [125]. Deadenylation of stable mRNA in the second phase is processive with irregular rates (asynchronous), mediated by the CCR4 and CAF1 deadenylases of the CCR4-NOT complex and *is* associated with decay of the mRNA body [125] **(see** [127,129]). In this model pathway, the PAN2-PAN3 and CCR4-NOT deadenylase complexes are presumed to operate in default mode, i.e., are not actively recruited to the stable mRNA by ancillary factors (below).

For unstable mRNA, the first phase of deadenylation is rapid and decay is faster than for stable mRNA [125,130–132] **(see** [124]). Unstable mRNAs with sequence elements that bind proteins (e.g., TTP [133]) or engage micro-RNAs actively recruit CCR4-NOT deadenylase which leads to asynchronous deadenylation early in their lifetime. The deadenylation kinetics for many/most mRNAs are complex as they bear sequences or use other mechanisms that recruit the PAN2-PAN3 and/or CCR4-NOT deadenylases **(see** [134] **and refs therein)** and do not fit the classic model of stable or unstable reporter, the half-lives of which are typically ≥8 h and ≤75 min, respectively, [124,135,136].

The CCR4-NOT complex carries two types of deadenylase activities (described below) as well as several stable subunits [137], and can interact with the PAM2 proteins TOB1 and TOB2, enabling it with regulatory potential. Indeed, phosphorylation-dependent control of the PAM2-mediated PABP-binding activity of TOB2 has been shown to direct mRNA deadenylation by CCR4-NOT1 [138].

In summary, analyses that employed reporters driven by pulsed or Tet-off transcription as well as certain endogenous transcripts, followed by assays including gel mobility to monitor poly(A) length and decay have led to a broad view of deadenylation kinetics and decay for stable and unstable mRNAs [131,134-136].

Poly(A)-PABP faces up to deadenylases

Studies show that PABP is intricately involved in deadenylation. Workers reconstituted poly(A) mRNPs with PABP and deadenylases using human or yeast proteins [129,143]. The yeast studies included high resolution structural analysis that revealed the importance of the RRM4 of PABP and its C-terminal extension in cooperative oligomerization of PABP–PABP interactions while bound to poly(A) [88]. Unique bending of the poly(A)-PABP-oligomer creates a docking site for Pan2/3 with the poly(A) 3′ end threaded into its active site [88]. The yeast Pan2/3 deadenylates long poly(A)-PABP oligomers but becomes progressively less active as poly(A) is shortened and the number of bound PABPs is reduced, at which point the Caf1 and Ccr4 deadenylases can continue deadenylation [88]. Using human proteins, Yi et al. showed that CAF1 and CCR4 have differential activities, dependent on PABP-binding. The CAF1 subunit of the CCR4-NOT complex can only deadenylate naked poly(A) or after PABP dissociates. The CCR4 subunit can also do so and additionally appears capable of actively dislodging PABP from poly(A). Similar conclusions came from using yeast Ccr4 and Caf1 enzymes and PABP [143]. These experiments were done in the absence of PAM2 proteins.

One type of active role in deadenylation reflects that PABP can recruit deadenylases. PABP directly recruits CCR4 activity to the poly(A) 3′ end [129,143]. PABP can also recruit the

CCR4-NOT1 deadenylase complex via the PAM2 proteins TOB1/2 [138,144–147], and the PAN2 deadenylase via its PAM2 protein partner PAN3 [87,134,145,148].

Webster et al. mapped PABP binding at the 5′ proximal region of poly(A), where its RRM4 meets the 3′ UTR sequence of the mRNA [143]. RRM4 could slide onto the UTR sequence with overlap binding on poly(A). The stability of this proximal mRNA-poly(A)-PABP was higher than PABP bound to poly(A) [143]. A consequent model predicts that the most proximal PABP-PABP dimers remaining after the poly(A) tail shortens to ~40-50 nucleotides might vary in dissociation kinetics in a sequence-specific, i.e., gene-specific manner (Fig. 3).

PABP stability is also dynamic at the poly(A) 3′ end. Its dissociation would appear to proceed in quantum steps that may correspond to dissociation of single RRM domains. The PABP molecule at the poly(A) 3′ end exhibits transitional dissociation states; the most 3′ distal of its RNA-binding domains, RRM1 can be dislodged or peeled off by CCR4 as if the poly(A) 3′ end can be digested away while under the RRM [143]. This mode of PABP dissociation from the poly-(A) 3′ end is presumably the way deadenylation occurs when poly(A) is short.

An active role for PABP in poly(A) metabolism would also include recruitment of factors that oppose deadenylation, for example, such as LARP4 [114,149] and presumably LARP1 [22,114]. Altered phasing of mRNA poly(A) tails in LARP4 knock-out cells suggest that transitional states of 3′-bound PABPs are sensitive to LARP4 stabilization [149] (Fig. 3, right). One can imagine that as different faces of the RRMs of PABP are revealed in their intermediate bound states [143], some may represent distinctive recruitment sites.

Critical transitions in poly(A) length may sensitize distinct PABP-binding states

Transcriptome-wide analyses point to PABP as contributing to the irregularity of deadenylation kinetics as mRNA poly(A) tails shorten to ≤150 nucleotides [129] **(reviewed in** [150]). This is reflected as 'phasing' in poly(A) sequencing profiles in which poly(A) tail length is plotted on the X-axis versus the fraction of total reads containing that length on the Y-axis. A simple phasing pattern appears as two-three peaks each reflecting a different number of PABP molecules associated with the peak poly(A) length [111]. A source of irregularity is a deadenylation rate difference related to how the CCR4 and CAF1 deadenylases respond to PABP.

As described, phased poly(A) length profiles are observed for a set of transcripts such as ribosomal protein mRNAs [111,149,150] **(see** [129]). Phasing reflects an *in vivo* state and is consistent with biochemical studies in which deadenylation rates accelerate as the number of bound PABPs decrease from three to one [129] **(see** [143]). This may result from the loss of intermolecular PABP-PABP contacts and RRM dissociation dynamics [88,143]. Accordingly, phasing was more prominent (and complex) after cellular CCR4 levels were decreased [129]. Gene-specific effects were also noted [129], suggesting 3′UTR sequence-specificity or involvement of transacting factors.

While phased poly(A) of relatively short length appear for ribosomal protein mRNAs, total mRNA poly(A) often resolves as one major peak **(reviewed in** [150]). In a study of mouse and human cells, poly(A) of ~75 nucleotides corresponding to three bound PABP proteins was most abundant [149]. As ~75 nucleotides poly(A) length appears to represent a majority in higher eukaryotes [150], it may reflect a dynamic state that is sensitive to regulation. For mRNPs in this state, loss of the 3′ PABP monomer might be expected to sensitize the remaining PABP-PABP dimer to dissociation and accelerated mRNA decay as reviewed above. Alternatively, such pruning may produce more efficiently translated ribosomal protein mRNPs [111,150]. Such fates may be programmed by 3′ UTRs.

LARP4 can alter the poly(A) phasing patterns of mRNAs

Transcriptome analysis by single molecule poly(A) tail-seq (SM-PAT-seq) confirmed effects of LARP4 on ribosomal protein mRNAs [149]. It revealed LARP4 as a general factor involved in mRNA poly(A) metabolism and led to insight into how its poly(A) 3′ length protection and mRNA stabilization activities are linked [149]. LARP4 slows deadenylation across a wide spectrum of poly(A) lengths [149]. Its prominent effects were resolved by comparing the poly(A) phasing of ribosomal protein mRNAs from LARP4 KO and wild-type cells, and their deadenylation kinetics during mRNA decay after transcription inhibition [149]. With regard to the latter, deadenylation of the peak mRNA poly(A) of ~75 nucleotides was observed to begin earlier in cells lacking LARP4 than in wild-type cells. The cumulative data suggested a model in which LARP4 is recruited to the poly(A) 3′-PABP complex or somehow slowed deadenylation while, in its absence the poly(A) 3′-PABP complex appeared to be more sensitive to dissociation by the CCR4-NOT1 deadenylases [88,129,143].

Although mechanisms remain unknown, the mutually exclusive poly(A) binding and PABP-binding by the LARP4 NTR seem likely involved[21]. The NTR binds poly(A) dependent on key residues in the PAM2w [21]. A proposed model is that LARP4 may stabilize PABP on short poly(A) in part by use of its poly(A) binding, and the resulting complex protects the RNA from deadenylases. We note again that the affinity of the LARP4 NTR is ~ 10-fold higher for poly(A) than the PAM2w affinity for the MLLE [21]. In any case, the mechanism is likely intricate because LARP4 also has another motif for PABP binding, the PBM [12] which remains relatively less well defined.

Northern blot mRNA mobility that monitors poly(A) length of reporters as well as endogenous mRNAs showed that LARP4 slows or generally decreases deadenylation and leads to mRNAs with longer poly(A) which are also more stable [114,149]. Time-resolved SM-PAT-seq analysis revealed that this activity should accurately be referred to as poly(A) 3′ length protection [22,114,149]. As described next, the mRNA mobility assays used to characterize poly(A) 3′ protection mRNA stabilization for LARP4 also show similar activity for LARP1 [22,114].

Similar mRNA poly(A) tail protection and stabilization activities for LARPs 1 and 4

The mRNA mobility assays for mRNA poly(A) length protection activity were positive for LARPs 1, 4 and 4B and negative for LARPs, 6, 7 and La protein [114]. Further analysis localized the poly(A) protection and mRNA stabilization activity of LARP1 to a 304 amino acid La-module fragment that was shown to bind PABP by co-IP [22]. A 230 amino acid La-module fragment with different terminal extensions had been shown to bind poly(A) as a recombinant protein [18]. These two LARP1 La-module fragments exhibited different qualitative and quantitative degrees of poly(A) length protection and mRNA stabilization activities in HEK293 cells [22]. Importantly, theirs and LARP1 activities were decreased upon point mutations to the PAM2[22]. In these assays, LARP1 exhibits activity on stable GFP mRNA and on β-globin-ARE unstable reporter mRNA, using LARP4 as a positive control [22]. Their dependency on PABP suggest that LARPs 1 and 4 harbour similar types of poly(A) length protection that confers mRNA stabilization.

Because the poly(A) length protection-mRNA stabilization occurred on standard reporter mRNAs, these activities were apparently uncoupled from the 5′TOP translation effects of LARP1 [114], and may be applicable to LARP1 activities that involve different sets of associated mRNAs [16,139–142,151–-,151–158]. Also however, the isolated La-module of LARP1 which lacks the 5′TOP-binding DM15 domain, was shown to exhibit PABP-dependent poly(A) length protection of endogenous TOP ribosomal protein-mRNAs as an ectopic protein in trans [22].

Converting 3' end protection activity to function: LARP4 contributes to immunity

SM-PAT-seq analysis included sets of mRNAs from interferon stimulated genes (ISGs), some of which have short half-lives [149]. ISG mRNAs accumulated to higher levels in response to LARP4 expression, dependent on its PABP interaction motifs [149].

Expression of LARP4 appears to be linked to a signalling branch in an innate immune pathway that involves the ARE in the 3′UTR of LARP4 mRNA that is recognized by TTP and negatively regulated by the proinflammatory cytokine, tissue necrosis factor-alpha (TNFα) [159]. TTP negatively regulates TNFα by binding the ARE in TNFα mRNA 3′UTR, recruits the CCR4-NOT1 deadenylase and mediates decay [160,161]. Exposure to TNFα led to a quick decline in the short-lived LARP4 [159]. In this pathway, a decrease in LARP4 would negate its stabilization of mRNA [114,149] and hypothetically contribute to tuning a balance of proinflammatory responses.

Investigations of another aspect of the immune system, involving a role for differential mRNA stability during activation of $CD4^+$ T cells led to discovery of intron-retention (IR) as a mechanism involved [162]. IR is a prominent form of alternative splicing, and can have pivotal outcomes on fundamental processes [163]. With the goal to screen for factors involved in mRNA stability-mediated activation of $CD4^+$ T cells, LARP4 emerged as the most influential RNA-binding protein [162]. This was substantiated by multiple approaches in human cells and confirmed using spleen-derived T cells isolated from LARP4 KO and WT mice [162].

SARS-CoV-2 may hijack or block LARP activities for mRNA 3' poly(A) metabolism

Of the seven human LARPs, 1, 4, 4B, and 7 were found to bind SARS-CoV-2 proteins and/or RNAs [164,165] or by a different system [166]. As an RNA genome at the upper length limit, coronaviridae differ from smaller RNA viruses as all of their mRNAs are polyadenylated [167] and required for replication [168]. Gordon et al. found 332 HEK293T cell proteins stably bind the 26 proteins of SARS-CoV-2 [165]. LARP4B binds the virus transcription and replication factor, RNA-dependent RNA polymerase (RdRp, Nsp12). LARP7 binds Nsp8, a RdRp complex subunit [169,170]. LARP1, PABP and PABPC4 (very similar to PABP) are 3 of 15 host proteins that interact with nucleocapsid N-protein which binds viral RNA and also modulates the host immune response [165,171,172]. In the most severe cases of COVID-19 disease, an unbridled proinflammatory response creates a cytokine storm led by TNFα [173]. Most of the other N-interacting host proteins are involved in mRNA metabolism and the modulation of SGs [165], common among RNA viruses [103] and in innate immune control [174]. Also notable is that while PABP is targeted by viral proteases of several viruses(see refs in [175]), coronaviruses preserve intact 70 kDa PABP on their mRNA poly(A) [168].

Schmidt et al. isolated 104 human proteins that UV-crosslink to the SARS-CoV-2 RNA in infected cells [164]. CNBP, required for expression of certain pro-inflammatory cytokines, and LARP4 are the two host proteins most significantly enriched with the viral RNA, followed by others including PABP and LARP1 [164]. The importance of distinguishing between protein and RNA interactions was emphasized by the authors noting that only 10 of the 332 human proteins bound to SARS-CoV-2 proteins [165] also bound directly to the SARS-CoV-2 RNA [164]. In another approach, proximity labelling by Nsp2-biotinylase was used to identify LARPs 1, 4 and 7 among 513 host proteins involved in early viral RNA metabolism [166] which occurs in replicase and transcription complexes (RTCs) [176].

LARPs 7, 4B and 1 were among 40 host proteins that were differentially phosphorylated over multiple time points after SARS-CoV-2 infection [177]. Phosphorylation at multiple sites changed on these LARPs although most complexly for LARP1 [177]. CRISPR/Cas knock out of LARP1 led to an increase in SARS-CoV-2 replication, and its overexpression led to a decrease in viral infection [164]. These results would indicate LARP1 as an anti-coronavirus factor in HEK293T cells, consistent with its UV-crosslinking to the 5′-cap pyrimidine-rich leader of the SARS-CoV-2 RNA [164] and its function as a repressor of mRNA translation [15,18]. Nonetheless, the complexity of its phosphorylation kinetics [177] may reflect effects on LARP1 activities during infection. Since LARP4 and 4B stabilize poly(A) mRNAs and promote translation, targeting of these factors along

with PABP by the viral proteins and/or RNA may also have functional effects [13,16,19,22,114,149,159,178].

LARP1 function includes mRNA stabilization and likely in pathways other than for TOPs

Multiple aspects of LARP1-mediated mRNA stabilization remain unclear although it likely involves the La-module which can bind poly(A) with 40 nM K_d affinity [18] and itself confer mRNA poly(A) length protection and stabilization [22]. TOP mRNAs are the best known class of transcripts that are controlled by LARP1, the key factor that represses their translation in the absence of mTORC1 signalling [14–-14–17,155,179,180]. In poor nutrient conditions, the DM15 domain of LARP1 binds the 5′TOP motif excluding eIF4E binding and averting mRNP assembly from entering into a pre-initiation complex [15]. When nutrients improve mTORC1 leads to release of the 5′TOP by the DM15 domain, eIF4E-5′cap binding and translation initiation [16]. Although ectopic expression of LARP1 stabilizes and knock-down leads to lower levels of TOP mRNAs including in nutrient-replete cells [13,14] the degree to which these mRNAs are stabilized/protected from decay during LARP1-mediated translational repression has not been thoroughly investigated..

Some data show that after starvation-mediated translational repression, TOP mRNA levels accumulate as their poly-(A) tails lengthen over a course of ~12 h, dependent on LARP1. Upon nutritional repletion, the TOP mRNAs that had accumulated with long poly(A) tails were loaded on polysomes and the poly(A) quickly shortened thereafter [181]. How this TOP mRNA poly(A) lengthening is related to other LARP1-associated mRNA stabilization processes remains to be determined.

A screen for kinases and phosphatases based on the formation of SGs revealed that the protein kinase CDK1 robustly promotes TOP mRNA translation in a LARP1-dependent manner [139]. That study demonstrated CDK1-dependent phosphorylation of LARP1 (although not as a direct target), and that CDK1 can regulate TOP translation independently of mTOR. Moreover, inhibition of CDK1 and of TOP mRNA translation occurred while LARP1 maintained an association with TOP mRNAs [139].

The LARP1 interactome of regulatable TOP mRNAs is distinctly larger than initially thought [155]. Beyond this, LARP1 binds hundreds to thousands of mRNAs involved in various pathways including related to cancer [140,141,182,183]. Some of the mRNA sets exhibit sequence specificity and other features although these have not been as well defined nor characterized as the 5' TOP motif [101,140,154,157,184–186].

A link from LARP1 to mitochondrial DNA replication during oogenesis in *Drosophila* has been observed to exist. LARP1 was reported to be required for translation of mRNAs localized to the mitochondrial outer surface whose protein products function in mitochondrial DNA replication [186]. Phosphorylation of *dr*LARP1 by PINK kinase inhibits an activity that promotes the translation of outer mitochondrial-located mRNAs [154]. Human LARP1 was found to be important for inner-mitochondrial translation required for oxidative phosphorylation (OXPHOS), groundbreaking and expanding the significance of the *Drosophila* link [153]. This functional link to energy production deepens the LARP1 connection to other aspects of mTORC1 metabolic control, and other factors [187].

LARP1 is conserved [2] and has been studied in a variety of organisms (**reviewed in** [33,142]). Although the yeast LARP1 homologs, Sro9p, Slf1p and spLARP [188] do not exhibit a DM15 domain [142], nor do yeast ribosomal protein mRNAs contain a 5′TOP motif, they are efficiently translated [85]. Slf1 is associated with an oxidative stress response and with cytoplasmic mRNAs that encode mitochondrial proteins [189]. Terminating ribosomes are proposed to efficiently reinitiate on closed-loop mRNPs critically involving the 40S ribosome-associated factor RACK1 [115,190]. That Sro9 and the RACK1 homolog Asc1 interact [191] is interesting because Asc1 can promote efficient translation of short ORF ribosomal protein mRNAs [115,192]. Sro9 and Slf1 associate with overlapping sets of mRNAs that include ribosomal protein mRNAs and although Slf1p is the less abundant it led to greater accumulation of its target mRNAs than Sro9 consistent with distinct stabilization activity [189]. The cumulative observations suggest conservation of LARP1-like activities.

The human LARP1 La-module can simultaneously bind poly(A) and pyrimidine-rich sequences comparable to TOPs but not requiring the m^7G(5′)ppp-cap which by contrast does contribute to DM15 binding [15,18]. Although part of a larger multimodular protein that functions in a signalling network, the relatively compact La-module with its PAM2 can confer PABP-dependent poly(A) length protection of ribosomal protein mRNAs and stabilization of reporter mRNAs as an ectopic protein expressed in trans [22]. As suggested by the different qualitative effects on poly(A) protection of this La-module bearing different extensions on its N- and C- termini [22], it might be expected that the activity of this unique La-module would be under regulatory control in the full length protein.

Concluding remarks

La and LARP7 are highly related nuclear proteins that bind and protect target RNAs with UUU-3′OH ends from exonucleases. Their La-modules bind RNA in a very similar manner that involves an unusual asymmetric use of their RRM1 domains. Both have a downstream RRM subtype unique to La and a diversity of LARP7 homologs with a similar unusual mode of asymmetric RNA recognition. Phylogenetics suggest the possibility that exchange between the two RRMs may have contributed to La-module evolution.

LARP4 and LARP1 contain divergent La-modules and other interaction regions but exhibit similar mRNA poly(A) 3′ protection-stabilization activities, and interact with PABP via PAM2 motifs [22]. Poly(A) tail profiling data suggests that LARP4 opposes deadenylation by interacting with PABP bound to 3′ poly(A). LARP 4 differs from LARP1 in RNA-binding characteristics, the position of its La-module relative to the PAM2, and in relative affinities for poly(A) and MLLE binding. The multiple differences suggest that these proteins likely act in mRNA poly(A) protection by employing distinct

molecular, targeting and perhaps regulatory mechanisms [11–13,18,21,22,114]. Future insights into the basic mechanisms of deadenylation and the roles of PABP [88,111,129,143] as well as comparison of similarities and differences between LARP1 and LARP4, and contributions of other PAM2 proteins should provide a more integrated view of poly(A) metabolism, mRNA translation and decay.

Acknowledgments

We thank LARP Society members, M. Bayfield (York University), A. Berman (University of Pittsburgh), M. Sasi Conte (Kings College London) as well as E. Valkov (NCI, NIH) and M. Hafner (NIAMS, NIH) for helpful discussion. This work was supported by the DIR of the *NICHD*, NIH.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development [ZIA HD000412-31 PGD].

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