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Review

The "tale" of poly(A) binding protein: The MLLE domain and PAM2-containing proteins

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ABSTRACT

The cytoplasmic poly(A) binding protein 1 (PABPC1) is an essential eukaryotic translational initiation factor first described over 40 years ago. Most studies of PABPC1 have focused on its N-terminal RRM domains, which bind the mRNA 3′ poly(A) tail and 5′ translation complex eIF4F via eIF4G; however, the protein also contains a C-terminal MLLE domain that binds a peptide motif, termed PAM2, found in many proteins involved in translation regulation and mRNA metabolism. Studies over the past decade have revealed additional functions of PAM2-containing proteins (PACs) in neurodegenerative diseases, circadian rhythms, innate defense, and ubiquitin-mediated protein degradation. Here, we summarize functional and structural studies of the MLLE/PAM2 interaction and discuss the diverse roles of PACs.

1. Introduction

PABPC1 is a key component of the translational machinery; it circularizes the mRNA and stimulates its translation into protein [1,2]. PABPC1 plays a direct role in 60S subunit joining and is integral to the formation of the translation initiation complex on the mRNA [2]. It is the most abundant of several cytoplasmic poly(A) binding proteins (PABPs) found in vertebrates and has been known for four decades [3]. The other isoforms express at lower levels with tissue specific distributions and likely function in specific developmental processes [4].

PABPC1 consists of four RNA-binding domains (RRM1-4) followed by a linker region and a conserved C-terminal MLLE domain (Fig. 1A). The RRM domains mediate the circularization of mRNA through the binding of the 3′ poly(A) tail and eIF4F complex on the mRNA 5′ cap [2,5–7]. The linker region appears to promote the self-association of PABPC1 on mRNA although the molecular details of the interaction are unknown [8,9]. The C-terminus of all cytosolic PABPs contains a MLLE domain that mediates binding of a peptide motif, PAM2, found in many PABP-binding proteins. Enigmatically, a MLLE domain is also found in an E3 ubiquitin ligase, EDD (also known as HYD or UBR5). In one case, the association appears to play a role in protein homeostasis but the broader significance of this connection remains unclear.

Here, we address the function of the MLLE domain and its interactions with PAM2-containing proteins (or PACs) involved in mRNA processing and translation. Some of the better characterized PACs are described along with a description of the functional importance of their PAM2 motifs and interactions with PABPC1. We conclude with a summary of structural studies of MLLE/PAM2 interactions and a discussion of the possibility that novel MLLE-binding proteins remain to be found.

2. The MLLE domain and PAM2

The 70-residue MLLE domain was first identified as a peptide-motif binding domain in 2001 [10]. The domain consists of a bundle of five α-helices and was named MLLE ("mademoiselle") due to the presence of a conserved four amino acid sequence, *MLLE*, in the heart of the peptide recognition site (Fig. 1B). In the literature, the domain has also been referred to as PABC, C2, and H2. It is highly conserved and is present in all eukaryotic kingdoms: animal, vegetal and fungal (Fig. 1C) [11,12].

The MLLE domain recognizes a conserved peptide sequence (L/P/F)X(P/V)XAXX(F/W)XP, which was named "*PA*BP-interacting *M*otif 2" or PAM2 (Fig. 1D) [10,13]. PAM2 sequences were first identified at the N- and C-termini of proteins that bind to PABPC1 [10]; subsequent studies confirmed their localization to unstructured regions of proteins [14,15]. Evolutionally, the motif is well conserved across eukaryotes from humans to *Schizosaccharomyces pombe* [14]. In *Saccharomyces cerevisiae*, the motif may have diverged. While the yeast protein, Pab1p, contains a bonafide MLLE domain [16], no PAM2 motifs were found in Pab1p-interacting proteins identified in a yeast two-hybrid screen [17] or by mass spectrometry [18]. Among the conserved residues, the two hydrophobic residues in the motif are the most critical

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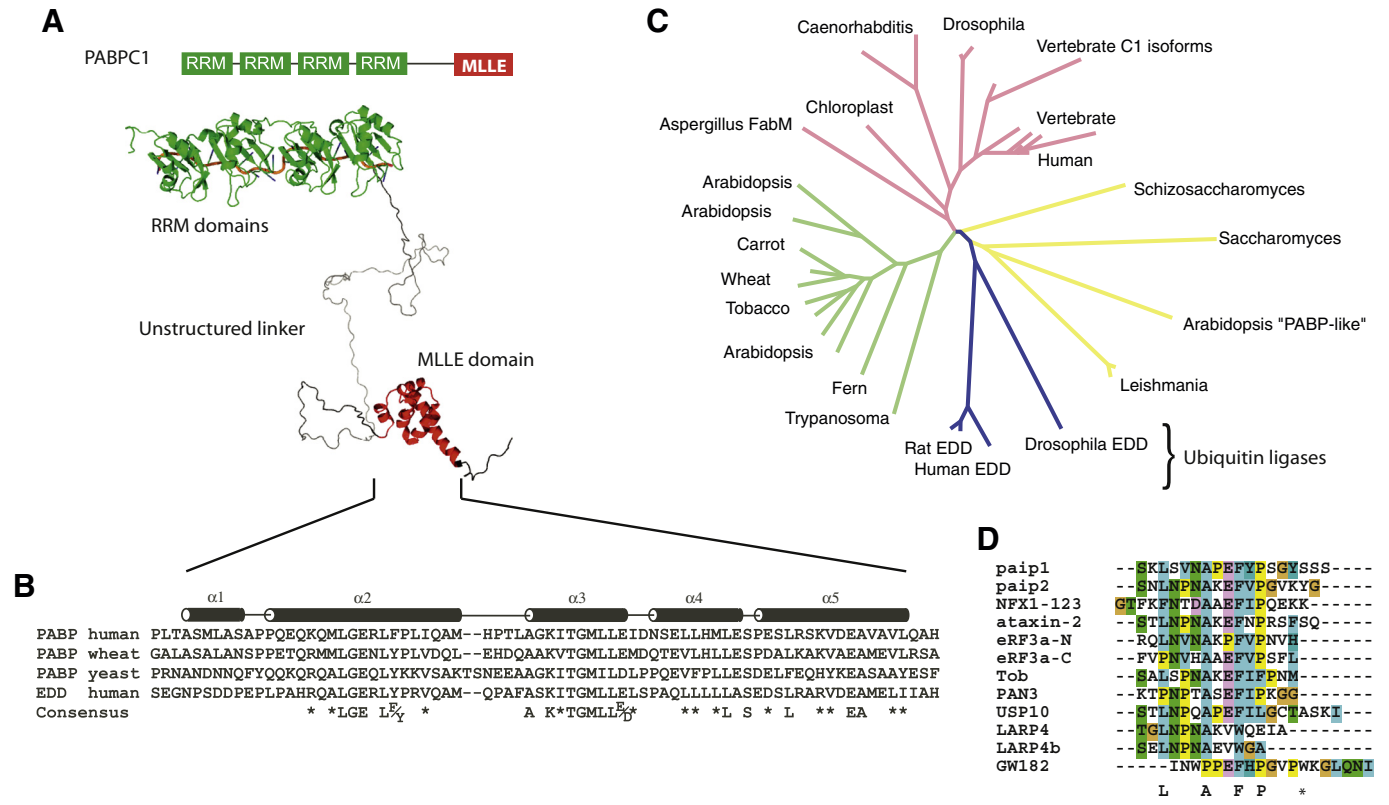

Fig. 1. PABPC1, MLLE domain, and PAM2. (A) Domain structure and model of full-length PABPC1 based on structures of the first two RRM domains with polyA RNA, the fourth RRM domain and the MLLE domain (PDB ID: 1CVJ [6], 1G9L [10], 2D9P and 4F02 [5]). (B) Sequence alignment of MLLE domains from human, wheat, and yeast PABPC1 and human EDD. The secondary structure and the helices are labeled according to the structure of human PABPC1. (C) Unrooted phylogenetic tree of MLLE domains showing the grouping of plant, vertebrate, and EDD sequences. Sequences labeled EDD are MLLE domains from HECT E3 ubiquitin ligases; all other sequences are poly(A)-binding proteins. The figure was generated with ClustalW [84] and TreeViewPPC [85]. (D) Sequence of MLLE-binding PAM2s. The consensus of conserved residues that contribute to the MLLE–PAM2 interaction is shown. The asterisk labels a tryptophan in GW182 that interacts with MLLE.

(Fig. 1D). Mutation of either the leucine or the aromatic phenylalanine residues to alanine decreased the affinity binding by over 1000-fold [19].

3. *PAM2*-containing proteins (PACs)

Since their first identification, PAM2 motifs have been found in some twenty human proteins; the best studied are listed in Table 1 [10,14]. The majority of studies have shown that PACs function in different mRNA-related processes by interacting with PABPC1 (Fig. 2). The function of PACs binding to the ubiquitin ligase EDD is less well understood and an area for future investigation.

3.1. PAN3, Tob1/2 and GW182 in mRNA decay

Deadenylation is an initial step for major mRNA decay pathways in higher eukaryotes. Several PACs regulate mRNA degradation via interactions with PABPC1. PAN3 is a PAC found in one of the two major cytoplasmic deadenylase complexes: PAN2–PAN3 and CCR4–NOT. The two complexes may function at different stages of deadenylation [20].

Table 1
List of PAM2-containing proteins (PACs).

Protein	Properties			
	Functions	Stress granule or P-body localization[a]	Affinity for PABPC1 (μM)[b]	Affinity for EDD (μM)
Paip1	Translation enhancement [46,54,55]	–	1.4	3.4
Paip2	Translation inhibition [47,49,50,52]	SG[c]	0.2	6
Ataxin-2	MicroRNA mediated gene silencing [43]; neurodegenerative diseases [37,40,41]; behavioral rhythm [44]	SG	0.7	10
eRF3	Deadenylation and mRNA decay [23,58,59]	–	1	5.2
GW182 proteins	Deadenylation [25]; miRISC assembly [30]	P-body	6	200[c]
Tob1/2	Deadenylation [22–24]	P-body	16	12
PAN3	Deadenylation [21]	P-body	40	No binding
LARP4	mRNA stability [61]	SG	22	–
NFX1	Increases mRNA level of telomerase catalytic subunit [86,87]	–	–	–
USP10	mRNA stability [64]	SG	26	33
TTC3	Neurodegenerative Down syndrome [70]	–	5.4	37

[a] Based on data from human cells following heat shock or sodium arsenite treatment.
[b] For details of affinity studies, refer to [19,21,29,61,72,82].
[c] Unpublished data.