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Review

Kiss your tail goodbye: The role of PARN, Nocturnin, and Angel deadenylases in mRNA biology ☆

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ABSTRACT

PARN, Nocturnin and Angel are three of the multiple deadenylases that have been described in eukaryotic cells. While each of these enzymes appear to target poly(A) tails for shortening and influence RNA gene expression levels and quality control, the enzymes differ in terms of enzymatic mechanisms, regulation and biological impact. The goal of this review is to provide an in depth biochemical and biological perspective of the PARN, Nocturnin and Angel deadenylases. Understanding the shared and unique roles of these enzymes in cell biology will provide important insights into numerous aspects of the post-transcriptional control of gene expression. This article is part of a Special Issue entitled: RNA Decay mechanisms.

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1. Introduction

This review covers three of an unexpectedly large contingent of deadenylase enzymes in eukaryotic cells: PARN, Nocturnin and Angel. Understanding the unique and shared roles of these enzymes in the cell is a major challenge to our working knowledge of the mechanistic details of the post-transcriptional regulation of gene expression. While each of these enzymes is generally widely conserved among eukaryotes, there are organisms that lack one or more these enzymes, providing evidence for functional redundancy among deadenylases.

From an enzymatic perspective, PARN is by far the best studied of the group. This versatile homodimeric enzyme plays a role in mRNA stability, the quality control of gene expression and the maturation of a class of small RNAs. Biochemical and structural studies have provided insight into the intriguing property of cap-mediated regulation of the activity of this enzyme, suggesting a potential mechanistic connection between deadenylation and translation. Nocturnin, on the other hand, may also participate in functions beyond poly(A) tail shortening. These include chaperoning the movement of factors to the nucleus by protein–protein interaction. Thus one needs to keep an open mind about the biochemical functions of these enzymes and consider them in the broader context of the networking of gene expression in the cell.

From a biological perspective, these three deadenylases have been associated with a variety of important developmental and metabolic responses. PARN-mediated deadenylation has been implicated in early development decisions in a variety of organisms. Furthermore, the enzyme has been linked with cancerous growth and DNA repair activities. Nocturnin is not only a key protein in the circadian rhythm of the cell, but also has been shown to have an interesting impact on adipogenic differentiation, metabolic processes related to obesity, and regulation of the inflammatory response. While less is known regarding the biological implication of the Angel deadenylase, it has been implicated as a regulator of cell cycle-specific genes. Therefore not only is this group of enzymes interesting from a molecular perspective, they also have significant impact on fundamental cell processes as well. Therefore the goal of this review is to provide an overview of work performed to date on these deadenylases to allow an in depth appreciation of their molecular and biochemical properties in an interdisciplinary context.

2. PARN (poly (A)-specific ribonuclease)

2.1. Overview

An activity in HeLa cells having the properties of PARN was first described by the Virtanen lab [1,2] and PARN was molecularly cloned in 1998 by Wahle and coworkers [3,4]. The enzyme is conserved and active in eukaryotic organisms from trypanosomes, through mosquitoes, plants and mammals [5,6]. However analysis of the sequenced genomes of *Saccharomyces cerevisiae* and *Drosophila melanogaster* has revealed

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that they lack PARN [7,8]. Similarly, PARN function appears to be largely dispensable in *Caenorhabditis elegans* and *Schizosaccharomyces pombe* [9] and PARN sequence is less conserved in these organisms compared to vertebrates (Fig. 1). Thus although PARN appears to play an important role in the biology of many organisms, presumably other redundant deadenylases fulfill the role of PARN in the handful of eukaryotes that to date have been shown to lack the enzyme. Furthermore, it is important to note that some presumed PARN proteins may not be true homologs. The AtPARN, CePARN and TbPARN polypeptides, for example, all appear to lack conserved R3H and RRM domains (Fig. 1).

In addition to recognizing the poly(A) tail as would be expected of an adenosine-specific 3′-to-5′ exonuclease, interestingly PARN can also simultaneously bind the 7-methyl-guanosine cap on the 5′ end of mRNAs [10–12]. This interaction influences the processivity of the enzyme, increasing the rate of poly(A) tail removal. Thus cap binding proteins such as eIF-4E may possibly serve to regulate PARN activity via competition for binding the cap structure. This cap-binding property may help to specifically target PARN to RNA Polymerase II-generated transcripts and coordinate PARN-mediated deadenylase activity with the translational competence of the mRNA. Interestingly, directing cells towards a quiescent state by serum-starvation results in a modest increase in phosphorylation of PARN and a modest increase in cap occupancy relative to eIF-4E in the cell, suggesting a dynamic interplay between PARN and other cap binding factors [13]. Furthermore, as our understanding of the regulation of cap-dependent and cap-independent translation evolves through sensitive global approaches such as ribosome profiling [14], the interplay between the cap-binding activity of PARN and the coordinate regulation of translation and mRNA deadenylation/decay should become clearer.

Enzymatically speaking, PARN is a member of the DEDD class of exonucleases that contain conserved Asp–Glu–Asp–Asp residues in their active site [15]. Mutation of any of these four amino acids substantially reduces the overall enzymatic activity of PARN. PARN exists as a dimer with the exonuclease domain acting to coordinate the two subunits [16]. The dimeric structure of PARN has been determined by X-ray crystallography of partial proteins [17], analytical ultracentrifugation [16], and in solution for the entire protein by atomic force microscopy and dynamic light scattering [18]. PARN requires divalent cations, particularly Mg^{2+}, for optimal enzymatic activity [19]. Two divalent cations specifically interact with the active site amino acids Asp^{28}, Asp^{292}, and Asp^{382} (numbers correspond to the human protein), with Asp^{28} being the most important for the binding of Mg^{2+} or other divalent cations like Fe^{2+} [19–21]. Mg^{2+} also plays a role in the structural stability of the PARN enzyme. Divalent cations protect the active site of PARN from thermal inactivation, causing retention of up to 90% of activity when the protein is incubated at 45 °C [22].

PARN appears to act both in the nucleus and in the cytoplasm of eukaryotic cells. In addition to the standard trimming of poly(A) tails of messenger RNAs, PARN has two interesting functions in the nucleus. First, PARN has recently been shown to be involved in the maturation of the 3′ end of snoRNAs [23]. Second, the deadenylase plays a role in the directed decay of transcripts following DNA damage [24]. PARN also plays a role in the quality control of gene expression in addition to assisting in the regulation of poly(A)$^+$ mRNA levels. The deadenylase is active in nonsense mediated mRNA decay (NMD) and can be immunoprecipitated with NMD factors Upf1, Upf2, and Upf3X. Knockdown of PARN by siRNAs to about 15% of wild-type levels led to the stabilization of a reporter transcript containing a premature nonsense codon [25].

PARN appears to play important roles in early development [4] as well as perhaps in certain cancers. A recent study found that PARN was 2.2-fold overexpressed in acute lymphocytic leukemia (ALL) and 2-fold overexpressed in acute myeloid leukemia (AML) compared to control patients [26]. In addition, PARN was phosphorylated to a greater extent in ALL patients compared to controls [26]. Similarly, PARN mRNA is expressed at significantly higher levels in growing follicles of the bovine oocyte compared to persistent follicles [27]. As PARN causes poly(A) tail removal at germinal vesicle breakdown, its expression silences maternal transcripts during oocyte maturation and embryogenesis. These studies are both consistent with a role for PARN in promoting cell growth.

PARN also plays a role in the regulation of mRNA turnover of a set of transcripts in mammalian cells. A recent study determined the effects of shRNA-mediated PARN knock-down in mouse C2C12 myoblasts using global mRNA expression and half-life analysis [28]. Forty stabilized mRNAs and 24 destabilized mRNAs were observed as a result of the PARN knock down. While this may appear to be a rather small subset of mRNAs in the cells, a similar small number of mRNAs (2%) was affected in cells depleted of other deadenylases (the CCR-like and CAF1-like enzymes) using RNAi technologies [29]. The effects of PARN on mRNA stability, at least on some mRNAs, is direct as analysis of the *Zfp36l2* transcript indicated an elongated poly(A) tail up to 2–3 fold the length in PARN knock down compared to control cells. Interestingly, many of the destabilized mRNAs in PARN knockdown cells encoded factors involved in cell migration and adhesion. This may represent a biologically relevant regulon as PARN KD cells migrated faster in a wound-healing assay consistent with the identities of the destabilized mRNAs.

The PARN deadenylase, therefore, is a multifunctional enzyme with interesting structural, subcellular localization and enzymatic properties that plays a role in a number of important biological phenomena. The sections that follow go into more depth into several of the aspects of this enzyme to provide further perspectives on its biochemical and biological properties.

2.2. Structural studies of PARN

PARN consists of three domains: a RNA recognition motif domain (RRM), a nuclease domain, and an R3H domain (a protein domain that contains an invariant arginine residue and a highly conserved histidine residue four amino acids downstream [30]). Crystal structures of PARN are available [16,31] and based on these crystal structures a structural model of PARN that visualizes the positions of the nuclease, R3H, and RRM domains has been reconstructed [31].

In addition to its expected role in single-stranded RNA binding, the RRM domain also plays a role in cap recognition. The m^7GpppG cap binds to the RRM domain in one subunit of the PARN homodimer and to a pocket formed by the RRM domain and the nuclease domain in the other subunit [33]. The relative position of the RRM domains differs by 30° in the two units, resulting in the two alternative modes of cap interaction. Crystal structures of the human and mouse PARN enzymes indicate that the RRM domain binds the 7-methylguanosine cap (m^7G) via a tryptophan residue (human Trp^{475} and mouse Trp^{468}) [17,32]. The m^7GTP is bound by coplanar stacking with human PARN Trp^{475} and by Trp^{456} hydrogen bonding with the m^7G N1 atom [17]. PARN was found to bind GTP with at least 100-fold less affinity than m^7GTP [17].

The R3H domain of one PARN subunit interacts with the active site of the other subunit as well as also being involved in RNA binding [16]. Deletion of either the R3H domain or the RRM domain revealed that both singly are able to bind RNA, but that both are required to be present for full enzymatic activity [33]. Crystallographic studies of a C-terminal truncation of human PARN (residues 1–430), lacking the cap binding domain, revealed that each subunit in the homodimer binds three adenosine residues of the poly(A) tail of the RNA substrate and that Glu^{30} specifically interacts with the 3′OH group [16]. Assays of a human PARN lacking the R3H domain revealed that 10^3 more protein was needed to achieve the same deadenylation activity as the C-terminal truncation (1–430 aa), further emphasizing the importance of the R3H domain [16].

In summary, significant progress has been made to date on the fundamental structure and interactions of this enzyme based on

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