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Abstract

In eukaryotic cells, the balance between the synthesis and the degradation decides the steady-state levels of messenger RNAs (mRNA). The removal of adenosine residues from the poly(A) tail, called deadenylation, is the first and the most crucial step in the process of mRNA degradation. Poly (A)-specific ribonuclease (PARN) is one such enzyme that catalyses the process of deadenylation. Although PARN has been primarily known as the regulator of the mRNA stability, recent evidence clearly suggests several other functions of PARN, including a role in embryogenesis, oocyte maturation, cell-cycle progression, telomere biology, non-coding RNA maturation and ribosome biogenesis. Also, deregulated PARN activity is shown to be a hallmark of specific disease conditions. Pathogenic variants in the *PARN* gene have been observed in various cancers and inherited bone marrow failure syndromes. The focus in this review is to highlight the emerging functions of PARN, particularly in the context of human diseases.

Key words

Deadenylase, Ribonuclease, PARN, mRNA degradation pathways, Telomere dysfunction, Bone marrow failures, Cancer

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Introduction

The central dogma of molecular biology postulates that the flow of genetic information proceeds from DNA through mRNA to protein. The control of gene expression in eukaryotic cells is mediated at multiple levels including the mRNA level, where synthesis, stability and turnover of the transcripts play vital roles [1]. In general, most eukaryotic mRNAs conform to a conserved architecture as illustrated in **Figure 1**. The modular functional segments of mRNA contribute towards stability and degradation and the process of mRNA degradation plays an important role in ensuring physiologically relevant mRNAs remain at steady-state levels in the cell. Any defect during this process could potentially contribute to a diseased condition with accumulation of mRNA long after they are required in a cell. Therefore, the fine balance between synthesis and breakdown of mRNA is critical in maintaining homeostasis. Specific recognition sequences in the untranslated regions of the mRNA allow the control over these processes, and are responsible for the appropriate stability profile to each mRNA molecule.

The enzymes that are involved in mRNA degradation are collectively called ribonucleases, and includes the deadenylases, the exonucleases and the endonucleases. These are hydrolytic enzymes that are responsible for cleaving phosphodiester bonds in RNA [2]. The RNase family comprises of eight distinct groups of genes [3] and are known to contribute to the recycling of ribonucleotides and the removal of defective RNAs [4]. Apart from RNA degradation, non-canonical functions of the RNases are also known. They are involved in functions as diverse as tumor suppression, stimulation of blood vessel formation to proliferation of motor neurons [1]. Broadly ribonucleases fall under two categories, namely endoribonucleases and exoribonucleases.

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The length of the mRNA poly(A) tail at the 3'end of the molecule is regulated by poly(A) polymerases and deadenylases. The existence of numerous deadenylases in eukaryotic cells suggests that regulation of the poly(A) tail is of vital importance to cellular homeostasis. Indeed, regulation of the mRNA poly(A) tail is known to control almost all functional aspects of mRNAs including transcription, translation, stability, intracellular transport and degradation. The regulatory function of deadenylases is governed by a complex series of factors including its sub-cellular location and association with specific regulatory RNA-binding proteins.

Pathways involved in mRNA degradation

Essentially, two types of mRNA degradation pathways exist in eukaryotic cells [1]. This include a) the deadenylation-dependent pathway involving removal of the poly(A) tail, and b) the endonuclease-dependent pathway, which is poly(A) tail independent. In the deadenylation pathway, degradation of mRNA proceeds by the initial removal of the poly(A) tail at the 3' end of the mRNA by specific deadenylases. This is followed by degradation of the mRNA from either the 5' end or the 3' end of the mRNA. In the 5' to 3' degradation event, the m7-G cap, a short stretch of sequence which gives stability to mRNA by protecting against 5' to 3' exonucleolytic degradation, is first removed by decapping enzymes, followed by degradation by exonucleases. The 3' to 5' degradation program of mRNAs on the other hand, involves degradation from the 3' end by poly(A) nucleases in the exosome complex (multi-protein intracellular complex which is capable of degrading various types of RNA molecules), followed by mRNA cap removal by de-capping enzymes like Dcp1-Dcp2 complex at the 5′ end. It is reported that the 3' to 5' deadenylation-dependent degradation pathway is the most encountered mechanism leading to mRNA degradation in mammals (**Figure 2**). It is worth mentioning that the rate limiting step in mRNA degradation occurs by the initiation of

poly(A) tail shortening or deadenylation [5-7]. Other mRNA degradation mechanisms include the endonuclease-dependent pathway (**Figure 3**). Here degradation is initiated by the RNA-induced silencing complex (RISC) that includes microRNAs (miRNAs) or short interfering RNAs (siRNAs) and endonucleases (such as Argonaute or Ago2) [8].

Conventional role of PARN – Poly (A)-specific ribonuclease

Poly(A)-specific ribonuclease (PARN) is an exoribonuclease [9] that is expressed ubiquitously in almost all tissues of eukaryotic organisms [10]. It is known to interact with single-stranded poly(A) mRNA [11] and is considered an important ribonuclease because of its pivotal role in mRNA degradation as a poly(A) tail remover. One of the most significant examples of translational silencing occurs during oocyte maturation and development when the poly(A) tail is removed from maternal mRNAs [12]. PARN is unique among ribonucleases due to its unusual ability to interact with the 5' cap end and poly(A) tail of mRNAs [13-17]. The interaction of PARN with the 5'-cap of mRNA is thought to enhance the rate of mRNA degradation [18].

PARN belongs to the DEDD (Death effector domain containing DNA binding) family of nucleases, which binds to a divalent metal ion [18] and consists of R3H domain and the RRM domain [19] **(Figure 4)**. A 54-kDa fragment of the total 74-kDa poly(A)- specific ribonuclease is an oligomeric, processive and cap interacting poly(A) specific 3' exonuclease [15], while the function of other 20KDa fragment is not known. From an evolutionary point of view, it is noteworthy to point out that PARN is not present in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. But in both these species POP2 protein, an enzyme involved in mRNA deadenylation, appears to be the homolog of PARN. Moreover, despite its presence in *Caenorhabditis elegans* and *Schizosaccharomyces pombe*, loss of PARN does not interfere with the survivability, suggesting a compensatory role by other redundant deadenylases in

these eukaryotes [17]. PARN and POP2 share 17% sequence similarity in their nuclear domains, POP2 does not belong to the DEDD family of nucleases [20]. Although PARN is known for its role in deadenylation (removal of Poly(A)), it can also degrade oligo(U) and oligo(G) but with ten-fold less efficiency [21]. Also, it is seen that PARN is involved in the degradation of Internal transcribed spacer 1 (ITS1) of human cells, which is highly GC-rich [22]. Another study on the crystal structure of PARN with m7GpppG cap analogue revealed the structural and functional overlaps of two domains (RRM domain and nuclease domain) in the cap binding site and the active site [23]. These reports provide the structural basis for studies to elucidate the mechanism of PARN-mediated deadenylation.

On the other hand, the role of PARN in NMD (Nonsense-mediated mRNA decay) is also well known. The matured transcript undergoes 5' end capping, splicing and 3'end processing by addition of adenosine monophosphate residues. All these processes are prone to error and the fidelity is taken care through various surveillance mechanisms. Nonsense-mediated mRNA decay (NMD) is one such surveillance mechanism where mRNAs with premature stop codons are detected and spontaneously degraded [24]. Deadenylation event begins with the decapping of mRNA and the NMD factors Upf1(ATP-dependent RNA helicase upstream frameshift 1), Upf2 (ATP-dependent RNA helicase upstream frameshift 1) and Upf3X (ATP-dependent RNA helicase upstream frameshift x) altogether forms a complex with PARN and initiate the process of deadenylation [24].

Evolutionary perspective of PARN

The stability of an mRNA poly (A) tail is regulated either by poly(A) polymerases (PAP) or by deadenylases. The major players in this regulation are poly(A)-specific ribonuclease (PARN), the CCR4–NOT complex, and the PAN2–PAN3 complex [25,26]. PARN is known to be structurally and functionally conserved in vertebrates, but invertebrates and even some

eukaryotes lack PARN presumably due to the fact that PARN belongs to CAF1 family of ribonucleases with RNA binding domains like RRM and R3H [27]. However, it is generally accepted that redundant deadenylases carry out the role of PARN in those eukaryotes that lack PARN. Indeed, homologs of PARN have been identified in eukaryotes like *Caenorhabditis elegans*, *Arabidopsis thaliana*, *Aedes albopictus*, *Apis mellifera*, *Schizosaccharomyces pombe* and *Neurospora crassa*. Intriguingly, PARN homologs are absent in *Saccharomyces cerevisiae* and *Drosophila melanogaster* [25,28-30].

The importance of PARN in maintaining mRNA stability in plants has also been highlighted through a number of studies. *Arabidopsis* has a single gene named *AHG2* that encodes the PARN homolog [31]. AHG2 mutant plants respond unusually to the phytohormones like salicylic acid and abscisic acid and show a dwarf phenotype [32]. Null AHG2 mutant plants are not viable, indicating the crucial role in the development [33,34]. AHG2-1 mutant *Arabidopsis* show accumulation of polyadenylated mitochondrial mRNA, suggesting that PARN also regulate the poly(A) stability of mitochondrial mRNAs in plants [34]. This unexpected discovery led to model that states that AHG2 and AGS1 regulatory system in *Arabidopsis* controls mitochondrial mRNA poly(A) stability and hypothesized that plants may have co-opted the cytosolic mRNA regulator PARN, for regulating the levels of mitochondrial mRNAs [34].

As described earlier, knock-out of PARN orthologue in *S. pombe and C. elegans* showed no obvious phenotype. In contrast, the T-DNA insertions in *Arabidopsis* PARN caused delayed embryo development and embryonic lethality, further indicating PARN appears to have contrasting roles in eukaryote viability [32,33]. However, it is important to note that the sequence identity between *Arabidopsis* PARN with its vertebrate orthologues is only ~ 20% with similarities only in the nuclease domain, suggesting that the presumed PARN proteins may not be true homologs across all species.

A study on *Xenopus* oocytes revealed that PARN controls the mRNA deadenylation, a process known to be triggered by progesterone-induced oocyte maturation [36]. Also, the process of deadenylation overlaps with breakdown of the nuclear envelope, allowing nuclear PARN to interact with the cytoplasmic substrates. Thus, the cytoplasmic polyadenylation element (CPE) with the CPE-binding protein (CPEB) maintains the poly(A) tail lengths of the RNAs that are important during the development of the oocytes [35]. Taking together, the above findings clearly indicate that the processes regulated by PARN and the biological consequences of loss of PARN differ considerably across evolution.

Diseases associated with PARN defects

1. Cancer

It is now becoming increasingly clear that there is a link between altered mRNA regulation and cancer**.** For instance, reduced activity of RNases specific for oncogenic mRNA or enhanced activity of RNases specific for tumor suppressor mRNAs can directly result in aberrant mRNA turnover or overexpression, thereby contributing to malignancy. Indeed, several RNases are now being considered to have tumor suppressive and/or tumor promoting roles [37]. Considering that PARN is involved in the first step of mRNA degradation, i.e., removal of the poly(A) tail, investigating the expression of PARN is of clinical significance in cancer. Interestingly, the expression of PARN is abnormal in many types of cancers including in solid tumors and in hematopoietic malignancies [38]. Additionally, PARN has been shown to regulate the stability of mRNAs that are involved in the DNA Damage Response, including the tumor suppressor TP53 and the oncogene c-MYC, by indirectly associating with their transcripts and keeping their levels low under normal non-stressed conditions.

In breast cancers, PARN activity appears to regulate PLD2 (phospholipase D2) in a reciprocal manner. Studies in breast cancer tissue revealed that silencing PARN using siRNA

led to an increase in levels of the PLD2 protein, while the overexpression of PARN decreased the concentration of PLD2 protein. Overexpression of PLD2, on the other hand, lead to upregulation of PARN protein. Simultaneous expression of PARN and PLD2 mimicked this observation in non-cancerous fibroblast cells, while PARN overexpression studies in breast cancer cells in contrast lead to the activation of PLD2 mRNA and its protein [39]. Alterations in phosphorylation status of the PARN protein can cause an increase in the rate of degradation of various mRNAs [40] including numerous mRNAs that code for oncogenes and tumor suppressors genes. It has been shown in some tumors that angiogenesis and cell proliferation-associated proteins including vascular endothelial growth factor (VEGF) and interleukin-8 [41-43] are negatively regulated by PARN. Also, PARN acts as a tumor suppressor by degrading IL-8 and VEGF mRNAs [44,45]. Other oncogenes such as c-JUN and *c*-FOS are also likely regulated indirectly by PARN through recruitment by RNA-binding proteins including KSRP, TTP, CUG-BP leading to destabilization of these oncogenic mRNAs [46-48]. Additionally, biallelic *PARN* mutations have been associated with severe bone marrow failure as well as in central hypomyelination. Knock down of *PARN* in human marrow cells was shown to result in impaired hematopoiesis and hypomyelination. PARN knockdown using morpholinos was confirmed to lead to defects in hematopoiesis in zebrafish model system [49]. Table 1 shows the expression of PARN in various types of cancer [50-54].

2.Aplastic anaemia

Aplastic anaemia (AA) is a bone marrow failure disorder resulting in a severe paucity of hematopoietic stem cells and is invariably life threatening. This illness can be treated with immunosuppressive drugs or hematopoietic stem cell transplantation (HSCT). The prolonged recovery process of haematopoiesis in patients after the stem cell transplant and their

response to immune suppressive therapies have suggested that the pathophysiology of AA is immune-mediated. Also, the alterations in the stromal microenvironment could account for the pathogenesis of AA [55].

In aplastic anaemia, early hematopoietic progenitor CD34+ cells are completely absent or found in reduced numbers [56,57]. Microarray analysis of AA-derived CD34+ cells show high levels of apoptosis and cell death [57-59] with shortened telomeres in white blood cells [60,61]. The telomere shortening may be due to mutation in various components of the telomerase complex resulting in low telomerase activity, telomere loss or decreased proliferation of hematopoietic stem cells [62]. Recent studies report that biallelic mutations in the *PARN* gene were found in several patients with AA, which was associated with hypomyelination. These results were supported by experiments done by Dhanraj et al., [49]where the authors demonstrated that knocking out PARN in human bone marrow CD34+ progenitor cells resulted in decreased hematopoietic colony formation similar to that observed in AA patients. Nevertheless, at this point draws a direct connection between the loss of PARN activity and AA is premature based on the existing evidence. Further experimentation will need to be done to confirm this correlation.

3.Idiopathic Pulmonary Fibrosis (IPF)

Idiopathic Pulmonary Fibrosis (IPF), a disease with progressive and irreversible decline in the pulmonary function, is characterised by the scarring of the pulmonary tissues. Having the appearance of dysplastic epithelial changes in fibrosis, the patients are prone to invasive malignancies [63]. IPF results in chronic damage to the alveolar epithelium, followed by impaired tissue repair and distorted structure of the alveoli. IPF appears to share several characteristics of malignancies, including poor response to treatment, as well as altered responses to signalling pathways, abnormal miRNA expression and activation of non-specific

signalling pathways. The survival rate is generally low in IPF [64-66]. The telomerase RNA component (TERC), which is responsible for regulating telomere length in eukaryotic cells a 3' H/ACA snoRNA-like domain, which is likely to be the target site for regulation by PARN and is involved in limiting telomerase activity and telomere elongation in mammalian cells [67-69]. Interestingly, several polymorphisms in TERC and telomerase reverse transcriptase (TERT) have been identified in both IPF and lung cancer patients. Many studies have revealed that patients with IPF are at a higher risk of progressing to lung cancer. This risk further increases with increasing age, smoking, combined pulmonary fibrosis and emphysema. Studies on IPF have also shown that about 75% cases have mutation in the tumor suppressor *TP53*. Mutations in *SMAD4* and *PTEN* were also common [70,71]. Interestingly, rare missense pathogenic variants in *PARN* have been identified in familial IPF, suggesting that defects in PARN might play a role in telomere dysfunction leading to IPF [72].

4.Dyskeratosis congenita

Mutations in the gene encoding dyskerin (*DKC1*) lead to the X-linked recessive form of dyskeratosis congenita (DC) [73]. Patients with this syndrome have defects in highly regenerative parts of the body like the skin and bone marrow. Chromosome instability is a hallmark of DC and hence patients are predisposed to malignancies. Many studies have shown that DC is caused by abnormal ribosomal RNA processing during ribosome biogenesis. Dyskerin is a pseudouridine synthase, important for the maturation of ribosomal RNA, and an essential part of the telomerase protein complex. Dyskerin interacts with TERC, as well as other small nucleolar RNAs (snoRNAs), via the highly conserved H/ACA RNA motif. Telomerase is involved in the addition of sequence repeats to telomere ends using RNA as a template, thereby preventing the induction of the DNA damage response

(DDR) and subsequent cell cycle arrest, senescence and/or apoptosis. In DC however, relatively lower levels of TERC and hence overall telomerase activity was observed in patients [74].

A recent study demonstrated bi-allelic mutations in the *PARN* gene in several unrelated cases of DC, suggesting that deadenylation deficiency may play a role in disease biology [75]. This deficiency was shown to result in an early induction of the DDR. DC cells when exposed to UV radiation demonstrated reduced survival, TP53 upregulation, and early cell cycle arrest. Additionally, the mRNA levels of several genes associated with telomerase maintenance including *TERC, DKC1,* Regulator of Telomere Elongation Helicase 1 (*RTEL1*) and Telomeric Repeat Binding Factor 1 (*TERF1*) were reduced in patients with biallelic *PARN* mutations [75]. Also, patients with Hoyeraal-Hreidarsson syndrome, a severe form of dyskeratosis congenita associated with short telomeres, exhibit mutations in DC-associated genes and *PARN* [76-78]. It was also observed that a subset of PARN deficient or dyskerin deficient cells, or cells expressing mutant TERC, accumulate TERC in cytoplasm. This observation suggests two important factors, first, that TERC can be exported from the nucleus to cytoplasm and second, that PARN deficiency and mutations in TERC or dyskerin can result in common features [79].

5.Do Rheumatoid arthritis and pulmonary fibrosis share common genetic risk factors?

Rheumatoid arthritis (RA) is a systemic, inflammatory autoimmune disorder, which predominantly affects adult populations. The worldwide prevalence is about 1% [80]. In 50% of rheumatoid arthritis patients lung involvement is observed leading to 10%-20% lung-related deaths [81-85]. Rheumatoid arthritis-Interstitial lung disease (RA-ILD) patients often present with interstitial pneumonia symptoms, which is also observed in patients with

pulmonary fibrosis [86]. Cigarette smoking has been shown to be a risk factor in RA-ILD [87].

Patients with familial pulmonary fibrosis (FPF) have been found to have mutations in genes associated with telomere maintenance [88]. The genes that are implicated in familial pulmonary fibrosis include genes encoding subunits of the telomerase complex (*TERT, TERC, DKC1*) [89-90] and associated proteins telomere-interacting factor-2 (*TINF2*) [91], *RTEL1*[92] and *PARN* [93]. The mechanistic association between telomerase complex associated gene mutations and FPF remains unclear [94]. Studies have also drawn some similarities between rheumatoid arthritis and interstitial lung disease and the common link appears to the telomere component [95-96]. Since PARN is a common player in IPF and telomere maintenance looking at its role in RA would be interesting. Table 2 shows the mutation status of PARN in various disease conditions.

Emerging roles of PARN

1.Regulation of noncoding RNAs by PARN

PARN apart from its role in mRNA degradation is also found to be involved in the maturation of H/ACA box snoRNAs. The snoRNAs are known for their role to induce chemical modifications in rRNAs, tRNAs and small nuclear RNAs. Generally, they are divided in two categories, the C/D box snoRNAs that are primarily involved in methylation and the H/ACA box snoRNAs that are associated with pseudouridylation [97].

Various lines of evidence suggest that PARN-mediated deadenylation is observed during processing of these noncoding RNAs. For instance, a study using HeLa cells showed that knockdown of PARN did not alter the poly(A) tail length or abundance of mRNAs, but instead played a major role in the maturation of diverse forms of noncoding RNAs like

snoRNAs and small Cajal body-specific RNAs (scaRNAs), TERC, specific miRNAs, and 18S rRNA [97,98].

From a microarray study, it was revealed that in both HEK293 and U2OS cells PARN knockdown contributed to the accumulation of H/ACA box RNAs, suggesting that PARN is involved in the maturation of H/ACA box snoRNAs, including TERC. Among all the H/ACA box RNAs, the small nucleolar RNA SNORA63 was strongly affected by PARN knockdown. This strongly suggested that the deadenylation associated with PARN is involved in the maturation of snoRNAs [99-101].

2.Role of PARN in miRNA maturation and stabilization

PARN is involved in trimming of the 3' end of the miRNA, either by dicer dependent or dicer independent pathways [102-104]. In dicer-independent pathway, Ago shRNAs (Argonaute short hairpin RNAs) or miRNA451 employees PARN to deadenylate the 3' end [105] while in dicer-dependent pathway, PARN trims the unused nucleotide recruited by the ncPAPs [103, 104] and deadenylate the extended 3' end of miRNA, which are post transcriptionally acquired or encoded by the genome. Additionally, deadenylation of oligo (A) at 3' end by PARN results in miR21 degradation [106] and de-stabilization of miR122 [107]. Also, two reports in 2011 suggest that Nibbler, a 3′-to-5′ exoribonuclease, seems to fill in for PARN in deadenylating miRNAs in Drosophila [108,109].

3.Role of PARN in telomere maintenance

Unlike an mRNA, a telomere RNA is non-polyadenylated. However, it has been shown that depletion of PARN in human cell lines results in a reduction in the levels of telomerase RNA. Although the exact mechanism still remains unclear, it is speculated that PARN is involved in the maturation of telomerase RNA, rather than its degradation [110]. Also**,** PARN deficiency

results in decreased expression of genes required in telomere maintenance leading to an aberrant DNA damage response including increased level of p53. On the other hand, PARN is involved in the post-transcriptional maturation of TERC [111]. These results confirm that PARN deficiency compromises telomere maintenance and indicate that PARN is rate-limiting factor for telomere maintenance in human cells, even in the presence of functional TERT. In humans, telomeres are elongated by the telomerase complexes that contains the reverse transcriptase hTERT, the pseudouridine synthase dyskerin and RNA template TERC/hTR. PARN is known to trim hTR precursors by removing poly(A)tail [112,113]. Also, the identification of mutations in *PARN* in telomere diseases including familial idiopathic fibrosis and DC, further consolidates the notion that PARN is involved in telomere maintenance.

4.Role of PARN in Ribosomal biogenesis

Studies in the human osteosarcoma (U2OS) cell line as well as in HEK293 cells showed that immunostaining with PARN- specific antibodies demonstrated localization of the PARN protein in nucleoli and Cajal bodies [114]. Furthermore, the nucleoli were found to be more densely stained compared to the nucleoplasm, suggesting that the concentration of PARN in the nucleoli was four times greater in comparison to that in the nucleoplasm. Loss of PARN immuno-staining in PARN knock-down cells confirmed nucleolar localization. It is noteworthy that the morphological structure of the nucleolus remained unaffected in the PARN knockdown cells [114].

Ribosome biogenesis is known to occur in a stepwise and ordered manner within several intracellular compartments including the nucleolus, the nucleoplasm and the cytoplasm [115,116]. The three sub-compartments of the nucleolus, the Nucleolar Organizer Regions consisting of active rDNA repeats, the dense fibrillar component (DFC), and the granular

component (GC), correspond respectively to sites of primary rRNA transcription and early and later maturation steps. Generally, ribosome biogenesis begins with the transcription of rRNA genes, which are transcribed into pre-rRNA precursor (47S) by RNA polymerase I. This is followed by a series of cleavage events aided by various nucleolar proteins to give rise to 18S, 5.8S, and 28S rRNA, while the other ribosomal components are transcribed in the nucleoplasm. The 5S rRNA is synthesized by Pol III from different loci located in the nucleolus vicinity. Later the ribosomal proteins along with the 5S rRNA are brought to the nucleolus, where they assemble to form 40S and 60S subunits. This assembly is then exported to cytoplasm to form the mature 80S ribosomes post modification. Ribosomes are ribonuclear protein supra-complexes which form the seat of mRNA translation. Two subunits comprise functional ribosomes (80S) in mammals, the large or 60S subunit and the small or 40S subunit [117,118]. Interestingly, PARN has been shown to be present as a component of pre-40s particles associated with the ribosome biogenesis [119]. Also, inhibition of PARN has shown accumulation of 3' extended 18S-E pre-rRNAs in the cytoplasm [120]. These data suggest that PARN is a key player in ribosome biogenesis, playing a role in the regulation of pre-rRNA processing at the later stages during the maturation of the 40S subunit of the ribosome.

5.TP53 and PARN: A feedback loop

TP53 is one of the most frequently altered genes in human cancer. Generally, wild type TP53 is a tetramer that binds to gene-specific cis elements in the promoters of responsive genes and in general, promotes expression of these genes which negatively influence cell growth and cell invasion [121]. Under normal physiologic conditions, PARN assists in keeping the levels of TP53 low. This occurs because PARN destabilizes the TP53 mRNA by binding to its AU-rich element (ARE) and subsequent deadenylation, causing it to turnover. When the

cell is stressed on the other hand (e.g. by UV radiation), levels of TP53 increase, which in turn increase expression of the PARN deadenylase leading to the destabilization of PARN target mRNAs [122]. Nevertheless, TP53 levels are not decreased by PARN under stressed conditions, likely in part because of the role of miR-125b-loaded miRISC and Ago-2 recruitment to TP53 mRNA, which normally contributes to deadenylation under non-stress conditions [123,124]. UV radiation is shown to downregulate miR-125b expression, preventing Ago-2 induced PARN activation, and to induce HuR binding to AREs, leading to stabilization of TP53 mRNA, explaining the increased TP53 expression in stressed conditions. Thus, PARN is known to regulate TP53 mRNA stability not only through ARE but also with miR-504 or miR-125b-targeting sites present in the 3′ UTR of the mRNA. The binding of PARN to the 3′ UTR is subject to both cis-acting signals and Ago-2 expression and also PARN deadenylase activity is triggered by the interaction of Ago-2 with the N-terminal domain of PARN.

TP53 has also been shown to interact with other PARN interacting proteins including CstF1 (cleavage stimulation factor 1), which blocks mRNA 3′ cleavage [125] and BRCA1-associated RING domain protein (BARD1), cross talk between PARN and CstF1/p53/BARD1 can have significant functional repercussions **(Figure 5)**. Taken together, PARN has an important role to play in cell proliferation by modulating the expression of p53 and p21 and depletion of PARN can induce cell death.

Regulation of PARN activity by cis-elements

Recent studies have revealed that three important classes of cis-acting elements in mRNA are involved in the binding of PARN to mRNA [125-127]. These elements include the cis element at the 5’ end located in the m^7 G cap structure, a 3’ cis-acting element located in the poly(A) structure and sequences in the 3’ UTR region of the mRNA including GU and AU

rich elements (GRE and ARE, respectively) [126]. The m^7-G cis-acting elements bind to PARN and upregulate PARN activity. This, however, does not happen if the competitive nuclear cap binding complex (CBC) is bound to the m^7 G Cap, which leads to inhibition of PARN activity [126]. PARN activity can also be inhibited in an m^7 G cap-independent way following PARN interaction with cap-binding protein 80 (CBP80). Even though CBC-m^7 G cap and CBP80 are known to inhibit PARN activity, the physiological context of this specific inhibition has not yet been clearly deciphered. Additionally, CBC-mediated inhibition of PARN is observed only when the mRNA to be degraded is located in the nucleus.

It is noteworthy that unlike in the nuclear compartment, PARN isolated from the cytoplasmic fraction was found to be inactive. It has been hypothesized that this observation could be the result of post-translational modification(s) of the PARN protein in the cytoplasm. In this context, studies have demonstrated that phosphorylation of PARN reduces its catalytic activity. The TP53 activity which is induced by UV radiation within the nucleus, PARN is thought to be activated in response to the UV radiation mediated DNA Damage Response (DDR) program. UV-induced changes in the nucleus are known to elicit a general downregulation of gene expression. Reduction in the activity of the nuclear mRNA polyadenylation machinery likely contributes to reduced gene expression under UV-induced stress. The involvement of PARN in UV-induced nuclear stress related polyadenylation inhibition is thought to occur by interaction with the tumor suppressor BRCA1-associated RING domain protein, BARD1. UV induced BARD1 interacts with the polyadenylation protein complex via the cleavage stimulatory factor (CstF) component CStF50 resulting in polyadenylation arrest [125].

Do TOE1, PNLDC1 compete with PARN?

TOE1, a DEDD family deadenylase found in the Cajal bodies of the nucleus is known to trim the 3' of small nuclear RNAs(snRNA) [128]. Previous reports suggest that depletion of TOE1 causes splicing defect and results in accumulation of adenylated snRNA [129]. This indicates that TOE1 may be involved in processing of the 3'end of snRNA. Since the canonical function of TOE1 and PARN are similar and both being the deadenylases, it can be speculated that they compete with each other for 3'end maturation of snoRNA, scaRNA and TERC. PNLDC1 is another deadenylase, strictly located in the cytoplasm. PNLDC1 is known to process the 3'end of piRNA (PIWI interacting RNAs, germ cell specific regulatory RNA) in *C. elegans* [130] and *B. mori* [131]. Also, in mice, loss of *PNLDC1* leads to accumulation of 3'end adenylated piRNAs resulting in impaired spermatogenesis [132]. Interestingly, PARN is also known to process the 3' end of piRNA in *C. elegans* [133]. Thus, it is conceivable that PARN may not be the only active deadenylase and there could be other redundant deadenylases that could come in play in absence of PARN. However, evidence for such a speculation is yet to be shown and the exact mechanism is yet to be still deciphered.

Epilogue

Although PARN has been primarily known as the regulator of the stability of mRNAs, recent studies indicate that PARN has multiple roles including the regulation of TP53 pathway, ribosome biogenesis and maturation of 3'-end of the telomerase RNA component. PARN has also been shown to be deregulated in many cancers. The identification of mutations in PARN in human diseases, primarily those that are linked to impaired telomere activity, suggests that PARN is not just involved in mRNA stock clearing, but has a much greater role in the cell that remains to be elucidated. Also, PARN has been confirmed as one of the exonucleases involved in the processing of the 3'-end of the human 18S rRNA and it is also known to trim oligo-adenylated tails of H/ACA box snoRNAs. Furthermore, our unpublished data suggest

that PARN is dispensable during the early embryonic development in zebrafish but appears to play a role in oogenesis as evidenced from the fact that homozygous null PARN mutant zebrafish turn out to be all males. Nevertheless, considering the recent discoveries pertaining to the non-canonical functions of PARN, it is becoming increasingly clear that PARN is not just about mRNA stock clearing but is essential for other important physiological activities in the cell namely telomere maintenance, and ribosome biogenesis **(Figure 6)**. A better understanding of the molecular pathways and mechanisms involved in the functions of PARN could provide crucial insights into deciphering the exact role of PARN in cells and the clinical relevance of PARN deficiency.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

DPN and NB did the literature search, organised the contents and prepared the first draft of the manuscript.

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AK-G and M-F O contributed to the review and editing of the manuscript.

PS, edited the first draft, revised the contents and edited the manuscript

AC conceptualized the manuscript, interpreted the data and wrote the final version of the manuscript.

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Figure with legends



Figure 1: A pictorial representation of a matured and fully processed eukaryotic mRNA showing 5'G-cap (green), untranslated regions (orange, red), coding regions(blue) and 3' Poly (A) Tail.

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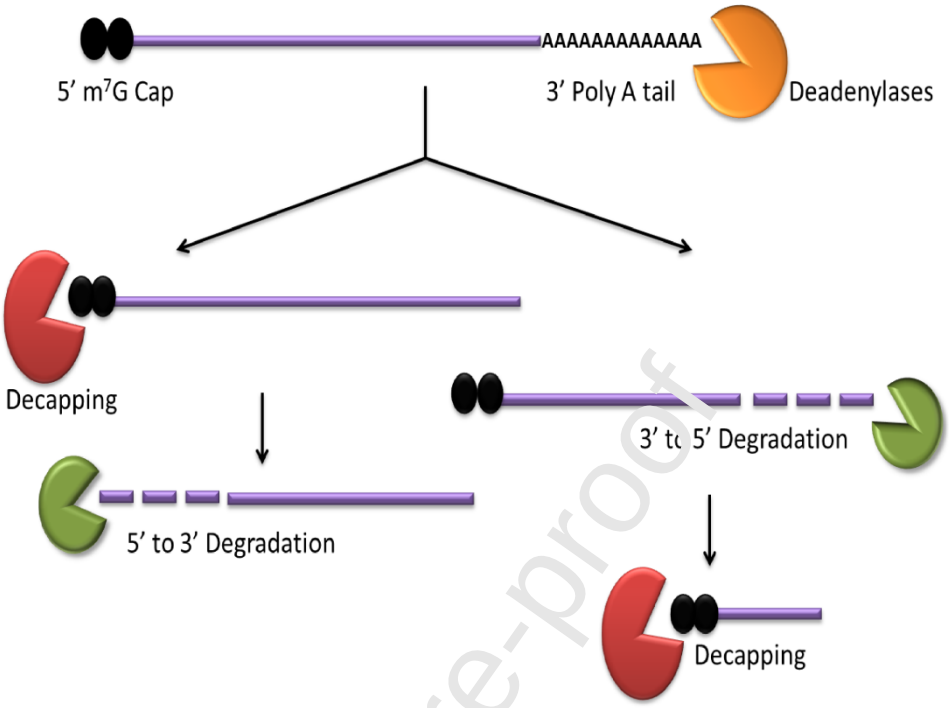


Figure 2: A schematics showing deadenylase dependent pathway of mRNA degradation in eukaryotes. mRNA degradation pathway is initiated by removal of 3' Poly A tail by the deadenylases. The shortening of Poly A tail may lead to 3'-5' exonucleolytic digestion of the mRNA, while decapping is initiated at the 5'-3' end by the exonucleases. The cap structure is cleaved later by the decapping enzymes

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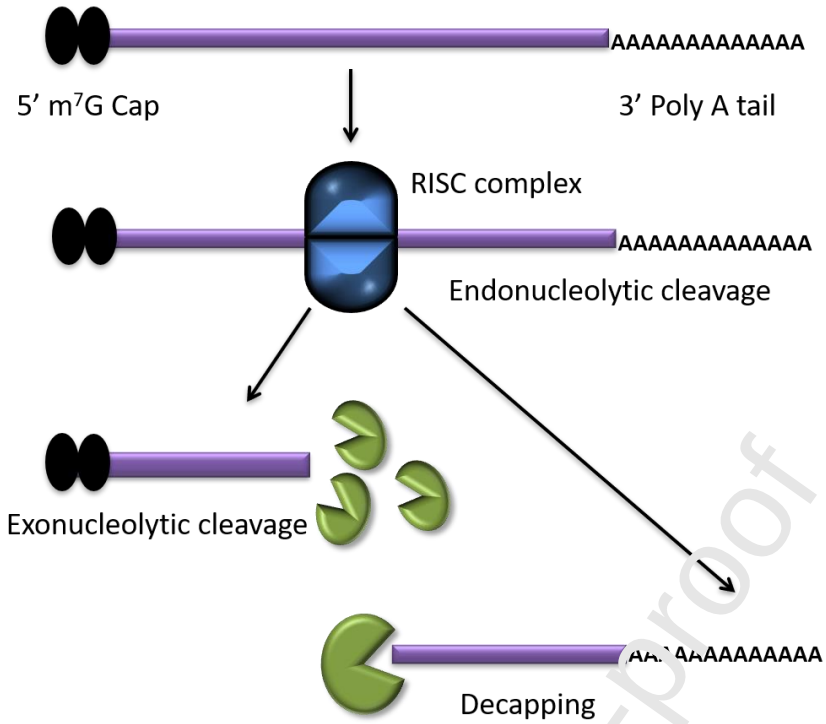


Figure 3: Endonuclease dependent pathway of mRNA degradation in eukaryotes. This process of degradation is initiated by the RNA induced silencing complex (RISC) that includes micro RNAs (miRNAs), short interfering RNAs (siRNAs) and endonucleases (such as Argonaute orAgo2).

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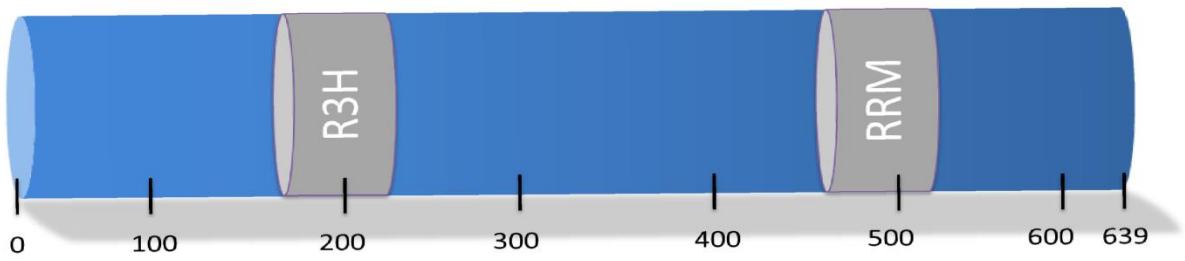


Figure 4: A schematic representation of regulatory regions of PARN. A full size PARN is encoded by 639 amino acids (shown 1-639), has two RNA binding domains, R3H(178-245 residue) and RRM(437-510residue).

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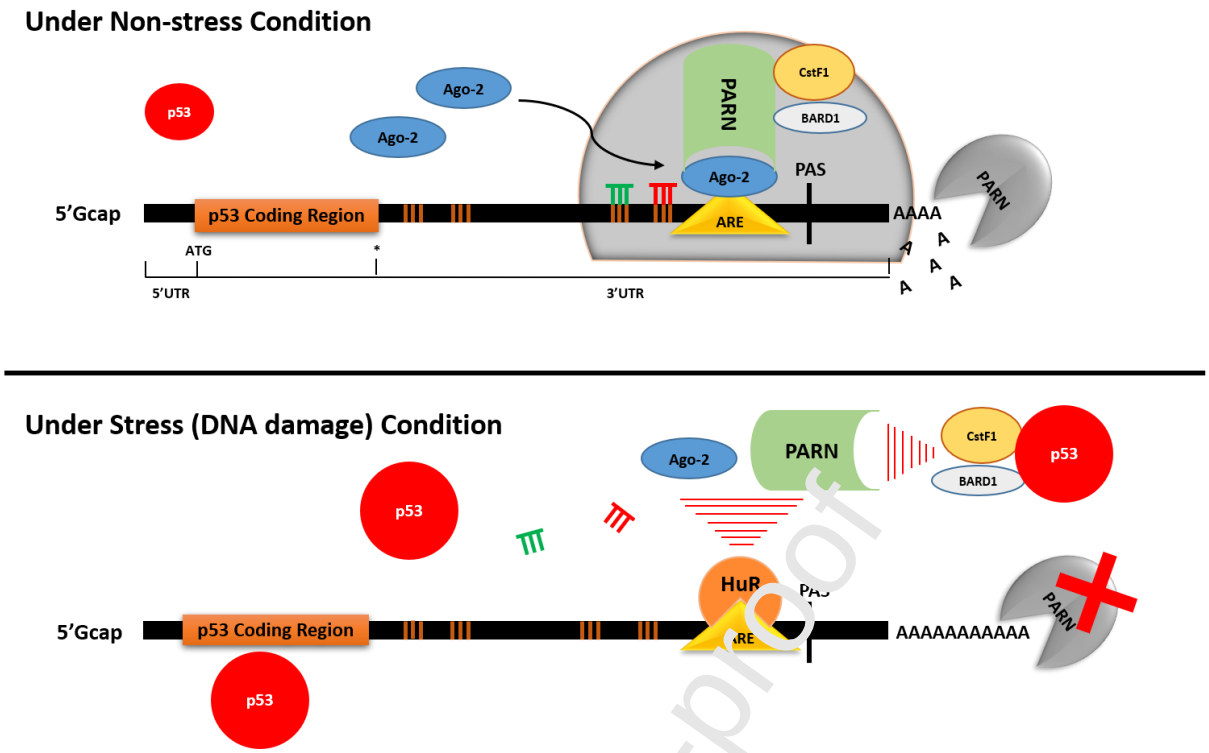


Figure 5: Regulation of TP53 by PARN. During non-stressed condition, PARN regulates the expression of TP53 mRNA through ARE-miRNA-Ago 2 complex (shown in grey)-mediated de-adenylation. PARN binds to its TP53 mRNA at the AU-rich elements (ARE) by associating with Ago2/RISC complex that includes specific miRNAs (miR125b, shown in red and miR-504, shown in green) to carry out the degradation process, thus keeping the level of TP53 low. The PARN binding proteins, cleavage stimulation factor-1 (CstF1), that inhibits mRNA 3' cleavage, and BRCA-1 associated RING domain protein (BARD1) also participate in this process. However, under stress conditions, due to the competitive binding of Human antigen R (HuR) to the ARE site of TP53, PARN is unable to bind to TP53 mRNA. In addition, TP53 interacts with CstF1 and BARD1 and prevent their binding with PARN. Thus the ARE-miRNA-Ago 2 complex doesn't form and it leads to a decreased deadneylation of TP53 mRNA, resulting in stabilization of TP53.

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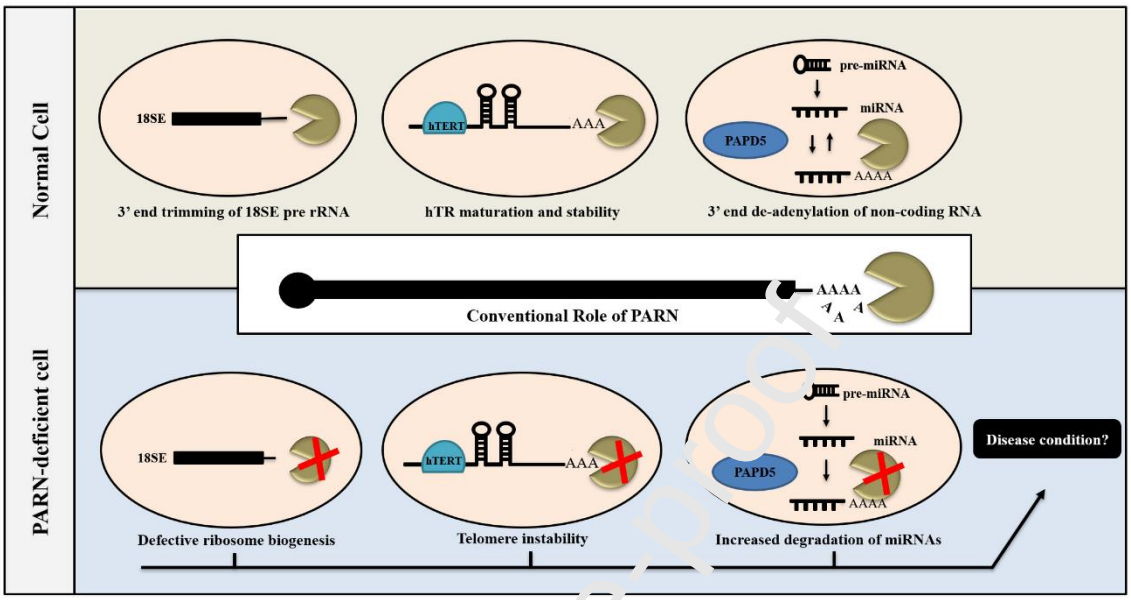


Figure 6: An overview of traditional and emerging functions of PARN. The conventional role of PARN in the removal of adenosine residues from poly-A tails of mRNAs in a 3'-5' direction is shown in the middle of the image. Recent data suggest three emerging roles of PARN, including the processing of the 3'-end of the human 18S rRNA, trimming of oligo-adenylated tails of H/ACA box snoRNAs and finally in miRNA processing. These emerging functions of PARN are linked to mRNA translation, telomere maintenance, and ribosome biogenesis, which are de-regulated in many disease conditions.

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Tables

Table1: PARN expression in cancer

CANCER TYPE	EXPRESSION OF PARN	REFERENCE
Follicular Lymphoma	No Change	Kloops et al., 2007(50)
Acute Leukemia	Upregulated	Maragozidis et al., 2012(51)
Small Cell Lung Carcinoma	Upregulated	Maragozidis et al., 2015(52)
Gastric cancer	Upregulated	Zhang, et al., 2015(38)
Breast Cancer	Deregulated	Miller et al., 2017(53)
Triple negative breast cancer	Downregulated	Cambronero et al., 2018(54)

Table 2: A consolidated table showing disease-associated alterations in *PARN*

DISEASE	MUTATION	REFERENCE
Idiopathic pulmonary fibrosis	Variants	Stuart et al., 2015 (92)
Dyskeratosis congenital	Biallelic mutations	Tummala et al., 2015 (75)
Haematological and neurological manifestation	Monoallelic and biallelic mutations	Dhanraj et al., 2015 (49)
Hoyeraal-Hreidarsson syndrome	Biallelic mutations	Dodson et al., 2019 (76) Benyelles et al., 2020(77)

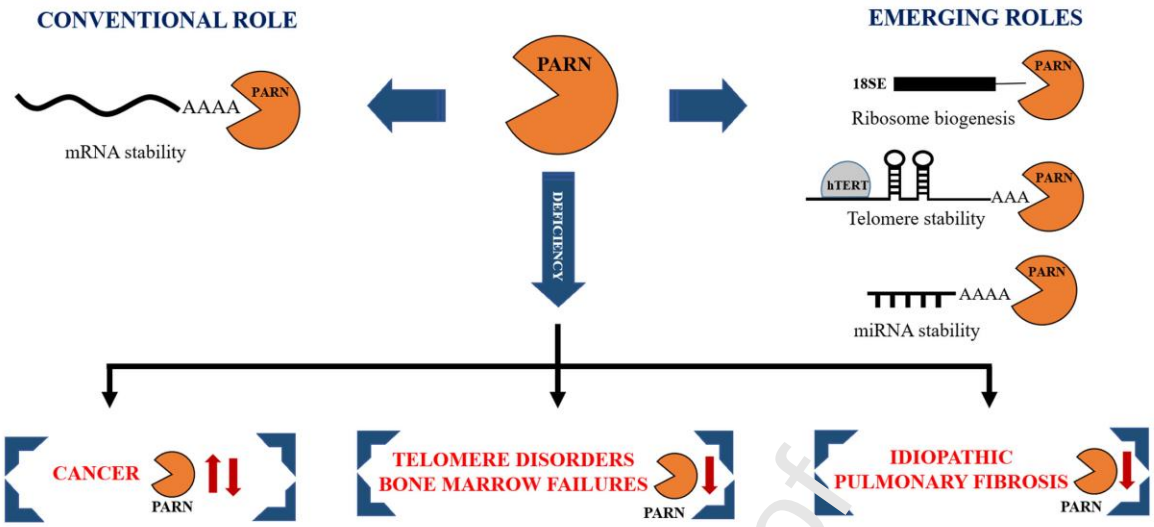
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Conflict of interest

The authors declare no conflict of interest.

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Graphical abstract

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