



HHS Public Access

Author manuscript

Oncogene. Author manuscript; available in PMC 2016 July 21.

Published in final edited form as:

Oncogene. 2011 April 14; 30(15): 1733–1743. doi:10.1038/onc.2010.572.

Human Polynucleotide Phosphorylase (*hPNPase^{old-35}*): An evolutionary conserved gene with an expanding repertoire of RNA degradation functions

Swadesh K. Das¹, Sujit K. Bhutia¹, Upneet K. Sokhi¹, Rupesh Dash¹, Belal Azab¹, Devanand Sarkar^{1,2,3}, and Paul B. Fisher^{1,2,3,*}

¹Department of Human and Molecular Genetics, Virginia Commonwealth University, School of Medicine, Richmond, VA 23298, USA

²VCU Institute of Molecular Medicine, Virginia Commonwealth University, School of Medicine, Richmond, VA 23298, USA

³VCU Massey Cancer Center, Virginia Commonwealth University, School of Medicine, Richmond, VA 23298, USA

Abstract

Human polynucleotide phosphorylase (*hPNPase^{old-35}*) is an evolutionary conserved RNA processing enzyme with expanding roles in regulating cellular physiology. *hPNPase^{old-35}* was cloned using an innovative “overlapping pathway screening” strategy designed to identify genes coordinately regulated during the processes of cellular differentiation and senescence. Although *hPNPase^{old-35}* structurally and biochemically resembles PNPase of other species, overexpression and inhibition studies reveal that *hPNPase^{old-35}* has evolved to serve more specialized and diversified functions in humans. Targeting specific mRNA or non-coding small microRNA (miRNA), *hPNPase^{old-35}* modulates gene expression that in turn plays a pivotal role in regulating normal physiological and pathological processes. In these contexts, targeted overexpression of *hPNPase^{old-35}* represents a novel strategy to selectively downregulate RNA expression and consequently intervene in a variety of pathophysiological conditions.

Keywords

hPNPase^{old-35}; Senescence; RNA degradation; c-myc; miRNA

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:http://www.nature.com/authors/editorial_policies/license.html#terms

*Corresponding Author: Dr. Paul B. Fisher, Professor and Chairman, Department of Human and Molecular Genetics, Director, VCU Institute of Molecular Medicine, VCU Massey Cancer Center, Virginia Commonwealth University, School of Medicine, 1101 East Marshall Street, Sanger Hall Building, Room 11-015, Richmond, VA 23298-0033, 804-828-9632; 804-827-1124 (Fax); pbfisher@vcu.edu.

Conflict of Interest

The authors declare no conflicts of interest.

1. Introduction

RNA degradation and/turnover are major processes controlling RNA levels and are important regulators of physiological and pathological processes (Parker and Song, 2004). Labile messenger RNAs to more stable non-coding RNAs (mostly rRNA and tRNA, but also the expanding class of small regulatory RNAs) are eventually degraded by a complex process involving the simultaneous or sequential interplay of multiple proteins. Many of these proteins are evolutionary conserved extending from prokaryotes to higher mammals and serving comparable functions. In eukaryotic cells, a variety of ribonucleases (RNases) act cooperatively, initially shortening the 3' poly (A) tail of an mRNA by deadenylases followed by the removal of the 5' cap structure by a decapping enzyme, which enables the degradation of the transcript by a 5'→3' exoribonuclease. Alternatively, the mRNA might be degraded from the 3' end by the cytoplasmic exosome, a multiprotein complex of diverse 3'→5' exoribonucleases. Polynucleotide phosphorylase (PNPase) is a 3'→5' exoribonuclease that uses the phosphorolytic mechanism to degrade RNA (Sarkar *et al.*, 2006; Mohanty *et al.*, 2000; Yehudai *et al.*, 2001). It is conserved evolutionarily and is expressed in different species including bacteria, plants, worms, flies, mice and humans. Our group cloned the human homolog of polynucleotide phosphorylase (*hPNPase^{old-35}*) in the unique contexts of differentiation and senescence, using an “overlapping pathway screening” scheme. We documented that *hPNPase^{old-35}* plays a key role in regulating both of these fundamental physiological processes (Leszczyniecka *et al.*, 2002, 2003, 2004; Sarkar *et al.*, 2003, 2004, 2005, 2006, 2007; Sarkar & Fisher, 2006). We presently review recent advances in our understanding of *hPNPase^{old-35}*-mediated RNA degradation, in particular its ability to target different classes of RNAs.

2. Cloning, expression and localization of *human polynucleotide phosphorylase (hPNPase^{old-35})*

hPNPase^{old-35} was cloned by using an overlapping pathway screening (OPS) approach (Leszczyniecka *et al.*, 2002) during a screen for genes up-regulated in the process of terminal cellular differentiation and senescence. Although terminal cell differentiation and cellular senescence represent two discrete phenomena, these processes have several common characteristics. Both are distinguished by irreversible growth arrest associated with marked inhibition of DNA synthesis, inhibition of telomerase activity, and modulation of discrete programs of gene expression, especially up-regulation of cyclin-dependent kinase inhibitors (CDKI) (Campisi, 1992; Fisher *et al.* 1985, 1986). Combined treatment of metastatic human melanoma cells HO-1 with recombinant human fibroblast interferon (IFN)- β and the protein kinase C activator mezerein (MEZ) induces irreversible growth arrest accompanied by morphological, biochemical, antigenic and gene expression changes culminating in a state of ‘terminal differentiation (Fisher and Grant, 1985; Fisher *et al.*, 1985, 1986; Guarini *et al.*, 1989, 1992; Jiang *et al.*, 1993, 1995). Screening of a temporal cDNA library generated from terminally differentiated HO-1 melanoma cells with cDNAs from senescent progeroid fibroblasts identified 75 genes, termed *old-1* to *-75*, that were upregulated during both terminal differentiation and senescence. Sequence analysis of one particular clone, *old-35*, confirmed its identity to the PNPase gene, resulting in the gene being renamed

hPNPase^{old-35} (Leszczyniecka *et al.*, 2002). The *hPNPase^{old-35}* gene consists of 28 exons and 27 introns spanning 54 kb in chromosome 2p15–2p16.1 (Leszczyniecka *et al.*, 2003). Of interest, this unstable genomic region is prone to cytogenetic alterations in human cancers and in various genetic disorders (Kirschner *et al.*, 1999) such as B-cell lymphoma (Fukuhara *et al.*, 2006), type I hereditary nonpolyposis colorectal cancer, familial male precocious puberty, Carney complex, Doyme's honeycomb retinal dystrophy and DYX-3, a form of familial dyslexia (Kirschner *et al.*, 1999). *hPNPase^{old-35}* mRNA expression could be detected in all normal tissues analyzed with the highest expression being detected in heart and brain (Leszczyniecka *et al.*, 2002). However, to date, there is no evidence linking expression and function of hPNPase to any of the aforementioned pathological processes.

In bacteria, PNPase autogenously regulates its expression by promoting the decay of PNPase mRNA by binding to the 5'-untranslated leader region of an RNase III-processed form of this transcript (Jarrige *et al.*, 2001). To date the only known regulators of *hPNPase^{old-35}* transcription are type I interferon (IFN- α and IFN- β) in both normal and cancer cells with diverse backgrounds irrespective of their p53 and Rb status (Leszczyniecka *et al.*, 2002). Double-stranded RNA and poly(I) poly(C), a known inducer of IFN- α and IFN- β , also stimulate *hPNPase^{old-35}* expression while IFN- γ and TNF- α have minimal or no effect, respectively. *hPNPase^{old-35}* is an early IFN response gene and its induction depends on the Janus-activated kinase ((JAK)/STAT (signal transducers and activators of transcription) signal transduction pathways. Analysis of the *hPNPase^{old-35}* promoter identified an IFN-stimulated response element (ISRE) that showed increased binding of ISGF3 complex upon IFN- β treatment (Leszczyniecka *et al.*, 2003). Mutation in this site abolished IFN- β induction of the promoter indicating that *hPNPase^{old-35}* is regulated at the level of transcription. In addition to the ISRE, the *hPNPase^{old-35}* promoter contains additional putative regulatory protein-binding sites, including a site for E2F transcription factor 3 (E2F3), a transcriptional repressor that is responsible for gene silencing during the G₁ to S phase transition (Gewartowski *et al.* 2006).

Studies using cellular fractionation and/or immunofluorescence showed that endogenous *hPNPase^{old-35}* and overexpressed C-terminal myc- tagged *hPNPase^{old-35}* localize only to the mitochondria (Chen *et al.*, 2006; Piwowski *et al.*, 2003). *hPNPase^{old-35}* has a typical mitochondrial localization signal (MTS) at the NH₂-terminal and it is imported into the mitochondria by *i*-AAA (ATPases associated with several diverse cellular activities) protease Yme1, localized into mitochondrial intermembrane space (IMS) and maintains mitochondrial homeostasis (Chen *et al.*, 2006). However, our studies document that overexpressed C-terminal HA-tagged *hPNPase^{old-35}* localizes both in cytosol and mitochondria (Sarkar *et al.*, 2005) indicating that *hPNPase^{old-35}* might reside within or outside mitochondria. In these contexts, the targets and the consequences of *hPNPase^{old-35}* expression in different cellular compartments may be distinct and diverse, thereby expanding the repertoire of activities of this interesting enzyme.

3. RNA degradation machinery: PNPase and Exosome

Ribonucleases (RNase) are enzymes that are master regulators of stability and decay of RNA (Deutscher *et al.*, 1993a, 1993b; Deutscher and Li, 2001). Depending upon their degradative

properties, RNases are divided into 2 functional classes, endoribonucleases that cleave RNA molecules internally and exoribonucleases that act at the end of RNA chains (Deutscher *et al.*, 1993b). RNA decay pathways in two of the most comprehensively studied model systems, the prokaryote *Escherichia coli* and the eukaryote *Saccharomyces cerevisiae*, are different. In eukaryotes exoribonucleases can degrade RNA both at 5' to 3' and 3' to 5' directions (Deutscher and Li, 2001), whereas in prokaryotes RNA degradation takes place only in the 3' to 5' direction. However, an interesting aspect of RNA decay in both prokaryotes and eukaryotes is the presence of multiprotein complexes known as the degradosome and the exosome, respectively.

In *E.coli*, PNPase is associated with the endonuclease RNase E, RNA helicase and the glycolytic enzyme enolase to form the degradosome and executes its processive 3' to 5' phosphorolysis or degradation of RNA species upon endonucleolytic cleavage by RNase E. PNPase acts as an integral component of degradosome like RNA-helicase and enolase. In contrast, in yeast, PNPase is absent and the exosome, a complex of multiple exoribonuclease performs RNA degradation in both the cytoplasm and the nucleus (Buttner *et al.* 2006; Mitchel *et al.* 1997; Hoof *et al.* 1999; Raijmakers *et al.* 2004). However, PNPase has been identified in higher animals such as mouse, rat and human indicating that the exosome and PNPase might serve specialized functions in these species (Leszczyniecka *et al.*, 2002, 2003, 2004; Raijmakers *et al.*, 2002).

In all species, PNPase contains five motifs that are conspicuously preserved through evolution extending from prokaryotes and plants to mammals (Leszczyniecka *et al.*, 2004; Almeida *et al.*, 2008). Two conserved catalytic RNase PH regions, a small domain of ~250 a.a. residues related to the *Escherichia coli* RNase PH enzyme and involved primarily in the 3' processing of transfer RNA (tRNA) precursors, are present at the N-terminus of *hPNPase^{Old-35}* (Leszczyniecka M *et al.* 2004). These RNase PH domains are separated by an α -helix that is unique to PNPase (Symmons *et al.*, 2002). The RNA binding property of *hPNPase^{Old-35}* is conferred by two C-terminal RNA binding domains, KH and S1 (Symmons *et al.*, 2000, 2002; Leszczyniecka *et al.*, 2002, 2004; Raijmakers *et al.*, 2002). In addition to the characteristic 5 motifs, plant PNPase contains an N-terminal target peptide allowing translocation to chloroplast and the mammalian PNPase contains an N-terminal mitochondrial localization signal facilitating its subcellular localization in mitochondria (Piwowarski *et al.*, 2003, Yehudai-Resheff *et al.*, 2003 and Sarkar *et al.*, 2005). The catalytic activity of PNPase enzyme in bacteria is located mainly in the second RNase PH domain (Jarrige *et al.*, 2002) while in spinach chloroplasts, both RNase PH domains have equal polyadenylation and exoribonuclease activity. Deletion and mutation analysis have identified the critical regions of bacterial PNPase activity. Deletion of the S1 or KH RNA binding domain reduces the enzymatic activity by 50- or 19-fold, respectively, while deletion of both S1 and KH domains results in 1% of the enzymatic activity (Stickney *et al.*, 2005). These deletions do not interfere with interaction with RNase E to form the degradosome. Site-directed mutagenesis reveals that the catalytic center of PNPase is located in the second RNase PH domain around the binding site for tungstate, a phosphate analogue (Jarrige *et al.*, 2002). Interestingly, a G454D mutation in the second RNase PH domain displays defective RNA binding and impairs the autogenous regulation of PNPase mRNA indicating that in

addition to S1 and KH domains, the catalytic domains are also capable of binding to RNA (Regonesi *et al.*, 2004).

The alignment of the proteins in eukaryotic exosome clearly points to a structural similarity with bacterial PNPase. The three-dimensional structure of the PNPase from the bacterium *Streptomyces antibioticus* has revealed that the enzyme is a ring (doughnut)- shape formed by a homotrimeric complex, with the hexameric PH-domains surrounding a central channel that can accommodate a single-stranded RNA molecule (Symmons *et al.*, 2002). Similarly, the same core structure is formed in the exosome: 6 RNase PH homologues comprise the core while 3 additional exosome subunits, which contain S1 RNA-binding domains, are positioned on the outer surface of the ring (Mitchell and Tollervey, 2000; Rajmakers *et al.*, 2002; Symmons *et al.*, 2002).

4. Specialized functions of *hPNPase^{old-35}*: RNA degradation

In eukaryotes, the poly(A) tail of mRNA first becomes deadenylated by 3 different enzyme complexes, the first composed mainly of Ccr4p and Pop2p, the second containing Pan2p and Pan3p and the third is a poly(A)-specific exonuclease called PARN (poly(A) ribonucleases) (Parker and Song, 2004). Following deadenylation, a decapping enzyme consisting of Dcp1p and Dcp2p removes the 5' cap structure thus facilitating degradation by the 5', 3' exoribonuclease Xrn1p (Tucker and Parker, 2000). Alternatively, after deadenylation, mRNA can be degraded in a 3' to 5' direction by the exosome complex and the oligonucleotide cap structure is hydrolyzed by the decapping enzyme DcpS (Wang and Kiledjian, 2001). In addition, mRNA can also be degraded by endoribonucleases and aberrant mRNA may be degraded by specialized pathways such as nonsense-mediated decay (NMD) or nonstop decay (NSD) that degrade mRNA containing a premature stop codon or lacking a stop codon, respectively (Dodson and Shapiro, 2002; Frischmeyer *et al.*, 2002 and Cao & Parker, 2003).

PNPase, as a phosphatase, it incorporates *P_i* and ADP in degradation and polymerization process, respectively (Littauer and Grunberg, 1999). Optimal degradation activity depends on the concentration of *P_i* and it varies from species to species (Portnoy *et al.*, 2008). The human PNPase is active in much lower concentration of *P_i* compared with bacterial PNPase (Portnoy *et al.*, 2008). The specificity of the enzyme for the polymerization reaction is, like that of the *E.coli* PNPase, is high for ADP, with much less activity for other NDPs and no activity for ATP/NTPs. More interestingly, *hPNPase* displays no preferential activity for polyadenylated RNA like bacterial or chloroplast PNPase (Portnoy *et al.*, 2008).

4.1. *c-myc* mRNA is the target of *hPNPase^{old-35}*

In the cytoplasm, adenoviral mediated overexpression of *hPNPase^{old-35}* could directly degrade *c-myc* mRNA by virtue of its 3'→5' exoribonuclease property and this degradation is specific for *c-myc* as compared with other mRNAs such as *c-jun*, GAPDH or GADD 34 (Sarkar D *et al.*, 2003). It is still not clear what confers the specificity of *hPNPase^{old-35}* for *c-myc* mRNA. There might be a specific sequence in *c-myc* mRNA that allows *hPNPase^{old-35}* binding and degradation. In *E. coli*, PNPase degrades a family of cold shock proteins (CSP) that do not show any sequence similarity (Yamanaka and Inouye, 2001). Considering this

observation, the secondary structure of the mRNA rather than its primary sequence might be the determining factor for specificity of *hPNPase^{old-35}* binding and degradation of *c-myc* mRNA and induction of morphological, biochemical and gene expression changes by *hPNPase^{old-35}* (Sarkar *et al.*, 2005) (described in next section of this review). While in bacteria, presence of the KH and the S1 RNA binding domains are required for functional activity of PNPase, *hPNPase^{old-35}* still retained its functional activity upon removal of KH and S1 domains (Sarkar *et al.*, 2005; Stickney *et al.*, 2005).

4.2. *hPNPase* might be involved in degradation of RNA in mammalian mitochondria

In mammals, mitochondrial RNA (mtRNA) degradation is not well defined as no RNA degrading complex has been identified. The current view is largely based on our understanding of the *E.coli* RNA degradosome and yeast mitochondrial exosome. Similar to cytoplasmic mRNAs, mtRNAs also require long poly (A) tails for recruitment of poly (A)-binding proteins for maintenance of stability (Temperley *et al.*, 2003). Human mitochondria-specific poly (A) polymerase (PAP) has been shown to synthesize mtRNA poly(A) tails. Knocking down PAP by siRNA decreases the length of poly (A) tail of mitochondrial mRNA and decreases their steady state levels (Nagaike *et al.*, 2005). The mitochondrial membrane potential (ψ) and oxygen consumption also decreased in PAP siRNA treated cells. In contrast, knocking down of *hPNPase* showed significantly extended poly(A) tails of mtRNA although it did not affect the steady-state levels of these mRNAs or their translational products (Nagaike *et al.*, 2005; Temperley *et al.*, 2003). In these studies, only the levels of full-length mtRNAs were quantified without considering the truncated and polyadenylated mtRNAs that normally occur in human mitochondria (Slomovic *et al.*, 2005). In normal mammalian mitochondria, truncated and polyadenylated transcripts do not accumulate and are rapidly degraded (See *et al.*, 1972). Recent studies suggest that knocking down hSUV3 (human Suppressor of Var1 3) helicase leads to an accumulation of shortened polyadenylated mtRNA species (Khidr *et al.*, 2008). hSUV3 makes a heteropentameric complex with *hPNPase* at a 2:3 molar ratio in a coordinated manner to degrade dsRNA substrates (Wang *et al.*, 2009). Considering this recent finding it is hypothesized that the hSUV3-*hPNPase* complex might be involved in removing truncated RNA species through *hPNPase*-mediated degradation. Further experimentation is required to validate this possibility.

4.3. Modulation of miRNAs by *hPNPase^{old-35}*

MicroRNAs (miRNAs), a subset of non-coding RNAs, are 22–25-nt long endogenously-initiated short RNA molecules that regulate gene expression at the post-transcriptional level and play important roles in a multiplicity of biological functions, including cell differentiation, tumorigenesis, apoptosis and metabolism (Ambros, 2004). An individual miRNA is able to control the expression of more than one target mRNA and each mRNA may be regulated by multiple miRNAs. The interaction between miRNA and mRNA are usually restricted to the “seed”- 6 to 8-nt at the 5' region of miRNA and partially complementary sites in the 3' UTRs of target mRNAs, resulting in either translational repression or target degradation (Kim, 2005). miRNAs are initially transcribed principally by either RNA polymerase II or RNA polymerase III as long primary transcripts (pri-

miRNAs), which are further processed by the nuclear RNase Drosha and cytoplasmic RNase Dicer to produce precursor miRNAs (pre-miRNAs) and mature miRNAs, respectively (Calin and Croce, 2006) (Figure 1). In principle, miRNA abundance could be controlled by developmental and tissue specific signaling (Landgraf, 2009). The steady state levels of miRNAs, crucial for its profound impact on a wide array of biological processes (Filipowicz *et al.*, 2008; Tsuchiya *et al.*, 2006) are presumably regulated by the opposing activities of miRNA biogenesis and degradation.

In the biogenesis process, miRNAs might be regulated both transcriptionally and post-transcriptionally. Numerous Pol II-associated transcription factors such as myogenin and MYOD1 are involved in transcriptional control of miR-1 and miR-133 genes during myogenesis (Chen JF *et al.*, 2006; Rao *et al.*, 2006). Some miRNAs are under the control of tumor suppressive p53 (reviewed by He *et al.*, 2007) or the oncogenic transcription factor *c-myc* (Chang *et al.*, 2008; He *et al.*, 2005). Epigenetic control also contributes to miRNA gene regulation (Bueno *et al.*, 2008). Several miRNAs expressions are also regulated at the post-transcriptional level. The primary transcript of let-7 (pri-let-7) is expressed in both undifferentiated and differentiated ES cells, whereas mature let-7 is detected only in differentiated cells, indicating that let-7a might be posttranscriptionally controlled (Suh *et al.*, 2004; Thomson *et al.*, 2006; Wulczyn *et al.*, 2007). Excluding this activity, turnover of miRNA also might contribute in maintaining its level, however, this remains a largely unexplored area. In *C.elegans* (Kennedy *et al.*, 2006) an exoribonuclease ERI1 (also known as THEX1) was previously shown to degrade siRNAs. Another exoribonuclease named small RNA degrading nuclease (SDN) proteins were reported to affect the stability of miRNAs in plants (Ramachandran and Chen, 2008). Very recently we demonstrated a role of *hPNPase^{old-35}* in human miRNAs degradation (Das *et al.* 2010). This novel way of regulating miRNA levels is being actively explored by our research group.

4.3.1. *hPNPase^{old-35}* downregulates specific miRNAs—miRNA microarray analysis between Ad.*hPNPase^{old-35}*- and Ad.vec-infected human melanoma cells identified specific miRNAs differentially regulated by *hPNPase^{old-35}*, which have been subsequently validated by qPCR and Northern Blot analyses. Of interest, robust downregulation of several miRNAs (e.g., miR-221, miR-222, miR-106b) by *hPNPase^{old-35}* was observed while a number of miRNAs were not affected (miR-184, miR-let7a) suggesting specificity and selectivity of this enzyme-mediated miRNA downregulation. Moreover, an inverse correlation between these miRNAs and *hPNPase^{old-35}* in primary melanocytes and different melanoma cell lines support the biological relevance of this protein in regulating specific miRNAs (Figure 2)

4.3.2. Post-transcriptional modification of miRNA biogenesis by *hPNPase^{old-35}*—miRNA genes are initially transcribed as long primary transcripts (pri-miRNAs), which are further processed to produce precursor miRNAs (pre-miRNAs) and mature miRNAs (Figure 1) (Calin and Croce, 2008). Ad.*hPNPase^{old-35}* did not show any effect on pri-miRNA and pre-miRNA. Only mature miRNAs were downregulated by *hPNPase^{old-35}*. *hPNPase^{old-35}* is an exoribonuclease and in vivo immunoprecipitation assays

clearly demonstrate specific binding of mature miRNAs to hPNPase^{old-35}, a pre-requisite step for enzymatic activity indicating that miRNAs undergo enzymatic degradation.

4.3.3. hPNPase^{old-35} preferentially degrades miR-221—Although the overexpression of hPNPase^{old-35} selectively downregulates specific miRNAs, the preferences to miRNAs varied significantly. In melanoma cells, degradation of miR-221 by hPNPase^{old-35} was more profound compared to other miRNAs tested. This preferential activity might be attributed to high binding affinity of hPNPase^{old-35} to the mature miR-221, a hypothesis that needs to be tested (Figure 3).

4.3.4. Role of human polynucleotide phosphorylase^{old-35} mediated miRNA degradation in interferon-β-mediated growth inhibition—hPNPase^{old-35} is an IFN-β-inducible early response gene and treating a panel of melanoma cells with IFN-β also downregulates hPNPase^{old-35}-target miRNAs. This effect was abrogated by knocking down hPNPase^{old-35}, thus establishing functional and mechanistic links between IFN-β-mediated hPNPase^{old-35} induction and miRNA downregulation. IFN-β is a potent growth inhibitor and one of the growth inhibiting mechanisms in melanoma is the induction of hPNPase^{old-35}, which in turn raises the level of p27^{Kip1}, a cyclin-dependent kinase inhibitor protein, by degrading *c-myc* mRNA (Sarkar *et al.*, 2006). However, forced degradation of *c-myc* by siRNA did not completely block IFN-β-mediated growth arrest indicating the existence of additional pathways potentially regulating this effect (Barnes and Karin, 2008; O'Byrne *et al.*, 1999; O'Hagan *et al.*, 2000). Since miR-221 directly targets p27^{Kip1} mRNA (Fornari *et al.*, 2008), IFN-β mediated hPNPase^{old-35}-dependent miR-221 downregulation might also be involved in inducing growth arrest.

5. Other biological functions of human polynucleotide phosphorylase^{old-35} protein

5.1 hPNPase^{old-35} and cellular senescence

Senescence is a state of irreversible growth arrest induced spontaneously in primary cells after a finite number of population doublings (replicative senescence) or induced by endogenous and exogenous acute and chronic stress signals (stress- or aberrant-signaling-induced senescence (Hayflick, 1976; Serrano *et al.*, 2004). hPNPase^{old-35} was first described as an induced gene during terminal differentiation and cellular senescence two end-stage processes sharing several overlapping features including irreversible growth arrest, marked inhibition of DNA synthesis and modulation of telomerase activity (Sarkar *et al.*, 2006). Overexpression of full length hPNPase^{old-35} or either one of its RNase PH domains in human melanoma cells and melanocytes induces distinctive changes associated with senescent phenotypes (Sarker *et al.*, 2003, 2005) including induction of SA-β-gal activity, cell cycle arrest in G₁ phase with inhibition of DNA synthesis followed by induction of apoptosis, and inhibition of telomerase activity. In total, these findings indicate that hPNPase^{old-35} might play an essential role in senescence- and differentiation-associated growth inhibition. Mechanistically, hPNPase^{old-35}-induced senescence is associated with an increase in p27^{Kip1} and a decrease in p21^{CIP1/WAF-1/MDA-6}. The increase of p27^{Kip1} is most likely secondary to the decrease in *c-myc* that controls p27^{Kip1} expression by multiple

mechanisms (Barnes and Karin, 2008; Obaya *et al.*, 1999; O'Hagan *et al.*, 2000). However, it is also a consequence of downregulation of miR-221 that specifically targets the mRNA of *p27^{kip1}*. Since oxidative stress mediates cellular senescence (Colavitti *et al.*, 2005; Passos *et al.*, 2006) the induction of reactive oxygen species (ROS) following upregulation of hPNPase^{old-35} could also be involved in induction of the senescent phenotype. Further studies are necessary to investigate the role of the interactive network of c-myc, miR-221, *p27^{kip1}* and ROS in regulating the senescence process.

5.2. Human polynucleotide phosphorylase^{old-35} and aging-associated inflammation

Aging represents a conundrum in the evolution of higher organisms that is associated with progressive degenerative diseases the underlying pathology of which is often chronic inflammation (Kiecolt-Glaser *et al.*, 2003). Oxidative stress plays an important role in induction of chronic inflammation. Reactive oxygen species (ROS) generated by mitochondria induce oxidative stress in DNA, protein and lipid and this activity is more prominent in tissues from aged individuals or aged experimental animals than their young counterparts (Finkel *et al.*, 2000; Harman, 1957; Chen, 2000; Hagen, 1997). A prominent mechanism by which ROS modulates diverse intracellular molecular processes is by turning on the expression of proinflammatory cytokines (Baldwin, 1996) and through regulating the activity of NF- κ B (Schreck *et al.*, 1992). hPNPase^{old-35} is localized in mitochondria and induces ROS that subsequently leads to NF- κ B activation, which is inhibited by anti-oxidant N-acetyl-L-cysteine (NAC). Activation of NF- κ B leads to increased production of pro-inflammatory cytokines such as IL-6, IL-8, RANTES and MMP-3, which could also be inhibited by treatment with NAC suggesting the involvement of hPNPase^{old-35} in producing pathological changes associated with aging by generating pro-inflammatory cytokines via ROS and NF- κ B (Sarkar *et al.*, 2004; Sarkar & Fisher, 2006)

5.3. Overexpression of hPNPase^{old-35} induced growth inhibition in different cancer cells and its molecular mechanism

Based on cell line studies it is observed that slow and sustained overexpression of hPNPase^{old-35} induces growth arrest ultimately culminating in apoptosis, whereas rapid overexpression of hPNPase^{old-35} directly promotes apoptosis without cell cycle changes. These observations imply that inhibition of cell cycle progression and induction of apoptosis by hPNPase^{old-35} involves multiple intracellular targets and signaling pathways. In the context of cell cycle, *hPNPase^{old-35}* overexpression induces growth arrest at both G₁/S and G₂/M phase depending on the cell types, although the molecular mechanism is quite similar (Sarkar *et al.*, 2004; Maerken *et al.*, 2009; Chan *et al.*, 2008). In all cases, *c-myc* is downregulated by hPNPase^{old-35} accompanied with upregulation of CDKI, p21^{CIP1/WAF-1/MDA-6} and p27^{KIP1} that play essential roles in cell cycle arrest either in the G₁ or G₂/M phase. Apoptosis-inducing activity of hPNPase^{old-35} is mediated by activation of double-stranded RNA-dependent protein kinase (PKR) (Sarkar *et al.*, 2007). Activation of PKR by hPNPase^{old-35} precedes phosphorylation of eukaryotic initiation factor-2A and induction of growth arrest and DNA damage-inducible gene 153 (GADD153) that culminates in the shutdown of protein synthesis and apoptosis. Activation of PKR by hPNPase^{old-35} also initiates down-regulation of the anti-apoptotic protein Bcl-xL. All of

these studies elucidate a novel pathway by which an evolutionary conserved RNA-metabolizing enzyme, hPNPase^{old-35}, regulates cell growth and viability.

5.4. Role of hPNPase^{old-35} in maintaining mitochondrial homeostasis

Based on the localization in the mitochondrial intermembrane space (IMS), hPNPase^{old-35} is predicted to play a major role in mitochondrial bioenergetics. Evidence for maintaining homeostasis comes from both loss-of-function and gain-of-function studies, although contradictory results also indicate that knocking down hPNPase^{old-35} does not affect mitochondrial morphology or the rate of oxygen consumption (Nagaike T *et al.*, 2005). Knocking down of hPNPase^{old-35} reduced mitochondrial Ψ and enzymatic activities of couple respiratory complexes compared with control cells resulting in mitochondrial dysfunction such as lactate accumulation and reduction of steady state ATP levels (Chen *et al.*, 2006, 2007). Concurrently, overexpression results in increased reactive oxygen species accumulation over time confirming a role for hPNPase^{old-35} in mitochondrial homeostasis (Sarkar *et al.*, 2004). However, it is not understood how mitochondrial homeostasis is precisely regulated by hPNPase^{old-35}, since no known substrates are located in the IMS. Accordingly, one must also consider potential non-enzymatic functions of hPNPase^{old-35} in maintenance of mitochondrial homeostasis. It is possible that hPNPase^{old-35} affects oxidative phosphorylation directly by impacting on components of the respiratory complexes or indirectly through interference in mitochondrial fusion. Consistent with this possibility, a very recent study (Wang et al. 2010) showed that components of the Electron Transport Chains (ETC) were reduced at both RNA and protein levels in PNPase RNAi transfected HEK293 cells as compared to controls. A similar result was also observed in liver mitochondria from a liver-specific PNPase knockout mouse model suggesting that the decrease in functional ETC complexes was responsible for decreased respiration. Furthermore, the ultrastructure of liver mitochondria from liver-specific knockout mice displayed disordered circular and smooth inner membrane criste, similar to mitochondria having impaired components of oxidative phosphorylation pathways. Citrate synthase activity, routinely used as a marker of aerobic capacity, also decreased in the liver of PNPase knockout mice compared with the wild type mice establishing a pivotal role of PNPase in mitochondrial morphogenesis and respiration *in vivo*. Additionally, a number of studies indicate that hPNPase^{old-35} potentially maintains high-fidelity translation by sequestering oxidative stress-damaged RNA (Hayakawa *et al.*, 2006; Wu *et al.*, 2008) supporting potential exoribonuclease-independent activity of this enzyme in maintaining normal mitochondrial function.

5.5. Role of PNPase in translocation of RNA in mitochondria

While the molecular mechanism of mitochondrial import of nuclear-encoded proteins is well defined, little is known about the factors that control mitochondrial RNA import. Wang and colleagues (Wang *et al.* 2010) have now demonstrated a direct involvement of PNPase in regulating specific cytosolic RNA import into the mitochondrial matrix. This function of PNPase is independent of its RNA processing function, as inactivation of RNA processing by mutation did not affect RNA import. Mammalian RNase P localizes in mitochondria and functions in the processing of tRNAs during the maturation of mitochondrial transcripts (Chang and Clayton, 1987). RNase P is encoded in the nucleus and PNPase is directly

involved in translocation of this enzyme. However, the exact mechanism by which PNPase augments RNA import is not yet known. It is hypothesized that PNPase imports RNAs from the cytosol into the IMS and then passes this RNA to another protein or complex that facilitates passage of the RNAs through the IM into the matrix. Interestingly PNPase showed specificity towards import of cytosolic RNAs based on their secondary structure. Consistent with *in vitro* findings, mitochondrial RNA import was severely compromised in the liver mitochondria from liver-specific PNPase (*pnpt1*) knock out mice (Wang *et al.* 2010).

6. Summary

The gradual unraveling of the multifaceted functions of hPNPase^{old-35} illustrates its prominent role in regulating diverse physiological and pathological processes (Figure 4). Since, hPNPase^{old-35} displays substrate specificity, either mRNA or miRNA, the elucidation of how it elicits such specificity will significantly improve our understanding of its RNA-processing functions. The preferential degradative effects on specific miRNAs might be applicable to selectively downregulate oncogenic miRNA in different malignancies and thus might be amenable for developing a novel potential anti-cancer therapy. miRNAs have recently been shown to be involved in developing drug resistance and thus combinatorial therapy of hPNPase^{old-35} and appropriate therapeutic drug might be an effective approach for combating cancer. The lack of expression in different cancer cells while the ubiquitous expression in multiple organ/tissues raises the relevant question whether hPNPase^{old-35} serves any specialized function in specific organs? Animal models with either conditional overexpression or knockdown of PNPase expression will be extremely valuable in answering these questions. Importantly, these models will facilitate studies on senescence and IFN action, including its anti-viral properties and role in inflammatory disease processes as well as maintaining mitochondrial homeostasis. Strategies using natural compounds and/or small molecules to block the enzymatic functions of hPNPase^{old-35} might provide effective therapeutic benefit for inflammation or even provide a means of retarding the aging process and enhancing longevity.

Overall, delineation of the nuances of how hPNPase^{old-35} induces its diverse effects in different organisms offers significant potential to comprehend the evolutionary relevance of this molecule and this will help delineate its quintessential role in both normal physiology and in disease pathology.

Acknowledgments

The present study was supported in part by NIH grants R01 CA097318, R01 CA127641, CA134721, and P01 CA104177, the Samuel Waxman Cancer Research Foundation and the National Foundation for Cancer Research (PBF) and NIH grant R01 CA134721 (DS). DS is the Harrison Endowed Scholar in Cancer Research. PBF holds the Thelma Newmeyer Corman Chair in Cancer Research and is a SWCRF Investigator.

References

- Allmang C, Petfalski E, Podtelejnikov A, Mann M, Tollervey D, Mitchell P. The yeast exosome and human PM-Scl are related complexes of 3'→5' exonucleases. *Genes Dev.* 1999; 13:2148–2158. [PubMed: 10465791]
- Almeida FC, DeSalle R, Leszczyniecka M, Fisher PB. Examining ancient inter-domain horizontal gene transfer. *Evolutionary Bioinformatics.* 2008; 4:109–119.

- Ambros V. The functions of animal microRNAs. *Nature*. 2004; 431:350–355. [PubMed: 15372042]
- Baldwin AS. The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol*. 1996; 14:649–683. [PubMed: 8717528]
- Barnes PJ, Karin M. Nuclear factor-kappaB: A pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med*. 1997; 336:1–10. [PubMed: 8970933]
- Bueno MJ, Pérez de Castro I, Gómez de Cedrón M, Santos J, Calin GA, Cigudosa JC, et al. Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1. *Cancer Cell*. 2008; 13:496–506. [PubMed: 18538733]
- Buttner K, Wenig K, Hopfner KP. The exosome: a macromolecular cage for controlled RNA degradation. *Mol Microbiol*. 2006; 61:1372–1379. [PubMed: 16968219]
- Calin GA, Croce CM. MicroRNA-cancer connection: the beginning of a new tale. *Cancer Res*. 2006; 66:7390–7394. [PubMed: 16885332]
- Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2008; 6:857–866. [PubMed: 17060945]
- Campisi J. Gene expression in quiescent and senescent fibroblasts. *Ann N Y Acad Sci*. 1992; 663:195–201. [PubMed: 1482053]
- Cao D, Parker R. Computational modeling and experimental analysis of nonsense-mediated decay in yeast. *Cell*. 2003; 113:533–545. [PubMed: 12757713]
- Chang DD, Clayton DA. A mammalian mitochondrial RNA processing activity contains nucleus-encoded RNA. *Science*. 1987; 235:1178–1184. [PubMed: 2434997]
- Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nature Genet*. 2008; 40:43–50. [PubMed: 18066065]
- Chen HW, Koehler CM, Teitell MA. Human polynucleotide phosphorylase: location matters. *Trends Cell Biol*. 2006; 17:600–608. [PubMed: 17983748]
- Chen HW, Rainey RN, Balatoni CE, Dawson DW, Troke JJ, Wasiak S, et al. Mammalian polynucleotide phosphorylase is an intermembrane space RNase that maintains mitochondrial homeostasis. *Mol Cell Biol*. 2006; 26:8475–8487. [PubMed: 16966381]
- Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nature Genet*. 2006; 38:228–233. [PubMed: 16380711]
- Chan I, Lebedeva IV, Su ZZ, Sarkar D, Valerie K, Fisher PB. Progression elevated gene-3 promoter (PEG-Prom) confers cancer cell selectivity to human polynucleotide phosphorylase (hPNPase(old-35))-mediated growth suppression. *J Cell Physiol*. 2008; 215:401–409. [PubMed: 17960560]
- Chen QM. Replicative senescence and oxidant-induced premature senescence. Beyond the control of cell cycle checkpoints. *Ann NY Acad Sci*. 2000; 908:111–125. [PubMed: 10911952]
- Colavitti R, Finkel T. Reactive oxygen species as mediators of cellular senescence. *IUBMB Life*. 2005; 57:277–281. [PubMed: 16036611]
- Das SK, Sokhi UK, Bhutia SK, Azab B, Su ZZ, Sarkar D, et al. Human polynucleotide phosphorylase selectively and preferentially degrades microRNA-221 in human melanoma cells. *Proc Natl Acad Sci U S A*. 2010; 107:11948–11953. [PubMed: 20547861]
- Deutscher MP, Li Z. Exoribonucleases and their multiple roles in RNA metabolism. *Prog Nucleic Acid Res Mol Biol*. 2001; 66:67–105. [PubMed: 11051762]
- Deutscher MP. Promiscuous exoribonucleases of *Escherichia coli*. *J Bacteriol*. 1993; 175:4577–4583. [PubMed: 8335617]
- Deutscher MP. Ribonuclease multiplicity, diversity, and complexity. *J Biol Chem*. 1993; 268:13011–13014. [PubMed: 8514741]
- Dodson RE, Shapiro DJ. Regulation of pathways of mRNA destabilization and stabilization. *Prog Nucleic Acid Res Mol Biol*. 2002; 72:129–164. [PubMed: 12206451]
- Filipowicz W, Bhattacharyya S, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*. 2008; 9:102–114. [PubMed: 18197166]
- Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature*. 2000; 408:239–247. [PubMed: 11089981]

- Fisher PB, Grant S. Effects of interferon on differentiation of normal and tumor cells. *Pharmacol Ther.* 1985; 27:143–66. [PubMed: 2412243]
- Fisher PB, Hermo H Jr, Solowey WE, Dietrich MC, Edwalds GM, Weinstein IB, et al. Effect of recombinant human fibroblast interferon and mezerein on growth, differentiation, immune interferon binding and tumor associated antigen expression in human melanoma cells. *Anticancer Res.* 1986; 6:765–774. [PubMed: 2944474]
- Fisher PB, Prignoli DR, Hermo H Jr, Weinstein IB, Pestka S. Effects of combined treatment with interferon and mezerein on melanogenesis and growth in human melanoma cells. *J Interferon Res.* 1985; 5:11–22. [PubMed: 3989332]
- Fornari F, Gramantieri L, Ferracin M, Veronese A, Sabbioni S, Calin GA, et al. MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene.* 2008; 27:5651–5661. [PubMed: 18521080]
- Frischmeyer PA, van Hoof A, O'Donnell K, Guerrerio AL, Parker R, Dietz HC. An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. *Science.* 2002; 295:2258–2261. [PubMed: 11910109]
- Gewartowski K, Tomecki R, Muchowski L, Dmochow Ska A, Dzwonek A, Malecki M, et al. Up-regulation of human PNPase mRNA by beta-interferon has no effect on protein level in melanoma cell lines. *Acta Biochim Pol.* 2006; 53:179–188. [PubMed: 16505900]
- Guarini L, Graham GM, Jiang H, Ferrone S, Zucker S, Fisher PB. Modulation of the antigenic phenotype of human melanoma cells by differentiation-inducing and growth suppressing agents. *Pigment Cell Res.* 1992; 2:123–131. [PubMed: 1357650]
- Guarini L, Temponi M, Edwalds GM, Vita JR, Fisher PB, Ferrone S. In vitro differentiation and antigenic changes in human melanoma cell lines. *Cancer Immunol Immunother.* 1989; 30:262–268. [PubMed: 2624919]
- Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, et al. Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. *Proc Natl Acad Sci U S A.* 1997; 94:3064–3069. [PubMed: 9096346]
- Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol.* 1957; 2:298–300.
- Hayflick L. The cell biology of human aging. *N Engl J Med.* 1976; 295:1302–1308. [PubMed: 980063]
- He L, He X, Lowe SW, Hannon GJ. microRNAs join the p53 network — another piece in the tumour suppression puzzle. *Nature Rev Cancer.* 2007; 7:819–822. [PubMed: 17914404]
- He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. *Nature.* 2005; 435:828–833. [PubMed: 15944707]
- Hoof A, Parker R. The exosome: a proteasome for RNA? *Cell.* 1999; 99:347–350. [PubMed: 10571176]
- Hayakawa H, Sekiguchi M. Human polynucleotide phosphorylase protein in response to oxidative stress. *Biochemistry.* 2006; 45:6749–6755. [PubMed: 16716086]
- Jarrige AC, Mathy N, Portier C. PNPase autocontrols its expression by degrading a double-stranded structure in the pnp mRNA leader. *EMBO J.* 2001; 20:6845–6855. [PubMed: 11726520]
- Jarrige A, Brechemier-Baey D, Mathy N, Duche O, Portier C. Mutational analysis of polynucleotide phosphorylase from *Escherichia coli*. *J Mol Biol.* 2002; 321:397–409. [PubMed: 12162954]
- Jiang H, Su ZZ, Boyd J, Fisher PB. Gene expression changes associated with reversible growth suppression and the induction of terminal differentiation in human melanoma cells. *Mol Cell Differ.* 1993; 1:41–66.
- Jiang H, Lin J, Young SM, Goldstein NI, Waxman S, Davila V, et al. Cell cycle gene expression and E2F transcription factor complexes in human melanoma cells induced to terminally differentiate. *Oncogene.* 1995; 11:1179–1189. [PubMed: 7566979]
- Kennedy S, Wang D, Ruvkun GA. Conserved siRNA degrading RNase negatively regulates RNA interference in *C. elegans*. *Nature.* 2004; 427:645–649. [PubMed: 14961122]
- Khidr L, Wu G, Davila A, Procaccio V, Wallace D, Lee WH. Role of SUV3 helicase in maintaining mitochondrial homeostasis in human cells. *J Biol Chem.* 2008; 283:27064–27073. [PubMed: 18678873]

- Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci U S A*. 2003; 100:9090–9095. [PubMed: 12840146]
- Kim V. MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol*. 2005; 6:376–385. [PubMed: 15852042]
- Kirschner LS, Taymans SE, Pack S, Pak E, Pike BL, Chandrasekharappa SC, et al. Genomic mapping of chromosomal region 2p15-p21 (D2S378-D2S391): integration of Genemap'98 within a framework of yeast and bacterial artificial chromosomes. *Genomics*. 1999; 62:21–33. [PubMed: 10585764]
- Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell*. 2007; 129:1401–1414. [PubMed: 17604727]
- Leszczyniecka M, Kang DC, Sarkar D, Su ZZ, Holmes M, Valerie K, et al. Identification and cloning of human polynucleotide phosphorylase, hPNPaseold-35, in the context of terminal differentiation and cellular senescence. *Proc Natl Acad Sci U S A*. 2002; 99:16636–16641. [PubMed: 12473748]
- Leszczyniecka M, Su ZZ, Kang DC, Sarkar D, Fisher PB. Expression regulation and genomic organization of human polynucleotide phosphorylase, hPNPaseold-35, a type I interferon inducible early response gene. *Gene*. 2003; 316:143–156. [PubMed: 14563561]
- Leszczyniecka M, DeSalle R, Kang DC, Fisher PB. The origin of polynucleotide phosphorylase domains. *Mol Phylogenet Evol*. 2004; 31:123–130. [PubMed: 15019613]
- Littauer, UZ.; Grunberg-Manago, M. Polynucleotide phosphorylase. In: Creighton, TE., editor. *Encyclopedia of Molecular Biology*. Vol. 3. 1999. p. 1911
- Mitchell P, Petfalski E, Shevchenko A, Mann M, Tollervey D. The exosome: a conserved eukaryotic RNA processing complex containing multiple 3'→5' exoribonucleases. *Cell*. 1997; 91:457–466. [PubMed: 9390555]
- Mitchell P, Tollervey D. Musing on the structural organization of the exosome complex. *Nat Struct Biol*. 2000; 7:843–846. [PubMed: 11017189]
- Mohanty BK, Kushner SR. Polynucleotide phosphorylase functions both as a 3' right-arrow 5' exonuclease and a poly(A) polymerase in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 2000; 97:11966–11971. [PubMed: 11035800]
- Nagaike T, Suzuki T, Katoh T, Ueda T. Human mitochondrial mRNA are stabilized with polyadenylation regulated by mitochondria-specific poly (A) polymerase and polynucleotide phosphorylase. *J Biol Chem*. 2005; 280:19721–19727. [PubMed: 15769737]
- Obaya AJ, Mateyak MK, Sedivy JM. Mysterious liaisons: the relationship between c-Myc and the cell cycle. *Oncogene*. 1999; 18:2934–2941. [PubMed: 10378690]
- O'Hagan RC, Ohh M, David G, de Alboran IM, Alt FW, Kaelin WG Jr, et al. Myc-enhanced expression of Cull1 promotes ubiquitin-dependent proteolysis and cell cycle progression. *Genes Dev*. 2000; 14:2185–2191. [PubMed: 10970882]
- Parker R, Song H. The enzymes and control of eukaryotic mRNA turnover. *Nat Struct Mol Biol*. 2004; 11:121–127. [PubMed: 14749774]
- Passos JF, von Zglinicki T, Saretzki G. Mitochondrial dysfunction and cell senescence: cause or consequence? *Rejuvenation Res*. 2006; 9:64–68. [PubMed: 16608398]
- Piwowski J, Grzechnik P, Dziembowski A, Dmochowska A, Minczuk M, Stepien PP. Human polynucleotide phosphorylase, hPNPase, is localized in mitochondria. *J Mol Biol*. 2003; 329:853–857. [PubMed: 12798676]
- Portnoy V, Evguenieva-Hackenberg E, Klein F, Walter P, Lorentzen E, Klug G, et al. RNA polyadenylation in Archaea: not observed in *Haloferax* while the exosome polyadenylates RNA in *Sulfolobus*. *EMBO Rep*. 2005; 6:1188–1193. [PubMed: 16282984]
- Ramachandran V, Chen X. Degradation of microRNAs by a family of exoribonucleases in *Arabidopsis*. *Science*. 2008; 321:1490–1492. [PubMed: 18787168]
- Raijmakers R, Egberts WV, van Venrooij WJ, Pruijn GJ. Protein-protein interactions between human exosome components support the assembly of RNase PH-type subunits into a six-membered PNPase-like ring. *J Mol Biol*. 2002; 323:653–663. [PubMed: 12419256]

- Raijmakers R, Schilders G, Pruijn GJ. The exosome, a molecular machine for controlled RNA degradation in both nucleus and cytoplasm. *Eur J Cell Biol.* 2004; 83:175–183. [PubMed: 15346807]
- Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF. Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc Natl Acad Sci USA.* 2006; 103:8721–8726. [PubMed: 16731620]
- Regonesi ME, Briani F, Ghetta A, Zangrossi S, Ghisotti D, Tortora P, et al. A mutation in polynucleotide phosphorylase from *Escherichia coli* impairing RNA binding and degradosome stability. *Nucleic Acids Res.* 2004; 32:1006–1017. [PubMed: 14963263]
- Sarkar D, Fisher PB. Molecular mechanisms of aging-associated inflammation. *Cancer Lett.* 2006; 236:13–23. [PubMed: 15978720]
- Sarkar D, Leszczyniecka M, Kang DC, Lebedeva IV, Valerie K, Dhar S, et al. Downregulation of Myc as a potential target for growth arrest induced by human polynucleotide phosphorylase (hPNPaseold-35) in human melanoma cells. *J Biol Chem.* 2003; 278:24542–24551. [PubMed: 12721301]
- Sarkar D, Lebedeva IV, Emdad L, Kang DC, Baldwin AS Jr, Fisher PB. Human polynucleotide phosphorylase (*hPNPaseold-35*): a potential link between aging and inflammation. *Cancer Res.* 2004; 64:7473–7478. [PubMed: 15492272]
- Sarkar D, Park ES, Emdad L, Randolph A, Valerie K, Fisher PB. Defining the domains of human polynucleotide phosphorylase (hPNPaseOLD-35) mediating cellular senescence. *Mol Cell Biol.* 2005; 25:7333–7343. [PubMed: 16055741]
- Sarkar D, Park ES, Fisher PB. Defining the mechanism by which IFN-beta downregulates c-myc expression in human melanoma cells: pivotal role for human polynucleotide phosphorylase (hPNPaseold-35). *Cell Death Differ.* 2006; 13:1541–1553. [PubMed: 16410805]
- Sarkar D, Park ES, Barber GN, Fisher PB. Activation of double-stranded RNA dependent protein kinase, a new pathway by which human polynucleotide phosphorylase (hPNPase(old-35)) induces apoptosis. *Cancer Res.* 2007; 67:7948–7953. [PubMed: 17804700]
- Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells. *Free Radic Res Commun.* 1992; 17:221–237. [PubMed: 1473734]
- See YP, Fitt PS. Partial purification and properties of rat liver mitochondrial polynucleotide phosphorylase. *Biochem J.* 1972; 130:343–353. [PubMed: 4352426]
- Serrano M, Blasco MA. Putting the stress on senescence. *Curr Opin Cell Biol.* 2001; 13:748–753. [PubMed: 11698192]
- Slomovic S, Slomovic S, Laufer D, Geiger D, Schuster G. Polyadenylation and degradation of human mitochondrial RNA: the prokaryotic past leaves its mark. *Mol Cell Biol.* 2005; 25:6427–6435. [PubMed: 16024781]
- Stickney LM, Hankins JS, Miao X, Mackie GA. Function of the conserved S1 and KH domains in polynucleotide phosphorylase. *J Bacteriol.* 2005; 187:7214–7221. [PubMed: 16237005]
- Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, Lee JY, et al. Human embryonic stem cells express a unique set of microRNAs. *Dev Biol.* 2004; 270:488–498. [PubMed: 15183728]
- Symmons MF, Jones GH, Luisi BF. A duplicated fold is the structural basis for polynucleotide phosphorylase catalytic activity, processivity, and regulation. *Structure Fold Des.* 2000; 8:1215–1226. [PubMed: 11080643]
- Temperley RJ, Seneca SH, Tonska K, Bartnik E, Bindoff LA, Lightowlers RN, et al. Investigation of a pathogenic mtDNA microdeletion reveals a translation-dependent deadenylation decay pathway in human mitochondria. *Hum Mol Genet.* 2003; 12:2341–2348. [PubMed: 12915481]
- Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev.* 2006; 20:2202–2207. [PubMed: 16882971]
- Tsuchiya S, Okuno Y, Tsujimoto G. Micro-RNA: Biogenetic and functional mechanisms and involvements in cell differentiation and cancer. *J Pharmacol Sci.* 2006; 101:267–270. [PubMed: 16921236]

- Tucker M, Parker R. Mechanisms and control of mRNA decapping in *Saccharomyces cerevisiae*. *Annu Rev Biochem.* 2000; 69:571–595. [PubMed: 10966469]
- Wang G, Chen HW, Oktay Y, Zhang J, Allen EL, Smith GM, et al. PNPase regulates RNA import into mitochondria. *Cell.* 2010; 142:456–467. [PubMed: 20691904]
- Wang Z, Kiledjian M. Functional link between the mammalian exosome and mRNA decapping. *Cell.* 2001; 107:751–762. [PubMed: 11747811]
- Wang DD, Shu Z, Lieser SA, Chen PL, Lee WH. Human mitochondrial SUV3 and polynucleotide phosphorylase form a 330-kDa heteropentamer to cooperatively degrade double-stranded RNA with a 3'-to-5' directionality. *J Biol Chem.* 2009; 284:20812–20821. [PubMed: 19509288]
- Wu J, Li Z. Human polynucleotide phosphorylase reduces oxidative RNA damage and protects HeLa cell against oxidative stress. *Biochem Biophys Res Commun.* 2008; 372:288–92. [PubMed: 18501193]
- Wulczyn FG, Smirnova L, Rybak A, Brandt C, Kwiedzinski E, Ninnemann O, et al. Post-transcriptional regulation of the let-7 microRNA during neural cell specification. *FASEB J.* 2007; 21:415–426. [PubMed: 17167072]
- Yamanaka K, Inouye M. Selective mRNA degradation by polynucleotide phosphorylase in cold shock adaptation in *Escherichia coli*. *J Bacteriol.* 2001; 183:2808–2816. [PubMed: 11292800]
- Yehudai-Resheff S, Hirsh M, Schuster G. Polynucleotide phosphorylase functions as both an exonuclease and a poly (A) polymerase in spinach chloroplasts. *Mol Cell Biol.* 2001; 21:5408–5416. [PubMed: 11463823]
- Yehudai-Resheff S, Portnoy V, Yogev S, Adir N, Schuster G. Domain analysis of the chloroplast polynucleotide phosphorylase reveals discrete functions in RNA degradation, polyadenylation, and sequence homology with exosome proteins. *Plant Cell.* 2003; 15:2003–2019. [PubMed: 12953107]

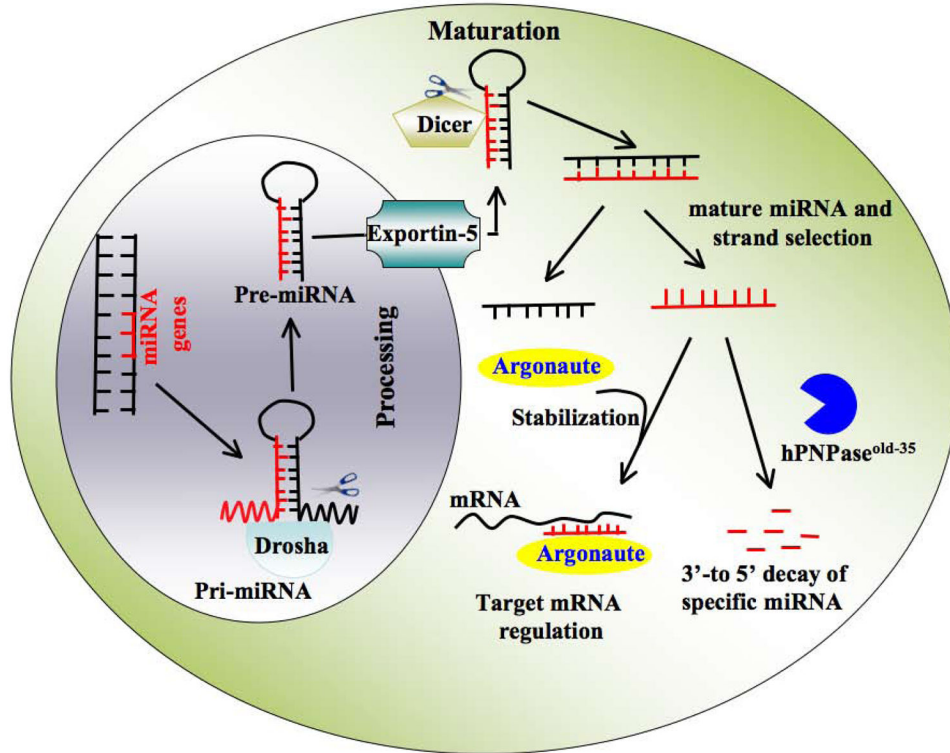


Figure 1. Schematic model of miRNA biogenesis and stability. After synthesis by RNA polymerase II, primary transcripts of (pri) miRNA are recognized by Drosha, which excises the hairpin precursor and released precursor (pre) miRNA. From nucleus, exportin five delivers the miRNA precursor to Dicer and its RNA binding partner in the cytoplasm for final processing to the mature 22-nt miRNAs. One strand is selected for stable association with Argonaute, where it serves as a guide to target and regulate specific mRNAs. By executing exonuclease activity *hPNPase^{old-35}* specifically degrades mature miRNAs. However, their substrate recognition mechanism is unknown.

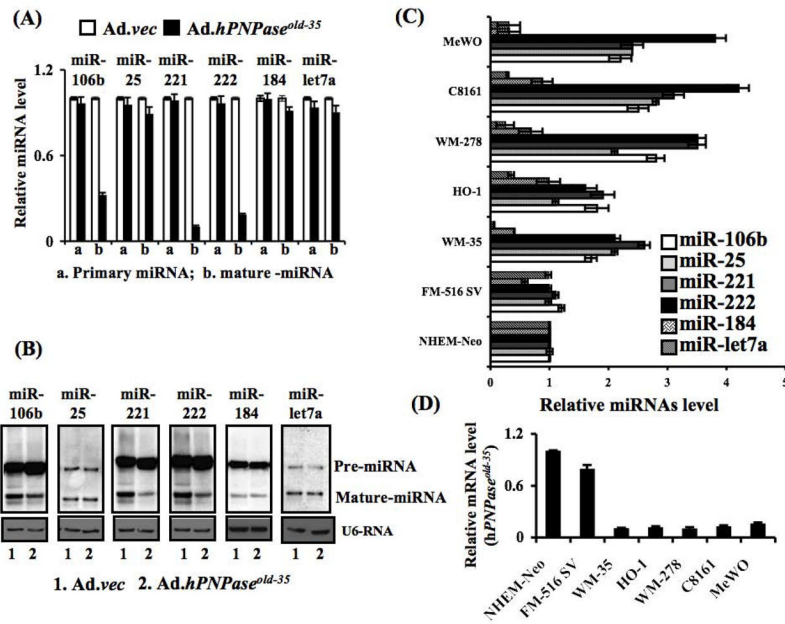


Figure 2. *hPNPase^{old-35}* target miRNAs

HO-1 cells were either infected with *Ad.vec* or *Ad.hPNPase^{old-35}* at a m.o.i. of 5000 vp/cells for three days and subjected to miRNA microarrays and potential target miRNAs miR-106b, miR-25, miR-221, miR-222, miR-let7a and miR-184 were validated for differential expression by using primary- and mature miRNA-specific Taqman® probes with qPCR. (B) Northern blotting was performed to detect mature miRNAs and its precursor species by using specific probes. Expression of *GAPDH*, miR-RNU44 and U6 RNA were used to normalize the pri- and mature miRNA in qPCR and Northern blotting data, respectively. (C & D) The basal level of different miRNAs (C) and mRNA for *hPNPase^{old-35}* (D) were evaluated by qPCR in different cell lines, including Normal human epidermal melanocytes (NHEM), Normal immortal human melanocytes FM-516-SV (referred to as FM-516), radial growth phase primary melanoma WM-35, vertical growth phase primary melanoma WM-278 and metastatic melanoma HO-1, C8161.9, and MeWo. (Taken from Das et al., PNAS, 2010)

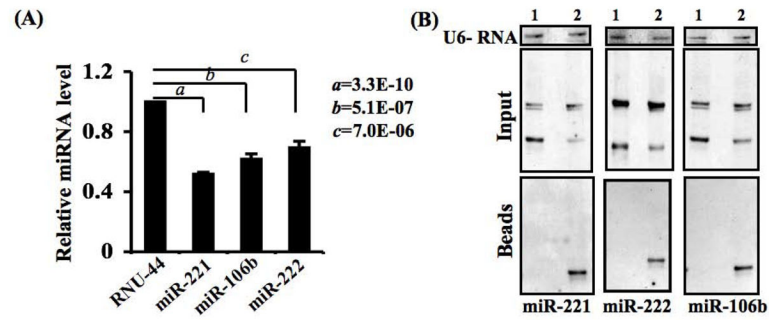


Figure 3. hPNPase^{old-35} preferentially degrades miR-221

(A) Comparative degradation between miR-221, miR-222 and miR-106b at a 2 h time point using *in vitro* degradation assays. P value was calculated using student's t-test by comparing the specific miRNA normalized by miR-RNU-44. The data represent mean \pm S.D. of two independent experiments each done in triplicate. (B) A direct interaction between targeted miRNAs with *in vitro* translated hPNPase^{old-35} was confirmed by immunoprecipitation and Northern blotting.

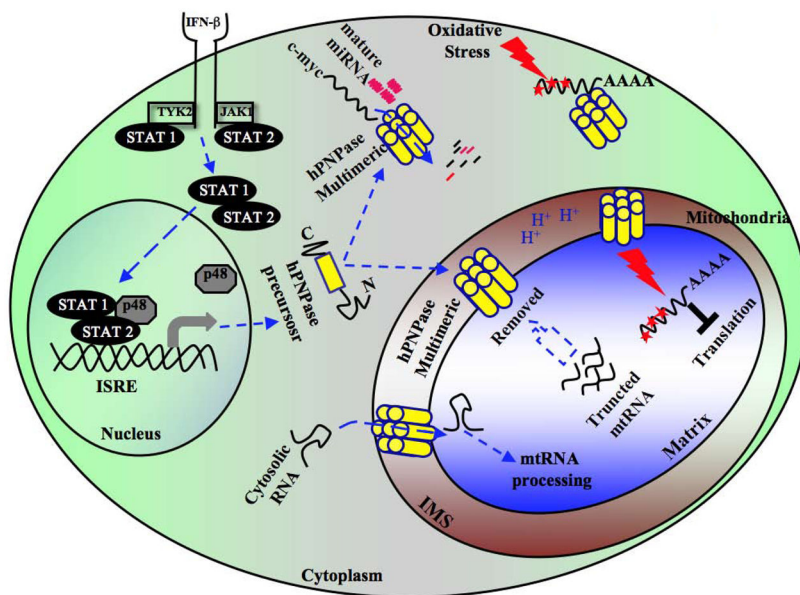


Figure 4. Schematic representation of the diversified functions of hPNPase^{old-35}

Upon Type I IFNs binding with its corresponding receptor, hPNPase^{old-35} transcription is promoted through activation of the JAK/STAT pathway. Once transcribed, hPNPase^{old-35} is either imported into the mitochondria IMS or mobilized into the cytosol by unknown mechanism(s). In the cytoplasm, by executing exoribonuclease activity it specifically targets mRNA/miRNAs thereby significantly affecting various biological functions, e.g., induction of cell cycle arrest by targeting *c-myc*. In addition to maintaining mitochondrial homeostasis, hPNPase^{old-35} actively participates to block the translation of oxidative stress-mediated damaged RNA by sequestering it. In addition, although not evident, hPNPase^{old-35} might also be involved in removal of truncated mtRNA from mitochondria.