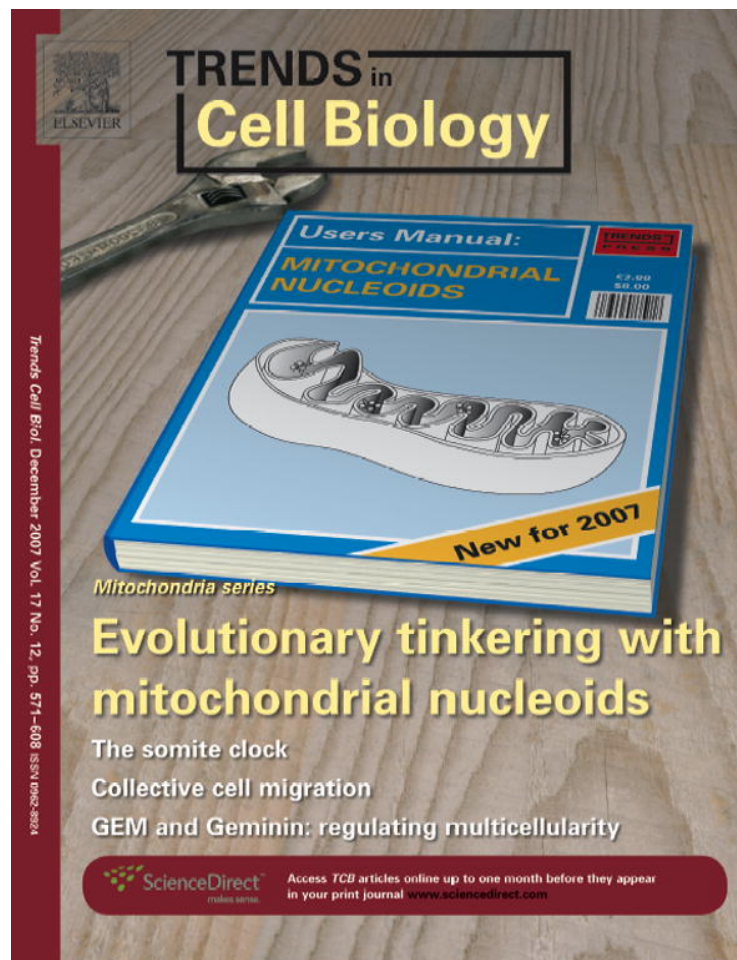


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# Human polynucleotide phosphorylase: location matters

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**Human polynucleotide phosphorylase (hPNPase) is an RNA-processing enzyme induced in response to type I interferons and during terminal differentiation and cellular senescence. hPNPase was thought to contribute to cellular senescence through its RNA-degrading activity in the cytosol; however, recent studies show that hPNPase localizes to the mitochondrial intermembrane space (IMS) and has a crucial role in maintaining mitochondrial homeostasis. Initial studies have also linked hPNPase to tumorigenesis and the cellular response to viral infection. Its surprising localization in the IMS, which is thought to be devoid of mRNA transcripts, raises questions about where and how hPNPase elicits its numerous suggested functions. Here, we discuss recent advances in understanding the various roles of hPNPase both within and potentially outside of the mitochondria.**

## Introduction

In eukaryotic cells, ribonucleases (RNases) reside in both the cytoplasm and nucleus and have an indispensable role in RNA-quality control and the regulation of gene expression [1]. RNA is degraded typically by hydrolysis to release nucleoside monophosphates, although some RNases use inorganic phosphate to catalyze phosphorolysis reactions, which generate nucleoside diphosphates [2]. A variety of RNases work cooperatively in eukaryotic mRNA turnover. In general, the initial shortening of the 3' poly(A) tail of a message by deadenylases is 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, following deadenylation, mRNAs may be degraded from the 3' end by the cytoplasmic exosome, a multiprotein complex of diverse 3' → 5' exoribonucleases. Degradation of mRNA can also be initiated within a transcript by endoribonucleases. For quality control, RNA-surveillance pathways in the cytoplasm and nucleus mediate the degradation of aberrant RNAs, many of which use the RNA-processing and -degrading exosome complex [1,3].

Polynucleotide phosphorylase (PNPase) is a 3' → 5' exoribonuclease that uses the phosphorolytic mechanism

to degrade RNA. Interestingly, it can also catalyze the reverse reaction and, thus, has RNA-polymerase activity [2,4–6]. PNPase is conserved evolutionarily and is expressed in bacteria, plants, worms, flies, mice and humans [7,8]. However, it is not present in yeasts, trypanosomes or the Archaea [2,9,10]. Bacterial and plant chloroplast PNPases, the family members studied most extensively, have been recognized as having important roles in normal RNA processing and turnover [5,6,11–13].

Human PNPase (hPNPase, encoded by the *PNPT1* gene) was first characterized 5 years ago as a 783-amino-acid exoribonuclease that was thought to contribute to cellular senescence through its RNA-degrading activity in the cytosol [14–16]. However, recent studies show that hPNPase localizes to the mitochondrial intermembrane space (IMS) and has a crucial role in maintaining mitochondrial homeostasis [17,18]. Additional studies suggest a potential role for hPNPase in combating viral infections [2,7,19] and show a potential interaction with the T-cell leukemia/lymphoma 1 (TCL1) oncoprotein [20]. There remain many unresolved issues regarding hPNPase, including the regulation of its expression, its subcellular localization and the mechanism(s) by which it performs the numerous cellular functions ascribed to it. Here, we examine how recent investigations have begun to unravel the mysteries behind this seemingly 'misplaced' RNase.

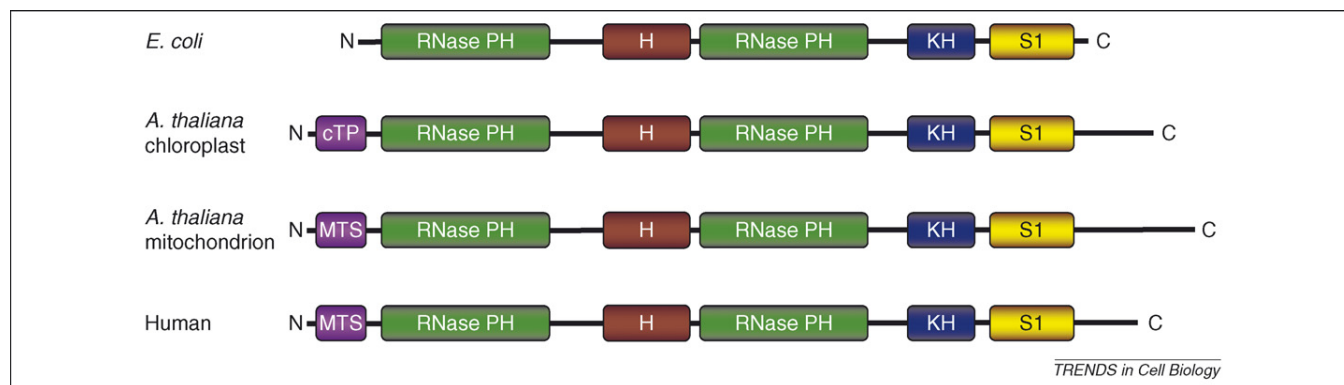
## PNPase structure and expression

*PNPT1* was cloned during a screen for genes up-regulated in both terminally differentiated melanoma cells and senescent progeroid fibroblasts, which are cells characterized by irreversible growth arrest [14]. The approximately 54 kb *PNPT1* gene consists of 28 exons and maps to 2p15–2p16.1, a notably unstable genomic region prone to deletions and amplifications that have been implicated in human cancers, such as diffuse large B-cell lymphoma [21], and in various genetic disorders [22]. To date, however, there is no evidence linking changes in hPNPase expression or function to any specific pathological process.

In all species, PNPase is comprised of five motifs. Two conserved catalytic RNase PH domains at the N-terminus are each related to the *Escherichia coli* RNase PH enzyme, which is involved primarily in 3' processing of transfer RNA (tRNA) precursors [9]. These RNase PH domains are

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**Figure 1.** Domains of PNPases. PNPases are conserved evolutionarily and consist of five motifs that include two RNase PH domains, which are separated by an  $\alpha$ -helical domain (H), and two RNA-binding domains, KH and S1, at the C-terminus [2,9,11]. In plants (*Arabidopsis thaliana* depicted here), two genes encode PNPase, one with a chloroplast-transit peptide (cTP) [11,13] and the other with a mitochondrial-targeting sequence (MTS) [26]. hPNPase also contains a MTS [17,18,31].

separated by an  $\alpha$ -helical domain that is unique to PNPases [23]. Conserved RNA-binding domains, K homology (KH) and S1, localize to the C-terminus [2,11] (Figure 1). In *E. coli*, PNPase either exists on its own, associates solely with RhlB, an RNA helicase that facilitates its degradation of double-stranded RNA, or exists within a multiprotein complex termed the RNA degradosome [2,6,24]. X-ray crystallographic analysis of *Streptomyces antibioticus* PNPase reveals that it forms a doughnut-shaped homotrimeric complex with a central channel that enables single-stranded RNA access to the enzyme's catalytic sites [25]. In plants, two genes encode PNPase: one encodes PNPase with a chloroplast-transit peptide (cTP) [11,13] and the other with a mitochondrial-targeting sequence (MTS) [26] (Figure 1). Chloroplast PNPase lacks known interacting partners and forms a homomultimeric complex that is predicted to be a dimer of ring-shaped trimers [11,23,27]. Analogously, hPNPase assembles into a multimeric complex, probably a trimer. Interestingly, although hPNPase is not a constituent of the exosome, the structures of the archaeal and eukaryotic exosomes resemble that of trimeric PNPase [3,6,8]. A brief comparison of the subcellular localization, characteristics of complex formation and function of the PNPases across species is provided in Table 1.

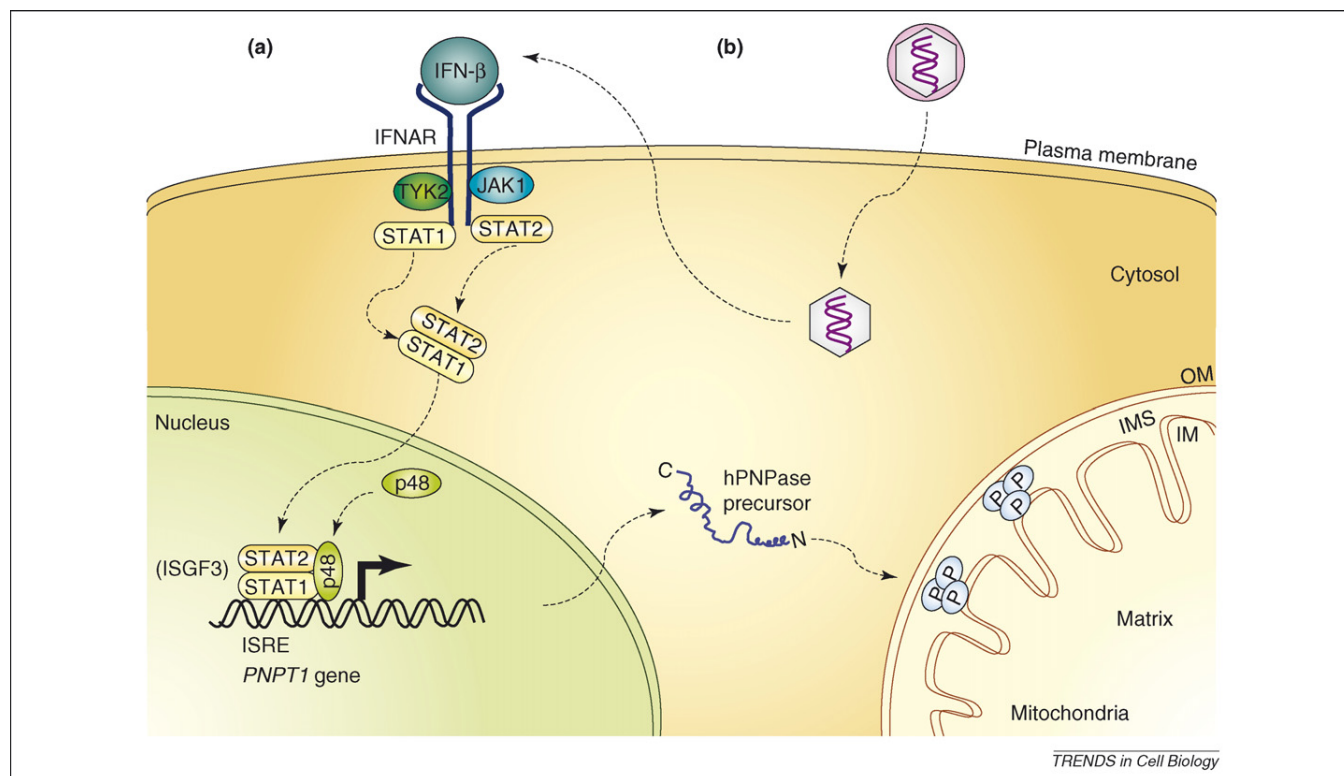
To date, the only known regulators of *PNPT1* transcription are type I interferons (IFN- $\alpha$  and - $\beta$ ). Induction of *PNPT1* by IFN- $\beta$  depends on the Janus-activated kinase

(JAK)/STAT (signal transducers and activators of transcription) signal-transduction pathway, which results in binding of interferon-stimulated gene factor 3 (ISGF3) to the IFN-stimulated response element (ISRE) within the *PNPT1* promoter [7] (Figure 2a). Type I IFNs are cytokines that are secreted typically by all cells in response to viral infection, suggesting that hPNPase might be involved in antiviral responses [2,19] (Figure 2b). In addition to the ISRE, the *PNPT1* promoter contains many other 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 G<sub>1</sub> to S phase transition [28]. Because *PNPT1* was identified initially as a gene up-regulated during senescence and terminal differentiation, processes characterized by cell-cycle arrest [14], it would be intriguing to speculate whether the repression of *PNPT1* by E2F3 is required for entry to S phase in normal cycling cells and whether changes in E2F3 repression of *PNPT1* correlate with or contribute to senescence.

The basal protein-expression level of hPNPase and whether up-regulation of *PNPT1* transcripts by IFN- $\beta$  reflects elevated protein expression remains unresolved. One study indicates an undetectable basal level but significant hPNPase protein induction following IFN- $\beta$  treatment in various human cell lines [29]. Other studies indicate the contrary, with significant basal hPNPase protein expression detected in many human cell lines [17,28,30]. Furthermore, although IFN- $\beta$  treatment

**Table 1. Cross-species comparison of PNPases**

Species	Organelle and sub-localization	Characteristics of formed complex	Functions	Refs
Bacteria	Not applicable	Ring-shaped homotrimer existing alone or associated solely with cytoplasm; degradosome	RNA phosphorolysis; polymerization of poly(A)-rich tails <i>in vitro</i> and under certain growth conditions or when poly(A) polymerase is inactivated <i>in vivo</i> ;	[2,4,6,24, 25,72–77]
		PNPase associated with cytoskeleton	regulation of virulence and persistence of <i>Salmonella enterica</i> ; cold temperature acclimation (for some species); modulation of <i>Yersinia</i> -type three secretion system	
Plants	Chloroplast, Stroma	Homomultimeric complex, probably a homohexamer	RNA phosphorolysis; polymerization of poly(A)-rich tails; acclimation to phosphorus limitation	[5,11,13, 27,78]
	Mitochondrion, Unknown	Unknown	RNA phosphorolysis	[26,49]
Human	Mitochondrion	Multimeric complex (trimer?)	Mitochondrial homeostasis maintenance; cellular senescence mediation; mtRNA poly(A) tail length alteration; antiviral response involvement?; potential interaction with TCL1 (role in cancer?)	[2,6,17– 20,35]
	Peripheral membrane protein in the intermembrane space			



**Figure 2.** Transcriptional regulation of *PNPT1* by type I interferons. (a) IFN- $\beta$  binds to the type I IFN receptor (IFNAR) to activate the JAK/STAT signaling pathway, resulting in binding of ISGF3 to the ISRE within the *PNPT1* promoter. The ISGF3 complex is composed of the STAT1/STAT2 heterodimer and p48 (IRF9/ISGF3 $\gamma$ ), a member of the interferon-regulatory factor (IRF) family [7]. Once hPNPase (P) is translated, it is imported into the mitochondrial IMS (see Figure 3a for details). (b) Type I IFNs are cytokines that are secreted typically by all cells in response to viral infection, suggesting that hPNPase might be involved in antiviral responses [2,19].

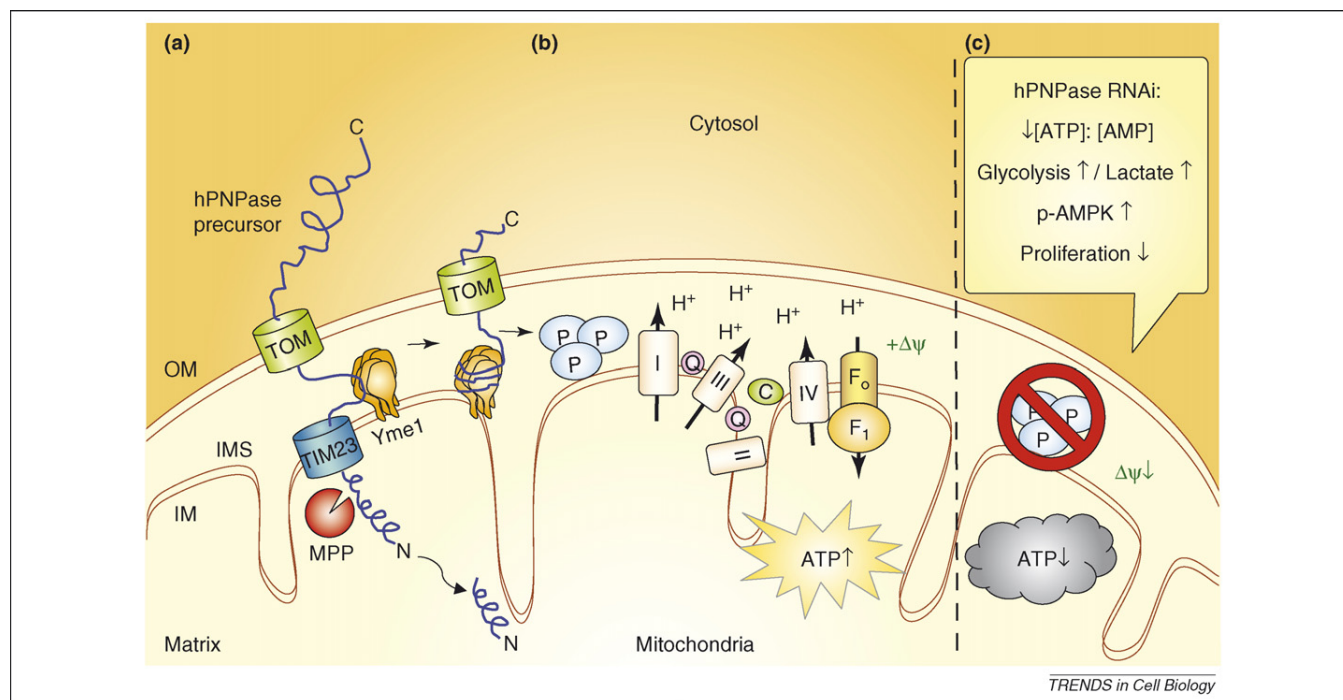
induces hPNPase protein levels in several lines, other lines show no difference in protein levels despite moderately increased mRNA transcripts [17,28]. These discrepancies might be a result of cell line-specific properties and/or differences in the sensitivities of the various hPNPase antisera used to detect the protein. It remains to be determined whether significant basal expression of hPNPase is necessary for all cell types. However, having a crucial role in maintaining mitochondrial homeostasis, as will be discussed later, hPNPase might prove to be both a house-keeping and a type I IFN-induced gene.

### Subcellular localization of hPNPase

hPNPase has a typical N-terminal MTS, which suggested strongly that it would be imported into mitochondria (Figure 1). This was confirmed by immunofluorescence and cell-fractionation studies [17,31]. The N-terminal-targeting sequence of hPNPase is crucial for its mitochondrial localization. When the N-terminus is blocked with a tag or truncated, hPNPase is not processed nor imported into mitochondria, resulting in its accumulation in the cytosol [17,31]. The human mitochondrial genome is located in the matrix and consists of a circular chromosome encoding 13 proteins, two ribosomal RNAs (rRNAs) and 22 tRNAs. Polycistronic RNAs are transcribed, initiating at three different promoter sites, and are further processed by RNase P and other unidentified RNases [32]. Because mitochondrial transcription and translation occur within the mitochondrial matrix, hPNPase was assumed to localize there to process mitochondrial

transcripts. Surprisingly, protease-protection assays with mitochondria from the yeast *Saccharomyces cerevisiae* expressing exogenous hPNPase and mitochondria isolated from mouse liver indicate that mammalian PNPase localizes specifically to the IMS of mitochondria as a membrane-associated protein. Furthermore, carbonate extraction reveals that hPNPase is a peripheral and not an integral membrane protein [17]. Although completely unexpected, these results might validate the isolation of PNPase from the inner membrane [33] and the detection of an obscure RNase activity in the IMS of rat-liver mitochondria [34] in two separate reports more than 30 years ago.

hPNPase is imported into the mitochondrial IMS by a novel pathway requiring the *i*-AAA (ATPases associated with several diverse cellular activities) protease, Yme1 [18]. As depicted in Figure 3a, after passing through the translocase of the outer mitochondrial membrane (TOM), the hPNPase precursor engages the translocase of the inner mitochondrial membrane (TIM)-23 complex in an electrochemical membrane potential ( $\Delta\psi$ )-dependent manner. The N-terminus of hPNPase extends through the TIM23 complex to enable the matrix-processing peptidase (MPP), consisting of a Mas1/Mas2 heterodimer, to cleave its 37-amino acid-targeting presequence. Then, IM-localized Yme1 facilitates the release of the mature hPNPase N-terminus into the IMS. Finally, to complete import, Yme1 pulls the C-terminus of hPNPase across the TOM complex into the IMS [18], where mature hPNPase assembles into a multimeric, probably trimeric, complex [17]. In this novel import pathway, Yme1 serves as



**Figure 3.** hPNPase import into the IMS by a novel Yme1 pathway and its maintenance of mitochondrial homeostasis. **(a)** After passing through the TOM complex, the N-terminus of hPNPase extends through the TIM23 complex to enable the matrix-processing peptidase (MPP), consisting of a Mas1/Mas2 heterodimer, to cleave its 37-amino-acid targeting presequence. With the facilitation of IM-localized Yme1, the mature N-terminus is then released into the IMS. Finally, to complete the import, Yme1 pulls the C-terminus of hPNPase from the cytosol, across the TOM complex and into the IMS where mature hPNPase assembles into a multimeric complex [18], probably a trimer [17], as depicted here (P, hPNPase monomer). In this novel import pathway, Yme1 acts as a translocation motor rather than a processing protease [18]. **(b)** Once imported, hPNPase has an important role in maintaining oxidative phosphorylation, electrochemical-membrane potential ( $\Delta\psi$ ) and ATP production in mitochondria [17]. **(c)** Reduction of hPNPase by RNAi results in decreased  $\Delta\psi$  and overall cell ATP levels, which leads to phosphorylation of AMPK and slowed growth [17].

a translocation motor rather than a processing protease [18]. It remains to be determined whether additional proteins gain entry to the mitochondrial IMS using this unique import mechanism.

There have been conflicting data on whether hPNPase also localizes to the cytosol and, if so, whether there are specific cellular conditions or cell types in which this occurs. Two studies using cellular fractionation and/or immunofluorescence showed that endogenous hPNPase and over-expressed C-terminal-tagged hPNPase localize only to the mitochondria [17,31]. Another study, using the same detection methods, showed over-expressed C-terminal-tagged hPNPase localizes to both mitochondrial and cytosolic compartments [16]. Because the over-expression of proteins can itself affect their distribution within the cell, studies of hPNPase in cells undergoing terminal differentiation or cellular senescence, in which hPNPase is over-expressed endogenously, are required to show the cellular distribution of hPNPase definitively.

### Role for hPNPase in maintaining mitochondrial homeostasis

The localization of hPNPase to the IMS prompted investigations of a potential role for hPNPase in mitochondrial bioenergetics. Although a recent study showed that RNA interference (RNAi) knockdown of hPNPase expression does not affect mitochondrial morphology nor the rate of oxygen consumption [35], another study provides contrary results and suggests that hPNPase maintains mitochondrial homeostasis [17] (Figure 3b). In the latter study, hPNPase RNAi generates fragmented and

granular-shaped mitochondria in comparison to the filamentous mitochondrial network of control cells. The mitochondrial  $\Delta\psi$  is also reduced by hPNPase knockdown and the enzymatic activities of coupled respiratory complexes I and III, II and III and individual complexes IV and V are each decreased to 30–40% that of control cells [17]. Therefore, this study concluded that hPNPase is required to support oxidative phosphorylation (Figure 3b). Furthermore, hPNPase deficiency causes protean cellular defects secondary to mitochondrial dysfunction such as lactate accumulation and a more than 50% reduction in steady-state ATP levels, leading to enhanced AMP-activated protein kinase (AMPK) phosphorylation and reduced cell growth [17] (Figure 3c). Because hPNPase is located in the IMS where, presumably, no known substrates for its exonuclease activity are present, the mechanism by which it maintains mitochondrial homeostasis remains unresolved and potential non-enzymatic functions need to be considered. An impairment of respiratory-chain function can result in the fragmentation of mitochondria [36] and, conversely, disruption of mitochondrial fusion can impair oxidative phosphorylation [37]. Therefore, it remains possible that hPNPase affects oxidative phosphorylation directly by impacting components of the respiratory complexes or that hPNPase is more directly involved in mitochondrial fusion and, thereby, affects oxidative phosphorylation indirectly.

Supporting loss-of-function study results, over-expression studies also suggest a role for hPNPase in maintaining mitochondrial homeostasis. Over-expressed hPNPase results in increased reactive oxygen species (ROS) accumulation over time [38]. Increased ROS in

hPNPase over-expressing cells leads to I $\kappa$ B- $\alpha$  degradation, nuclear translocation of the p65 subunit of NF- $\kappa$ B and increased p50/p65 NF- $\kappa$ B DNA binding, which increases proinflammatory cytokine production. These effects are downstream of ROS accumulation, as shown by the inhibition of these processes with the antioxidant N-acetyl-L-cysteine (NAC) [38,39]. Although ROS can be generated by cytosolic enzymes, most ROS are produced by mitochondria, with superoxide radicals being produced primarily at complex I and complex III of the respiratory chain [40,41]. It is thought that damaged mitochondria release increased levels of ROS, although this might be dependent on the nature of the mitochondrial defect [40,42]. Thus, it is possible that excess hPNPase leads to mitochondrial dysfunction, as does reduced levels of hPNPase. Over-expressed hPNPase might perturb mitochondrial gene expression, resulting in impaired mitochondrial function and, in turn, increased ROS production. IFN- $\alpha/\beta$  treatment leads to reduced expression of certain mitochondrial-encoded genes [43]. Because hPNPase expression is induced by IFN- $\alpha/\beta$ , we and others postulate that it acts as the mediator of this effect [31]. Alternatively, because knockdown of hPNPase resulted in fragmented mitochondria, it might be interesting to speculate that hPNPase might regulate mitochondrial dynamics, with its over-expression leading to sustained elongation of mitochondria, thereby leading to increased ROS production. It has been shown recently that, in hFis1 (a mitochondrial-fission mediator)-depleted cells, mitochondria exhibit an elongated phenotype with increased production of ROS [44]. However, over-expressed hPNPase also localizes to the cytosol and potential hPNPase cytosolic effects, such as degrading antioxidant protein transcripts, should not be overlooked.

#### hPNPase and mitochondrial transcript poly(A) tail lengths

Bacterial and chloroplast PNPases have exoribonuclease and poly(A) polymerase activities [4,5] (Table 1). *In vitro*, hPNPase also has both activities [14,20,30,35] and degrades polyadenylated GAPDH RNA preferentially over GAPDH RNA lacking poly(A) tails in the absence of ADP substrates [20]. In human mitochondria, all 13 protein-encoding genes, transcribed and translated in the matrix, are subunits of the respiratory complexes [32] and are essential to mitochondrial homeostasis. Therefore, determining whether hPNPase alters the poly(A) tail length and stability of these transcripts despite its IMS localization is crucial to understanding the mechanism by which it maintains mitochondrial homeostasis.

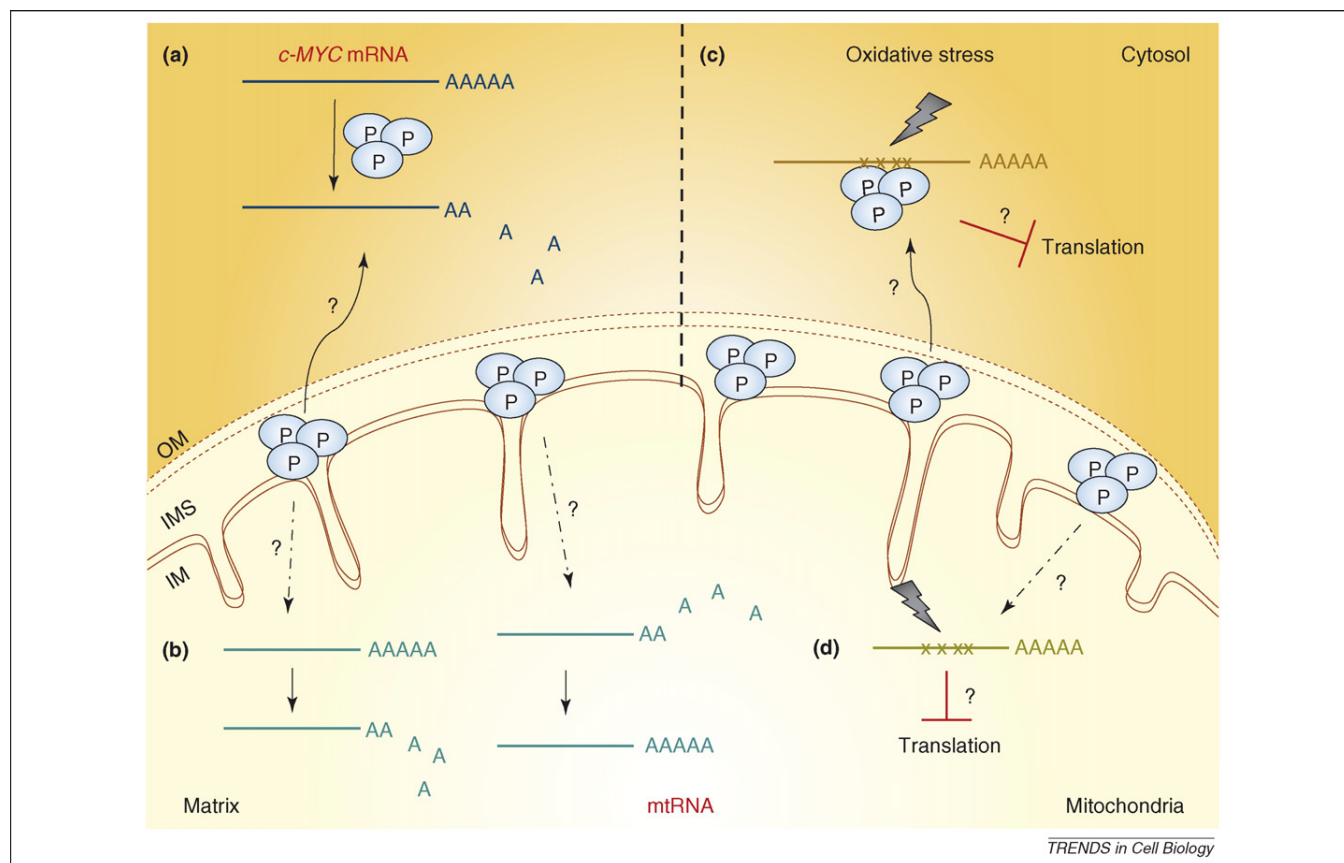
Although polyadenylation has a role in stabilizing cytoplasmic mRNA in eukaryotic cells, it also promotes the degradation of RNA in the eukaryotic nucleus and in bacteria, chloroplasts and plant mitochondria [3,32,45–51]. Interestingly, human mitochondria possess both transiently polyadenylated truncated transcripts and stably polyadenylated mature transcripts, suggesting the potential coexistence of paradoxical roles for RNA polyadenylation, either in promoting stabilization or degradation, in mitochondria [3,10,51,52]. Whether the polyadenylation of human mitochondrial RNA (mtRNA) has a role in

regulating stability remains under debate. However, an established function of stable poly(A) tails is to generate complete translation stop codons that are missing from some mtRNAs after processing from polycistronic units [32]. Human mitochondrial poly(A) polymerase (mtPAP) has recently been described to synthesize mtRNA poly(A) tails. However, the effect of shortened mtRNA poly(A) tails in cells with reduced mtPAP expression on mtRNA stability remains unresolved [35,53].

Knockdown of hPNPase results in the lengthening of the poly(A) tails of some mtRNAs compared with control cells, as determined by mitochondrial poly(A) tail-length assays (MPATs) [35,54]. However, hPNPase knockdown does not alter the steady-state levels of these transcripts [35]. Because hPNPase localizes to the IMS, whereas mtRNAs reside in the matrix, rather than degrading mtRNAs directly, hPNPase might modulate mtRNA poly(A) tail lengths indirectly through the maintenance of ATP levels. Consistent with this notion, studies have shown that mitochondrial-transcript polyadenylation and synthesis are sensitive to ATP levels [55,56]. Furthermore, although the precise mechanisms of mtRNA turnover and processing are not clear, it is possible that sufficient ATP levels are required for enzymatic activities that regulate poly(A)-tail reduction or addition. Alternatively, because there is a detection limit for protease-protection studies in determining submitochondrial localization, it remains possible that trace amounts of hPNPase are in the matrix that could impact mtRNA poly(A)-tail lengths directly. Along similar lines, endonuclease G has been proposed to reside in both the IMS and matrix [57]. If, indeed, trace amounts of hPNPase reside in the matrix, elongated mtRNA poly(A) tails in hPNPase-knockdown cells would suggest that hPNPase acts primarily as an exoribonuclease rather than an RNA polymerase in mitochondria [35], although the possibility that hPNPase also performs RNA-polymerase activities in certain circumstances *in vivo*, as shown *in vitro*, cannot be excluded completely (Figure 4b). Repression of plant mitochondrial PNPase expression leads to the accumulation of unprocessed RNAs and rRNA and tRNA-maturation byproducts in mitochondria, suggesting a role for PNPase in the matrix [49]. However, thorough submitochondrial localization studies for plant-mitochondrial PNPase have not been performed and this effect could also be secondary to PNPase activity in the IMS as proposed for hPNPase. Clearly, further studies are required to support or invalidate these possibilities and provide a mechanism by which hPNPase maintains mitochondrial homeostasis.

#### hPNPase and cellular senescence

As mentioned earlier, *PNPT1* was described as an induced gene during terminal differentiation and cellular senescence. These end-stage processes in general share overlapping features, including irreversible growth arrest and marked inhibition of DNA synthesis with induction of cyclin-dependent kinase inhibitors (CDKIs) and inhibition of telomerase activity [2]. Over-expression of full-length hPNPase or either one of its RNase PH domains results in senescent phenotypes [15,16]. hPNPase over-expression decreases cell-colony formation, induces G<sub>1</sub> arrest, decreases S phase, induces apoptosis and decreases telomerase



**Figure 4.** Hypothetical models of hPNPase activity. (a) hPNPase is mobilized into the cytosol by an unknown mechanism [17] and might be exposed to and degrade cytosolic RNAs, such as *c-MYC* [15,16,29]. (b) hPNPase has exonuclease and RNA polymerase activity *in vitro* [14,20,30,35]. Reduction of hPNPase by RNAi leads to elongated mtRNA poly(A) tails, suggesting that hPNPase might function as a ribonuclease in the mitochondrial matrix [35]. Nevertheless, it is also possible that hPNPase performs RNA polymerase activities *in vivo*, as shown *in vitro*. However, submitochondrial localization studies [17] suggest that hPNPase might modulate mtRNAs indirectly from the IMS (see text for discussion). (c) and (d) hPNPase binds specifically but does not degrade RNA damaged by oxidative stress *in vitro* and thus, potentially, maintains high-fidelity translation by sequestering damaged RNA [30]. Whether this interaction occurs *in vivo* and where it occurs is unknown.

activity. Furthermore, hPNPase over-expression leads to alterations of various cell cycle-regulatory proteins, including the induction of the CDKI p27<sup>KIP1</sup> and the repression of the CDKI p21<sup>CIP1/WAF-1/MDA-6</sup>, cyclin A, cyclin E, E2F transcription factor 1 (E2F1) and the hyperphosphorylated form of the retinoblastoma (Rb) protein [15]. Interestingly, *c-MYC* [58,59] is repressed when hPNPase is over-expressed [15]. This repression provides a potential mechanism for hPNPase-induced senescence because *c-MYC* promotes cell-cycle progression to S phase and shortens G<sub>1</sub> phase by multiple actions, including repression of p27<sup>KIP1</sup> transcription. *In vitro* degradation assays indicate that hPNPase degrades *c-MYC* mRNA selectively [15], although a mechanism for this selectivity remains unclear. A correlation between hPNPase induction and *c-MYC* repression is also observed in IFN-β-treated melanoma cells. Therefore, hPNPase might have a role in IFN-β-mediated *c-MYC* repression and growth inhibition, which is observed in multiple cell types, including human lymphoblastoid and colon carcinoma cells [29]. However, evidence that hPNPase degrades *c-MYC* mRNA *in vivo* is still lacking and, because over-expressed hPNPase localizes to both mitochondria and the cytosol [16], it remains to be shown under what physiological conditions endogenous hPNPase could gain access to cytosolic RNAs, such as *c-MYC*. As mentioned briefly, hPNPase-localization studies in cells undergoing terminal

differentiation or cellular senescence could provide crucial evidence for or against an essential role for increased hPNPase in targeting cytosolic *c-MYC* in these key physiological processes (Figure 4a).

The senescent phenotype of hPNPase-over-expressing cells might be linked alternatively to the function of hPNPase within mitochondria rather than selective *c-MYC* mRNA turnover. Oxidative stress mediates cellular senescence [60,61]. Therefore, induction of ROS following upregulation of hPNPase could be a potential mechanism. Whether ROS accumulation has a causative role in hPNPase-induced senescence could be assessed with ROS scavengers following selective upregulation of hPNPase expression. Interestingly, alterations in mitochondrial-gene expression, which might be caused by hPNPase over-expression and potentially lead to ROS accumulation, as discussed earlier, occur in terminal differentiation and replicative senescence [2,62,63]. Moreover, the knockdown of hFis1, which leads to increased ROS production, by affecting mitochondrial dynamics (as described earlier) also triggers cellular senescence [44]. We propose a testable model for hPNPase-induced senescence: following its over-expression, hPNPase mediates an increase in ROS production either by perturbing mitochondrial-gene expression, perhaps indirectly from the IMS, or by affecting mitochondrial fission and fusion, which eventually results in growth arrest.

### Future directions and concluding remarks

In summary, hPNPase, an RNA-processing enzyme characterized initially as induced during terminal differentiation and cellular senescence, surprisingly localizes to the IMS of mitochondria and maintains mitochondrial homeostasis by an unknown mechanism. hPNPase may be an IMS protein that functions both within and outside mitochondria. However, further work is required to clarify which signaling pathways, if any, lead to hPNPase activity in the cytosol (Figure 4a and c). New, more sensitive techniques are also needed to determine whether there are trace amounts of endogenous hPNPase in the mitochondrial matrix or cytosol. The identification of hPNPase targets, in addition to the potential target *c-MYC*, and the elucidation of how hPNPase elicits substrate specificity will improve our understanding of its RNA-processing function(s) significantly.

Importantly, the mechanism by which hPNPase maintains oxidative phosphorylation remains a challenging but crucial area of study. Are the alterations in poly(A) tail lengths of mitochondrial transcripts observed in hPNPase knockdown cells secondary to its enzymatic activity in the IMS? Alternatively, does hPNPase maintain mitochondrial homeostasis by a mode of action independent of its exoribonuclease and RNA-polymerase activities? By *in vitro* RNase protection assays, a recent study showed that hPNPase binds specifically but does not degrade RNAs containing 8-oxo-7,8-dihydroguanine (8-oxoGua), which is an indication of RNA that has been damaged by oxidative stress. Thus, hPNPase potentially maintains high-fidelity translation by sequestering damaged RNA [30]. It is unclear if this occurs *in vivo* and, if so, when or where in the cell this occurs. Despite this and the observation that oxidative stress appears to reduce rather than induce hPNPase expression [30], it remains possible that hPNPase maintains normal mitochondrial function by preventing the translation of damaged cytosolic or mitochondrial mRNAs in situations that enable hPNPase to sequester them (Figure 4c and d).

In addition to its crucial role in maintaining mitochondrial homeostasis, potential roles of hPNPase in antiviral responses and tumorigenesis are also two emerging research areas that are of particular biomedical significance. Because hPNPase expression is regulated by type I IFNs (Figure 2a), hPNPase might be involved in combating viral infections either by degrading viral RNA or by inducing senescence to prevent viral propagation (Figure 2b). If this is so, hPNPase would add to the growing evidence that mitochondria have a role in signaling in innate immunity, which was first shown by the signaling protein mitochondrial antiviral signaling (MAVS), a mitochondrial outer membrane protein that activates type I IFN expression in response to viral infections [64,65]. It would be interesting to ascertain whether IFN expression in response to MAVS or other virus-mediated signaling is sufficient to induce *PNPT1* expression.

A link between hPNPase and cancer has also been hypothesized following the identification of the TCL1 oncoprotein as an interacting partner for hPNPase [20]. Intriguingly, TCL1 is a 14 kDa non-enzymatic adaptor molecule that augments AKT (protein kinase B) and protein

kinase C-mitogen-activated protein kinase (PKC-MAPK) signaling pathways to enhance cell survival and proliferation [66,67]. Transgenic mice that over-express TCL1 develop mature lymphoid malignancies [68–70], although the tumorigenic mechanism remains incompletely resolved [71]. However, TCL1 does not possess an identifiable MTS and is not imported into energized mitochondria in a standard import assay [17]. Therefore, it remains an open question how an hPNPase-TCL1 interaction occurs.

Overall, whether from its home in the IMS or within the cytosol, linking location to function will prove crucial to understanding how hPNPase mediates effects on energy metabolism, RNA processing, senescence and, potentially, antiviral responses and tumorigenesis.

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