Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta xx (2008) xxx–xxx
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbaexp

Review

Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases

Shimyn Slomovic, Victoria Portnoy, Shlomit Yehudai-Resheff, Ela Bronshtein, Gadi Schuster *

Department of Biology, Technion — Israel Institute of Technology, Haifa 32000, Israel

Received 18 September 2007; received in revised form 29 November 2007; accepted 6 December 2007

Abstract

The addition of poly(A)-tails to RNA is a phenomenon common to almost all organisms. Not only homopolymeric poly(A)-tails, comprised exclusively of adenosines, but also heteropolymeric poly(A)-rich extensions, which include the other three nucleotides as well, have been observed in bacteria, archaea, chloroplasts, and human cells. Polynucleotide phosphorylase (PNPase) and the archaeal exosome, which bear strong similarities to one another, both functionally and structurally, were found to polymerize the heteropolymeric tails in bacteria, spinach chloroplasts, and archaea. As phosphorylases, these enzymes use diphosphate nucleotides as substrates and can reversibly polymerize or degrade RNA, depending on the relative concentrations of nucleotides and inorganic phosphate. A possible scenario, illustrating the evolution of RNA polyadenylation and its related functions, is presented, in which PNPase (or the archaeal exosome) was the first polyadenylating enzyme to evolve and the heteropolymeric tails that it produced, functioned in a polyadenylation-stimulated RNA degradation pathway. Only at a later stage in evolution, did the poly(A)-polymerases that use only ATP as a substrate, hence producing homopolymeric adenosine extensions, arise. Following the appearance of homopolymeric tails, a new role for polyadenylation evolved; RNA stability. This was accomplished by utilizing stable poly(A)-tails associated with the mature 3′ ends of transcripts. Today, stable polyadenylation coexists with unstable heteropolymeric and homopolymeric tails. Therefore, the heteropolymeric poly(A)-rich tails, observed in bacteria, organelles, archaea, and human cells, represent an ancestral stage in the evolution of polyadenylation.

Keywords: RNA polyadenylation; RNA degradation; PNPase; Exosome; Heteropolymeric tails

Contents

1. Polynucleotide phosphorylase (PNPase)

PNPase (EC 2.7.7.8) was discovered in 1955 by Grunberg-Manago et al. [1] and was the first enzyme to be identified that catalyzes the formation of RNA using ribonucleotides. It was

* Corresponding author. Tel.: +972 4 8293171; fax: +972 4 8295587.
E-mail address: gadis@tx.technion.ac.il (G. Schuster).

1874-9399/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagrm.2007.12.004

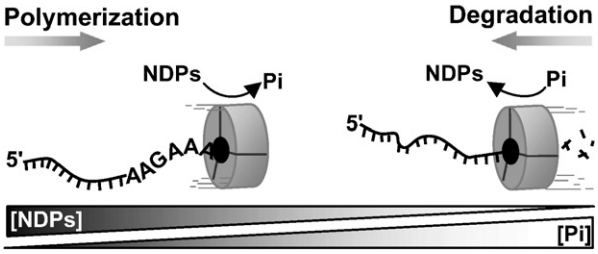

Fig. 1. PNPase acts as both a polymerase and a 3′ to 5′ exoribonuclease. PNPase is presented schematically as a homotrimer. When polymerizing RNA (left side of figure), PNPase consumes nucleotide diphosphates (NDPs) and produces inorganic phosphate (Pi). During exoribonucleolytic RNA degradation (right side of figure), the opposite occurs. Because the equilibrium of this reaction lies close to unity, PNPase is extremely sensitive to Pi and NDP concentrations (gray wedges at bottom). Therefore, the reaction catalyzed by PNPase can theoretically be dictated by local concentrations of each substrate.

later characterized by Littauer and Grunberg-Manago in studies of the nature of ribonucleotide incorporation into nucleic acids in *E. coli* [2].

Unlike RNA polymerases, it does not require a template and cannot copy one and when supplied with a mixture of ribonucleotide diphosphates (NDPs), the ensuing polymerization reaction forms a random copolymer. PNPase not only catalyzes 5′→3′ RNA polymerization but processive 3′→5′ phosphorolysis as well [2–4]. As a phosphorylase, it uses phosphate (Pi) to cleave phosphodiester bonds (Fig. 1). The phosphorolysis reaction, unlike hydrolysis, is close to equilibrium and therefore, mostly reversible. The direction of PNPase activity, either degradation or polymerization, can be controlled by the relative concentrations of NDPs and Pi (Fig. 1).

In *E. coli*, PNPase is mostly active in 3′→5′ phosphorolysis during RNA degradation and 3′ end processing, but a substantial degree of activity in the polymerization of heteropolymeric tails has also been reported [5,6]. Part of the PNPase population in *E. coli* is associated with the endoribonuclease RNase E, an RNA helicase, enolase, and possibly other proteins in a high molecular weight complex termed the degradosome [7]. In spinach chloroplasts, cyanobacteria, and gram-positive bacteria, PNPase is suggested to be the major polyadenylating enzyme [8–11]. It

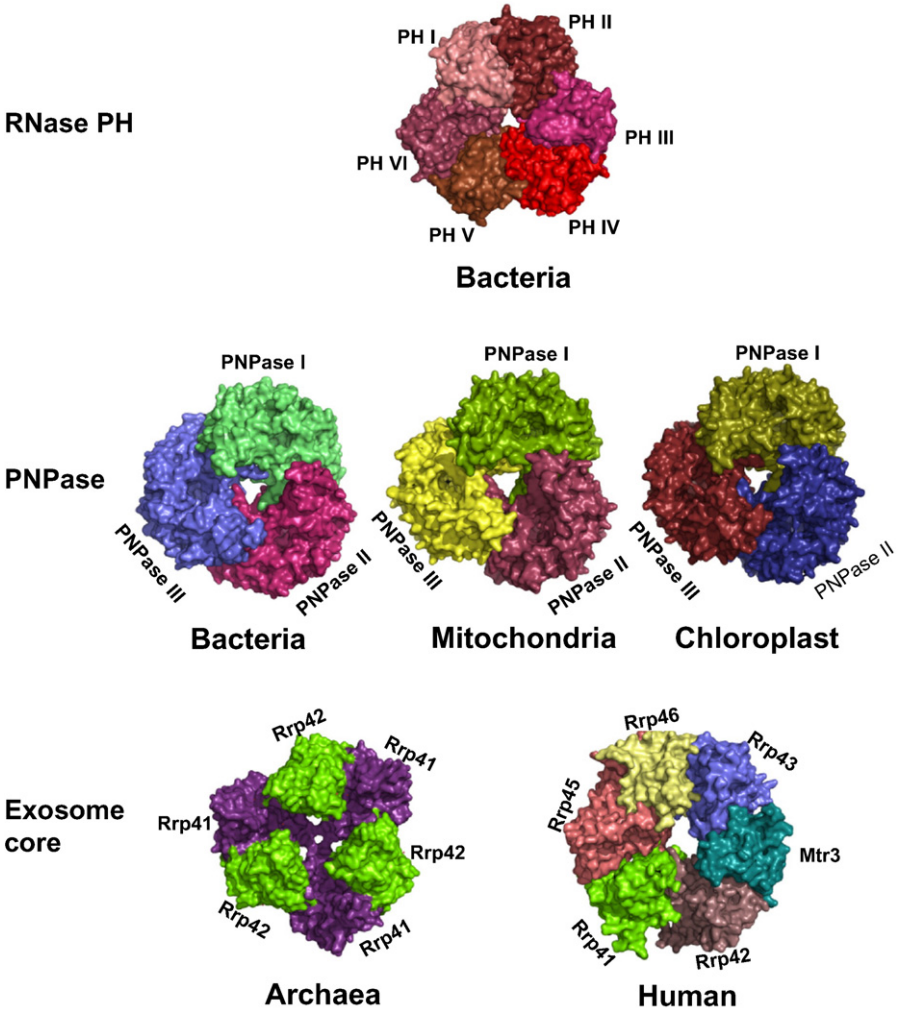

Fig. 2. Structural similarities between RNase PH and the 3′→5′ RNA degradation machines. The structures of RNase PH [19,20], the bacterial PNPase [24], archaeal [29,33] and eukaryotic [41] exosomes, as well as the predicted structure of the chloroplast PNPase [27], are shown in order to compare their ring-shape compositions. The structures were generated using Pymol (http://pymol.sourceforge.net/).

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

was also reported to be a global regulator of virulence and persistence in *Salmonella enterica* [12]. In Chlamydomonas, the chloroplast PNPase was recently presented to be a key regulator in the adaptation to Pi starvation [13]. In human cells, PNPase was identified in an overlapping pathway screen to discover genes displaying coordinated expression as a consequence of terminal differentiation and senescence of melanoma cells [14]. Although it harbors a mitochondrial target peptide, the human PNPase was recently localized to the mitochondrial inter-membrane space (IMS), not to the matrix and therefore, unlike its homologues in bacteria, chloroplasts, and plant mitochondria, may not play a direct role in the processing and degradation of RNA [15–17].

RNase PH (EC 2.7.7.56) is a second Pi-dependent 3′→5′ exoribonuclease in bacteria that functions in the trimming of the tRNA precursors at their 3′ ends [18]. In bacteria, six RNase PH polypeptides form a homohexameric structure that is similar to that of PNPase and the exosome core (Fig. 2) [19,20]. Indeed, RNase PH homologues have been observed in some archaea and eukaryotes in which they form the core of the exosome complex (see below) [21].

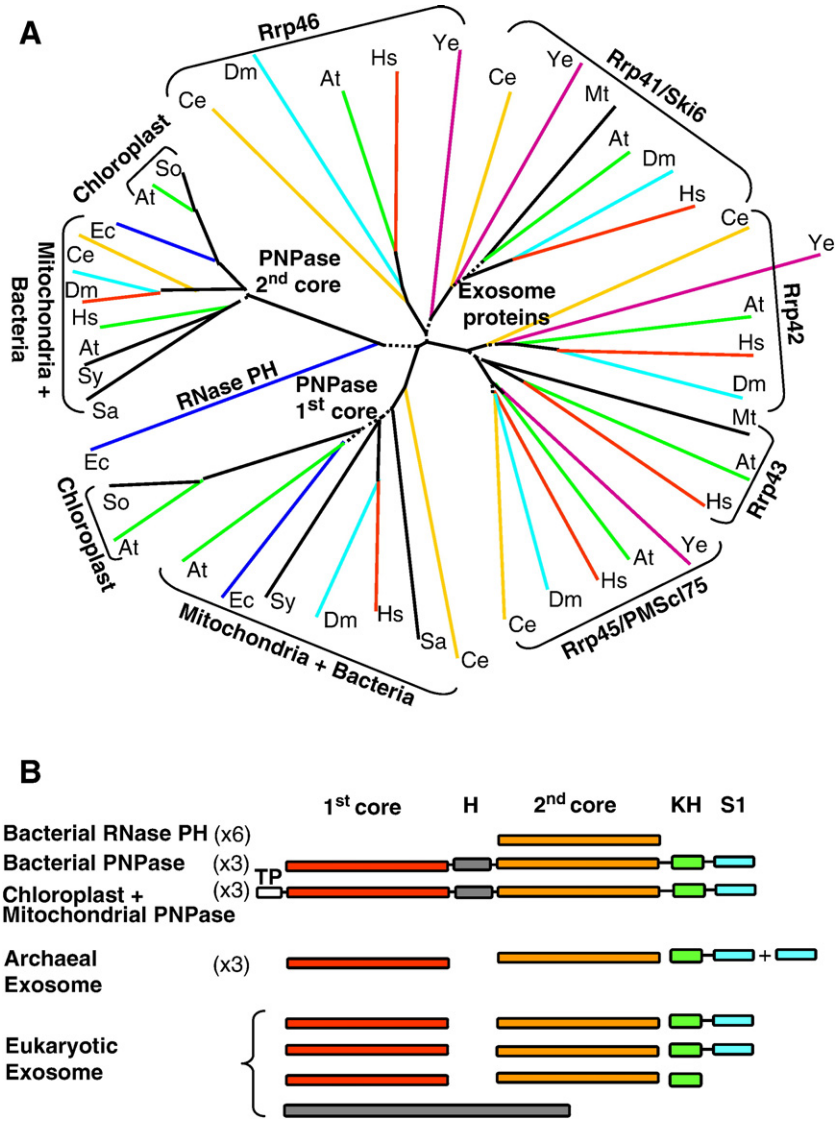

Fig. 3. A. Phylogenetic tree of the RNase PH-domains of bacterial and organellar PNPases and exosome proteins. The 1st and 2nd core domains of PNPases, related exosome proteins and *E. coli* RNase PH were aligned using the Clustal X multiple sequence alignment tool, and a phylogenetic tree was constructed as described [27]. Proteins from the same organism have the same color. The dotted lines indicate regions of the tree in which the bootstrap value was less than 50%; the validity of these regions is therefore low. The organisms are: At, *Arabidopsis thaliana*; So, *Spinacia oleracea*; Hs, *Homo sapiens*; Ec, *Escherichia coli*; Sa, *Streptomyces antibioticus*; Sy, *Synechocystis sp. PCC6803*; St, *Staphylococcus aureus*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Ye, *Saccharomyces cerevisiae*; and Mt, *Methanobacterium thermoautotrophicum*. [Reproduced with permission of the American Association of Plant Biology from Ref. [27].] B. Domain analysis comparison of RNase PH, the bacterial and organellar PNPases, and the archaeal and eukaryotic exosomes. The two core RNase PH-domains of PNPase are shown in red and orange and the KH and S1 domains are shown in green and light blue, respectively. The exosome protein harbors the hydrolytic RNA degradation activity is shown in gray. Note that the PNPase enzyme is a homotrimer, as indicated by the (×3) label, which makes a complex with the same number of RNase PH core domains.

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

2. Structural similarities between PNPase, RNase PH, and the exosome complexes

The amino acid sequences of PNPases from bacteria, as well as from the nuclear genomes of plants and mammals, display a high level of homology and feature a similar composition of five motifs (Fig. 3) [22–27]. The protein contains two domains that are related to the RNase PH enzyme termed the phosphorolytic domains, 1st and 2nd core domains, or RNase PH-like domains. An additional two domains are homologous to the K1 and S1 domains characterized in RNA binding proteins and the fifth, an α-helix domain, separates the two core domains. The PNPases of plants and animals are directed to the chloroplast and mitochondrion by an N-terminal transit peptide that is removed from the protein upon translocation.

The three-dimensional structure of the PNPase from the bacterium, *Streptomyces antibioticus*, has been deciphered by X-ray crystallographic analysis. The enzyme forms a ring-shaped homotrimeric complex, with the hexameric PH-domains surrounding a central channel that can accommodate a single-stranded RNA molecule (Fig. 2) [24,28].

PNPase has not been found in any members of the archaeal domain. However, several hyperthermophilic and methanogenic archaea contain a protein structure that is composed of two proteins, Rrp41 and Rrp42, which are homologous to RNase PH and one protein, containing a KH/S1 domain (Fig. 3). Three copies of these proteins form a nine-subunit complex termed the archaeal exosome that, like PNPase, is responsible for the polyadenylation and degradation of RNA [29–34]. Crystallographic analysis of the archaeal exosome revealed a structure very similar to that of the bacterial PNPase (Fig. 2). The eukaryotic exosome, which is active in 3′ → 5′ RNA degradation in the cytoplasm and nucleus, is composed of 10–11 subunits and its alignment with the archaeal exosome and the various PNPases clearly points to a striking similarity between both the number and characteristics of the different domains [34–40]. The same type of core structure is formed: a ring-shaped complex created by six different PH-like polypeptides, in which three different subunits each contribute a KH and an S1 domain [41]. The yeast exosome core, in addition to RNase PH-like polypeptides, contains a novel subunit with hydrolytic 3′ exonuclease activity, termed Rrp44 or Dis3, that is homologous to members of the bacterial RNase R family [21,42,43]. Interestingly, however, both the yeast and human exosomes were recently shown to lack RNA phosphorolysis activity [41,42]. It could be suggested that this activity was lost during the evolution of the eukaryotic exosome, despite the fact that it preserved the ring-shaped PNPase-like core structure (Fig. 2).

These observations imply that the bacterial and organellar PNPases, as well as the archaeal and eukaryotic exosomes, constitute a family of ring-shaped machines, which has evolved to phosphorolytically (in bacteria and organelles) and/or hydrolytically (in eukaryotes) polymerize and degrade RNA. The hypothetical ancestral complex, from which these enzymes evolved, was already present before the separation of bacteria, archaea, and eukaryotes (Fig. 3). It could be hypothesized that the RNase PH, S1, and KH domains were functionally assembled into these ring-structured RNA phosphorolysis machines as the result of evolutionary processes and necessities. It is likewise interesting to postulate why this machinery separated into several different proteins in archaea and eukaryotes while remaining comprised of repetitions of one protein in bacteria and organelles.

3. The polyadenylation-stimulated RNA degradation pathway and PNPase as a polymerase

The molecular mechanism of RNA polyadenylation and degradation in prokaryotes and organelles has been extensively studied, mostly by analyzing *E. coli*. The general scheme of this pathway consists of three sequential steps initiated with endonucleolytic cleavage of the transcript. In the second stage, the cleavage product is polyadenylated and thereby targeted for rapid exonucleolytic degradation which comprises the final step (Fig. 4). In *E. coli*, polyadenylation is carried out mainly by a nucleotidyl transferase-type poly(A)-polymerase (Ntr-PAP), producing homopolymeric poly(A)-tails, and to a certain extent, by PNPase, which extends heteropolymeric poly(A)-rich tails containing all four nucleotides [5]. Hfq, a protein that resembles the eukaryotic Sm polypeptide, was found to be involved in modulating the polyadenylation activity between Ntr-PAP and PNPase [44,45]. The exonucleolytic degradation, is performed by PNPase, RNase II, and possibly, also by RNase R [46]. Research in *E. coli* led to the study of related mechanisms in other systems, revealing polyadenylation-stimulated RNA degradation pathways in other bacteria, chloroplasts, both plant and human mitochondria, archaea, and for nuclear encoded transcripts in yeast and human cells. The detection of non-abundant, truncated polyadenylated RNA molecules, which are

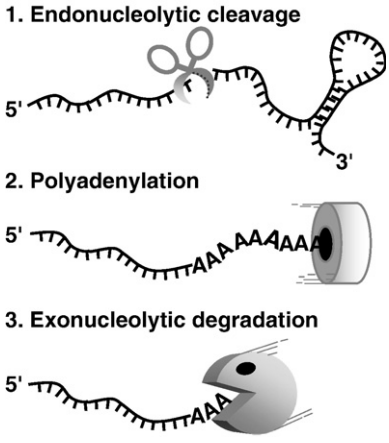

Fig. 4. The polyadenylation-stimulated RNA degradation pathway. The three stages of polyadenylation-stimulated RNA turnover are: (1) endonucleolytic cleavage, (2) polyadenylation, and (3) exonucleolytic digestion. The first endonucleolytic cleavage is believed to be performed by RNase E in *E. coli* and related bacteria. RNase J has been implicated to this function in *Bacillus subtilis*. The polyadenylation is performed by Ntr-PAP, producing homopolymeric poly (A)-tails or by PNPase, producing heteropolymeric poly(A)-rich tails. In hyperthermophilic and several methanogenic archaea, the heteropolymeric tails are synthesized by the archaeal exosome. The exonucleolytic degradation step is carried out by PNPase and RNase II/R in bacteria and by the archaeal exosome, in the exosome-containing archaea. In those in which the exosome is not present, neither is polyadenylation and degradation is carried out by RNase R.

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

Table 1
Examples of heteropolymeric tails

Organism	Polymerase	Example of a representative tale
B. subtilis [48]	PNPase	$GA_3UA_6CUA_4CACA_{18}UA_8CAACA_3UACGA_4CA_3U_2A_{17}CA_3GA_9U_2A_8$ (23S rRNA)
S. colelicolor [65]	"	$A_3UA_2U_2A_3UA_5UA_4U_2A_4GA_5UA_{17}$ (23S rRNA)
E. coli [66]	"	$A_6GCUACAUGA_{12}GCUGACGUGA_6GAGACUAU_2CGA_4CAG_2A_3GUA_8U_{21}$ (lpp)
Synechocystis [9]	"	$G_2UGACAGA_2G_3A_5UAUACAGAGA_2GAUGA_3GUA_2GUA_2GAGAGAUCUGAGCGA_4CU_2A_5GA_5CUA_6$-$CAAG_2A_2GA_3GA_2U_2GA_4G_3$ $CA_9U_2AGACACA_6$ (*rbcL*)
Spinach chloroplast [47]	"	$A_2GAGAG_2AG_2CA_6G_2A_2GA_5GAGA_3G_2A_2GUA_3GA_2GCA_4GA_6G_2CAGAGA_5G_2A_3GA_3GA_4GAGAGA_3$-$G_2ACA_7UA_8GAGA_3G_2A_4G_2AG_2ACAG_2A_4GAG_2A_3CA_5GAGC_2AGA_5G_2AG_2A_4G_2CGAG_2AGA_5GAGA$-$GAUA_2C_4A_{13}GAG_2AG_2A_3C_3A_3G_2A_3UA_9G_2A_3GUG_2A_{14}$ (*psbA*)
Archaea (*Sulfolobus*) [32]	Archaeal exosome	$AGAUA_3CUGA_2GACAGA_7G_2A_4GA_2UA_4GAUAGAGAUA_4UAGUAGAG_3AUGA_3GACUA_{12}G_2AUA_{17}$ (16S rRNA)
Human [54]	?	$A_5GA_4GA_7GA_3GA_3GA_3GAG_2A_2GA_2GAG_2AGA_5G_2AGA_2G_2AGA_6G_3AG_2A_4GAG_2AG$ $A_3GA_2GUA_5GA_{19}$ (28S rRNA)

the degradation intermediates caught between the second and third stages of the pathway, serves as a tell tale sign of the presence of a poly(A)-stimulated RNA degradation mechanism [11].

4. Heteropolymeric poly(A)-rich tails are added by PNPase or the archaeal exosome

For clarification, the terms "homopolymeric" and "heteropolymeric" tails refer here to post-transcriptionally added extensions that were analyzed by DNA sequencing and found to contain either only adenosines or any of the other nucleotides as well, respectively. Examples of heteropolymeric tails are presented in Table 1. Short tails, containing only several non-A nucleotides, or tails produced by a non-specific polymerization enzyme but not analyzed by DNA sequencing are not referred to here as heteropolymeric tails.

Heteropolymeric poly(A)-rich tails were first discovered when spinach chloroplast transcripts were analyzed for polyadenylation [47]. However, the identity of the polymerizing enzyme was unknown and likewise, the biological meaning of these extensions was not understood. Several years later, such heteropolymeric tails were detected in *E. coli* strains, in which the poly(A)-polymerase gene was inactivated, and PNPase was identified as the enzyme responsible for this activity [5]. Following these initial observations, experiments in cyanobacteria, the gram-positive bacteria, *Streptomyces coelicolor* and *Bacillus subtilis*, as well as spinach chloroplasts, determined that in all cases in which heteropolymeric poly(A)-rich tails were detected, PNPase was the polymerizing enzyme [8–10,48]. Therefore, once detected, the nature of the post-transcriptionally added extensions, concerning their nucleotide composition, can indicate the identity of the polymerizing enzyme (Tables 1 and 2).

To date, only few organisms or organelles have been described in which RNA does not undergo any form of polyadenylation and is, therefore, metabolized by other means. Among these are the halophilic, and several methanogenic archaea, as well as the small genome parasitic bacteria, mycoplasma ([32,49] and Portnoy and Schuster, submitted). In addition, polyadenylation does not occur in yeast mitochondria, perhaps reflecting a general rule in fungus (Fig. 5) [50–52]. In these organisms which lack poly(A), there is no PNPase and in the case of polyadenylation-lacking archaea, the exosome is absent. However, a correlation between the absence of PNPase and of polyadenylation does not exist in trypanosomal mitochondria, where no PNPase has been identified yet polyadenylation is present [53]. As described, the archaeal exosome is very similar to PNPase and therefore, it was logical that the RNA tails detected in the hyperthermophilic and methanogenic archaea containing the exosome were found to be heteropolymeric (Table 1) [32,49]. Likewise, biochemical analysis of the exosome in the hyperthermophilic archaea, *Sulfolobus*, demonstrated that the exosome is indeed responsible for the polyadenylation activity and produces, similar to PNPase in bacteria and organelles, heteropolymeric tails [32].

5. Heteropolymeric tails in human cells

Heteropolymeric tails were recently discovered in human cells [54]. Using oligo(dT)-primed reverse transcribed PCR amplification, truncated, non-abundant polyadenylated transcripts related to ribosomal RNA were detected (Table 1). Surprisingly, approximately 50% of the isolated and sequenced RNA fragments harbored heteropolymeric tails while the other half contained homopolymeric extensions. Similar results were obtained upon the application of a bioinformatic tool designed

Table 2
Poly(A)-polymerases, their substrates, and the nature of the tails which are produced

	Enzyme	Location	Tails	Substrate	References
1.	PNPase	Bacteria, organelles	Hetero	NDPs	[5,8,10,44,47,48]
2.	Archaeal exosome	Archaea	Hetero	NDPs	[32,49]
3.	Ntr-PAP	Bacteria, organelles?	Homo	ATP	[61,62]
4.	mtPAP	Animal mitochondria	Homo	ATP	[67,68]
5.	Trf4, trf5	Nucleus	Homo	ATP	[59,69–71]
6.	GLD-2		Homo	ATP	[59,72,73]
7.	Pol II PAP	Nucleus	Homo	ATP	[74]
8.	Pol II PAP	Cytoplasm	Homo	ATP	[74]
9.	?	Mammals: –Nucleus? –Cytoplasm?	Hetero	?	[54]

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

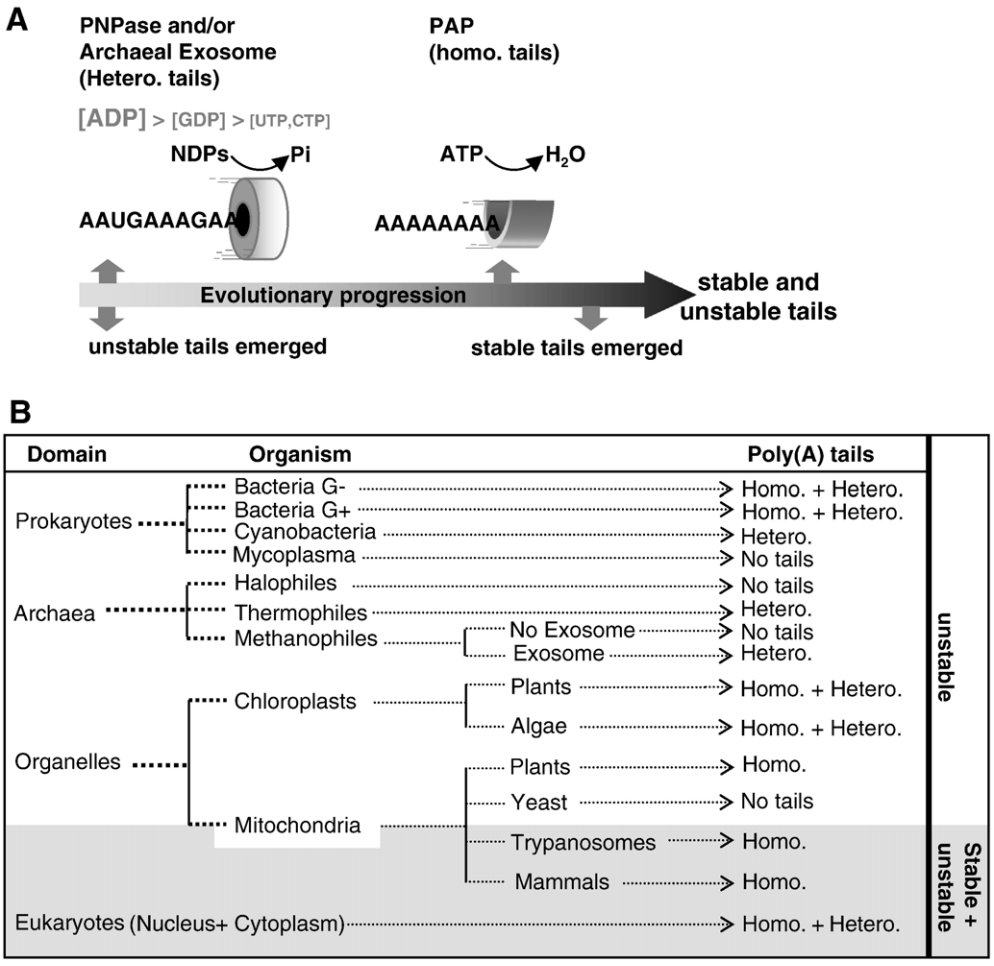

Fig. 5. A suggested scenario for the evolution of polyadenylation. A. First, polyadenylation functioned as a mechanism for RNA degradation in a polyadenylation-stimulated RNA degradation pathway. The polymerizing enzyme was PNPase and/or the archaeal exosome, whichever existed first, and the tails were heteropolymeric and unstable (left side). Later, PAP arose and with it, homopolymeric unstable tails. The stable poly(A)-tails, which characterize animal mitochondria and nucleus encoded mRNAs, were established by stabilization of the homopolymeric unstable tails. At present, various organisms contain both stable and unstable tails, as shown in part B. B. The distribution of homopolymeric (Homo.), heteropolymeric (Hetero.), stable, and unstable tails between different organisms and organelles. When "No tails" is indicated, no polyadenylation takes place in this organism/organelle.

to screen the human EST data bank in search of similar ribosomal sequences [54]. Heteropolymeric extensions, added to truncated mRNA molecules in human cells, have been detected as well (Bronshtein and Schuster, unpublished data). In addition to the relatively long heteropolymeric tails described above (Table 1), RNA with very short tails, containing as few as one non-A (guanosine) residue, were found in the nucleus of human cells [55]. It should be noted that a quality control mechanism, involving the polyadenylation-stimulated degradation pathway, which degrades improperly folded, mutated, and intergenic transcripts, has been described in yeast and human cells [21,38,55]. The discovery of heteropolymeric tails in human cells opened the question of whether these are part of this quality control mechanism. A second question concerns the identity of the enzyme responsible for the polymerization of these tails. One possibility is that a poly(A)-polymerase, unidentified as of yet, which is not exclusively specific to adenosine and therefore, introduces all four nucleotides, like PNPase and the archaeal exosome, is responsible for this activity. A second option is that these heteropolymeric tails, similar to their prokaryotic and archaeal counterparts, are polymerized by the human exosome or PNPase. However, as described in the previous paragraph, the human exosome was recently shown to lack phosphorolytic activity and PNPase is located in the mitochondrial inter-membrane space and therefore, neither are likely candidates [15,56].

6. Polyuridylation

Recently, several enzymes that preferentially polymerize RNA using UTP as a substrate and therefore, could potentially produce poly(U)-tails, were reported [57,58]. These enzymes, like the Ntr-PAP, are template-independent members of the DNA β-like superfamily and their homologues seem to be present throughout Eukaria [59]. Indeed, polyuridylation of reporter transcripts [57], decay intermediates of miRNA-directed cleavage [60], as well as the insertion of U in the poly(A)-tail of mRNA [58], has been reported. Similar to homo- and heteropolymeric poly(A) extensions, in which the identity of the enzyme, PAP or PNPase, determines the nature of the tail

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

composition, the activity of poly(U)-polymerases seems to produce poly(U)-tails and perhaps incorporate single uridine residues into poly(A)-tails. It is too early to assess how common polyuridylated RNA is, compared to polyadenylated RNA and also, how long the average poly(U)-tails are and whether they function in stabilization, destabilization, or both.

7. Are the heteropolymeric tails evidence of an ancestral polyadenylation stage in evolution?

The almost ubiquitous presence of polyadenylation across the life kingdoms, the heteropolymeric poly(A)-rich tails, and the structural and functional similarities between the various PNPases and human and archaeal exosomes, suggest the following possible scenario for the evolution of RNA polyadenylation (Fig. 5): The first role of polyadenylation to evolve was for the purpose of targeting RNA to degradation. This occurred very early in evolution, prior to the separation of the prokaryotic and archaeal domains. Furthermore, it is suggested that the first enzyme active in polyadenylation was either PNPase or the archaeal exosome. Although PNPase can utilize all NDPs as substrates, thereby synthesizing heteropolymeric RNA extensions, the tails were mostly composed of adenosines, as the relative concentration of ADP was much higher than that of the other nucleotides. Later, the poly(A)-polymerases appeared, already specific to ATP and therefore, produced homopolymeric poly(A)-tails. It is logical that the poly(A)-polymerases evolved after the PNPases and evolutionarily, quite recently as, for example, in bacteria and plant organelles, the poly(A)-polymerases evolved from nucleotidyl transferases (Ntr) (the enzyme which adds the CCA tail to the 3′ ends of tRNA). Indeed, their amino acid sequences remain almost identical [61,62]. Cyanobacteria, *S. coelicolor*, and hyperthermophilic and some methanogenic archaea represent the ancient polyadenylation stage in evolution when only heteropolymeric tails existed (Fig. 5 and Table 1). The heteropolymeric tails detected in many other organisms, from bacteria to human cells, in which, in some cases, these extensions coexist with homopolymeric tails, are remnants of this early evolutionary stage.

Assuming that RNA degradation-stimulating polyadenylation was the first form of polyadenylation to appear in evolution, this hypothesis could be taken a step further. It could be suggested that the stable poly(A)-tails that characterize the 3′ ends of most nucleus-encoded and mitochondrial mRNAs in mammalian cells originated and evolved from the destabilizing tails of the degradation-stimulating poly(A) pathway (Fig. 5). For example, during the drastic condensation of the animal mitochondrial genome which eliminated almost all intergenic regions, the addition of stable poly(A)-tails at the mature 3′ ends of several mRNAs became essential, as they generated complete and functional translational stop codons in cases in which only partial stop codons of U or UA remained encoded in the gene sequence [51,63,64]. Since a mechanism for the polyadenylation of truncated endonucleolytically cleaved transcripts, as part of the polyadenylation-stimulated degradation pathway, already existed at that point, the stabilization of these tails would seem possible and may have been the turning point at which the poly (A)-tails were stabilized and even used to grant stability to mRNAs, as in the case of nucleus-encoded mRNA (Fig. 5) [11]. Alternatively, it could be argued that the stable poly(A)-tails of nucleus-encoded mRNA evolved upon nuclear membrane formation, in order to create a mechanism for the export of mRNA from the nucleus. It could be further hypothesized that the stable poly(A) extensions of nucleus-encoded mRNAs coincided with the evolution of poly(A)-binding proteins, thereby preventing digestion via exosomal degradation activity. However, in the case of animal mitochondria, no poly(A)-binding proteins are known to be present. Finally, the recently observed poly(U)-polymerases could fit into this evolutionary scenario if they evolved from a modification of the Ntr-PAPs, at a relatively late stage, in order to establish specific functions yet to be revealed.

Together, this evolutionary hypothesis implies that the reason that nucleus-encoded mRNA molecules contain 3′ end extensions composed exclusively of adenosines resulted from the ancient fact that adenosine evolved as the energy coin of life and therefore, ADP was (and remains) present at a higher concentration than the other nucleotides. Hence, PNPase, the first poly(A)-polymerase, although able to utilize all NDPs, produced tails rich with adenosines, which laid the foundations for the conversion to homopolymeric tails, that occurred later in evolution.

8. Note added in proof: A recent publication describes the oligouridilation of histone mRNA as a stimulator of degradation [1, 2]

[1] T.E. Mullen and W.F. Marzluff, Degradation of histone mRNA requires oligouridylation followed by decapping and simultaneous degradation of the mRNA both 5′ to 3′ and 3′ to 5′, Genes Dev 22 (2008) 50–65.

[2] C.J. Wilusz and J. Wilusz, New ways to meet your (3′) end oligouridylation as a step on the path to destruction, Genes Dev 22 (2008) 1–7.

Acknowledgements

Work in the author's lab was supported by grants from Israel Science Foundation (ISF), United States-Israel Binational Science Foundation (BSF) and the United States-Israel Binational Agricultural Research and Development Fund (BARD).

References

[1] M. Grunberg-Manago, P.J. Oritz, S. Ochoa, Enzymatic synthesis of nucleic acidlike polynucleotides, Science 122 (1955) 907–910.

[2] U.Z. Littauer, M. Grunberg-Manago, in: T.E. Creighton (Ed.), The Encyclopedia of Molecular Biology, John Willy and Sons Inc., New York, 1999, pp. 1911–1918.

[3] U.Z. Littauer, H. Soreq, Polynucleotide Phosphorylase, vol. 15, Academic Press, New York, 1982.

[4] M. Grunberg-Manago, Messenger RNA stability and its role in control of gene expression in bacteria and phages, Annu. Rev. Genet. 33 (1999) 193–227.

[5] B.K. Mohanty, S.R. Kushner, Polynucleotide phosphorylase functions both as a 3′ to 5′ exonuclease and a poly(A) polymerase in *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 11966–11971.

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

[6] M.P. Deutscher, Z. Li, Exoribonucleases and their multiple roles in RNA metabolism, Prog. Nucleic Acid Res. Mol. Biol. 66 (2001) 67–105.
[7] A.J. Carpousis, The RNA degradosome of *Escherichia coli*: an mRNA-degrading machine assembled on RNase E, Annu. Rev. Microbiol. 61 (2007) 71–87.
[8] S. Yehudai-Resheff, M. Hirsh, G. Schuster, Polynucleotide phosphorylase functions as both an exonuclease and a poly(A) polymerase in spinach chloroplasts, Mol. Cell. Biol. 21 (2001) 5408–5416.
[9] R. Rott, G. Zipor, V. Portnoy, V. Liveanu, G. Schuster, RNA polyadenylation and degradation in cyanobacteria are similar to the chloroplast but different from *Escherichia coli*, J. Biol. Chem. 278 (2003) 15771–15777.
[10] B. Sohlberg, J. Huang, S.N. Cohen, The *Streptomyces coelicolor* polynucleotide phosphorylase homologue, and not the putative poly(A) polymerase, can polyadenylate RNA, J. Bacteriol. 185 (2003) 7273–7278.
[11] S. Slomovic, V. Portnoy, V. Liveanu, G. Schuster, RNA polyadenylation in prokaryotes and organelles; different tails tell different tales, Crit. Rev. Plant Sci. 25 (2006) 65–77.
[12] M.O. Clements, S. Eriksson, A. Thompson, S. Lucchini, J.C. Hinton, S. Normark, M. Rhen, Polynucleotide phosphorylase is a global regulator of virulence and persistency in *Salmonella enterica*, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 8784–8789.
[13] S. Yehudai-Resheff, S.L. Zimmer, Y. Komine, D.B. Stern, Integration of chloroplast nucleic acid metabolism into the phosphate deprivation response in *Chlamydomonas reinhardtii*, Plant Cell 19 (2007) 1023–1038.
[14] M. Leszczyniecka, D.C. Kang, D. Sarkar, Z.Z. Su, M. Holmes, K. Valerie, P.B. Fisher, Identification and cloning of human polynucleotide phosphorylase, hPNPase old-35, in the context of terminal differentiation and cellular senescence, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16636–16641.
[15] H.W. Chen, R.N. Rainey, C.E. Balatoni, D.W. Dawson, J.J. Troke, S. Wasiak, J.S. Hong, H.M. McBride, C.M. Koehler, M.A. Teitell, S.W. French, Mammalian polynucleotide phosphorylase is an intermembrane space RNase that maintains mitochondrial homeostasis, Mol. Cell. Biol. 26 (2006) 8475–8487.
[16] S. Slomovic, G. Schuster, Stable PNPase RNAi silencing; its effect on the processing and adenylation of human mitochondrial RNA, RNA (in press).
[17] V. Portnoy, G. Palnizky, S. Yehudai-Resheff, F. Glaser, G. Schuster, Analysis of the human polynucleotide-phosphorylase (PNPase) reveals differences in RNA binding and response to phosphate compared to its bacterial and chloroplast counterparts, RNA (in press).
[18] Z. Li, M.P. Deutscher, Maturation pathways for *E. coli* tRNA precursors: a random multienzyme process in vivo, Cell 86 (1996) 503–512.
[19] R. Ishii, O. Nureki, S. Yokoyama, Crystal structure of the tRNA processing enzyme RNase PH from *Aquifex aeolicus*, J. Biol. Chem. 278 (2003) 32397–32404.
[20] J.M. Choi, E.Y. Park, J.H. Kim, S.K. Chang, Y. Cho, Probing the functional importance of the hexameric ring structure of RNase PH, J. Biol. Chem. 279 (2004) 755–764.
[21] S. Vanacova, R. Stef, The exosome and RNA quality control in the nucleus, EMBO Rep. 8 (2007) 651–657.
[22] M. Leszczyniecka, R. DeSalle, D.C. Kang, P.B. Fisher, The origin of polynucleotide phosphorylase domains, Mol. Phylogenet. Evol. 31 (2004) 123–130.
[23] R. Raijmakers, W.V. Egberts, W.J. van Venrooij, G.J. Pruijn, 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. 323 (2002) 653–663.
[24] M.F. Symmons, G.H. Jones, B.F. Luisi, A duplicated fold is the structural basis for polynucleotide phosphorylase catalytic activity, processivity, and regulation, Structure 8 (2000) 1215–1226.
[25] A.J. Callaghan, J.G. Grossmann, Y.U. Redko, L.L. Ilag, M.C. Moncrieffe, M.F. Symmons, C.V. Robinson, K.J. McDowall, B.F. Luisi, Quaternary structure and catalytic activity of the *Escherichia coli* ribonuclease E amino-terminal catalytic domain, Biochemistry 42 (2003) 13848–13855.
[26] Y. Zuo, M.P. Deutscher, Exoribonuclease superfamilies: structural analysis and phylogenetic distribution, Nucleic Acids Res. 29 (2001) 1017–1026.
[27] S. Yehudai-Resheff, V. Portnoy, S. Yogev, N. Adir, G. Schuster, Domain analysis of the chloroplast polynucleotide phosphorylase reveals discrete functions in RNA degradation, polyadenylation, and sequence homology with exosome proteins, Plant Cell 15 (2003) 2003–2019.
[28] M.F. Symmons, M.G. Williams, B.F. Luisi, G.H. Jones, A.J. Carpousis, Running rings around RNA: a superfamily of phosphate-dependent RNases, Trends Biochem. Sci. 27 (2002) 11–18.
[29] E. Lorentzen, P. Walter, S. Fribourg, E. Evguenieva-Hackenberg, G. Klug, E. Conti, The archaeal exosome core is a hexameric ring structure with three catalytic subunits, Nat. Struct. Mol. Biol. 12 (2005) 575–581.
[30] E. Lorentzen, E. Conti, Structural basis of 3′ end RNA recognition and exoribonucleolytic cleavage by an exosome RNase PH core, Mol. Cell 20 (2005) 473–481.
[31] E. Lorentzen, E. Conti, The exosome and the proteasome: nano-compartments for degradation, Cell 125 (2006) 651–654.
[32] V. Portnoy, E. Evguenieva-Hackenberg, F. Klein, P. Walter, E. Lorentzen, G. Klug, G. Schuster, RNA polyadenylation in Archaea: not observed in *Haloferax* while the exosome polyadenylates RNA in *Sulfolobus*, EMBO Rep. 6 (2005) 1188–1193.
[33] K. Buttner, K. Wenig, K.P. Hopfner, Structural framework for the mechanism of archaeal exosomes in RNA processing, Mol. Cell 20 (2005) 461–471.
[34] K. Buttner, K. Wenig, K.P. Hopfner, The exosome: a macromolecular cage for controlled RNA degradation, Mol. Microbiol. 61 (2006) 1372–1379.
[35] P. Mitchel, E. Petfalski, A. Shevchenko, M. Mann, D. Tollervey, The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′ to 5′ exoribonucleases, Cell 91 (1997) 457–466.
[36] A. van Hoof, R. Parker, The exosome: a proteasome for RNA? Cell 99 (1999) 347–350.
[37] R. Raijmakers, G. Schilders, G.J. Pruijn, The exosome, a molecular machine for controlled RNA degradation in both nucleus and cytoplasm, Eur. J. Cell Biol. 83 (2004) 175–183.
[38] J. Houseley, J. LaCava, D. Tollervey, RNA-quality control by the exosome, Nat. Rev. Mol., Cell Biol. 7 (2006) 529–539.
[39] J.A. Chekanova, J.A. Dutko, I.S. Mian, D.A. Belostotsky, *Arabidopsis thaliana* exosome subunit AtRrp4p is a hydrolytic 3′→5′ exonuclease containing S1 and KH RNA-binding domains, Nucleic Acids Res. 30 (2002) 695–700.
[40] A.M. Estevez, T. Kempf, C. Clayton, The exosome of *Trypanosoma brucei*, Embo J. 20 (2001) 3831–3839.
[41] Q. Liu, J.C. Greimann, C.D. Lima, Reconstitution, activities, and structure of the eukaryotic RNA exosome, Cell 127 (2006) 1223–1237.
[42] A. Dziembowski, E. Lorentzen, E. Conti, B. Seraphin, A single subunit, Dis3, is essentially responsible for yeast exosome core activity, Nat. Struct. Mol. Biol. 14 (2007) 15–22.
[43] S. Lin-Chao, N.T. Chiou, G. Schuster, The PNPase, exosome and RNA helicases as the building components of evolutionarily-conserved RNA degradation machines, J. Biomed. Sci. 14 (2007) 523–532.
[44] B.K. Mohanty, V.F. Maples, S.R. Kushner, The Sm-like protein Hfq regulates polyadenylation dependent mRNA decay in *Escherichia coli*, Mol. Microbiol. 54 (2004) 905–920.
[45] M. Folichon, F. Allemand, P. Regnier, E. Hajnsdorf, Stimulation of poly (A) synthesis by *Escherichia coli* poly(A)polymerase I is correlated with Hfq binding to poly(A) tails, FEBS J. 272 (2005) 454–463.
[46] Z.F. Cheng, M.P. Deutscher, An important role for RNase R in mRNA decay, Mol. Cell 17 (2005) 313–318.
[47] I. Lisitsky, P. Klaff, G. Schuster, Addition of poly(A)-rich sequences to endonucleolytic cleavage sites in the degradation of spinach chloroplast mRNA, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 13398–13403.
[48] J. Campos-Guillen, P. Bralley, G.H. Jones, D.H. Bechhofer, G. Olmedo-Alvarez, Addition of poly(A) and heteropolymeric 3′ ends in *Bacillus subtilis* wild-type and polynucleotide phosphorylase-deficient strains, J. Bacteriol. 187 (2005) 4698–4706.
[49] V. Portnoy, G. Schuster, RNA polyadenylation and degradation in different archaea; roles of the exosome and RNase R, Nucleic Acids Res. 34 (2006) 5923–5931.
[50] A. Dziembowski, J. Piwowarski, R. Hoser, M. Minczuk, A. Dmochowska, M. Siep, H. van der Spek, L. Grivell, P.P. Stepien, The yeast mitochondrial degradosome. Its composition, interplay between RNA helicase and

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004

RNase activities and the role in mitochondrial RNA metabolism, J. Biol. Chem. 278 (2003) 1603–1611.
[51] D. Gagliardi, P.P. Stepien, R.J. Temperley, R.N. Lightowlers, Z.M. Chrzanowska-Lightowlers, Messenger RNA stability in mitochondria: different means to an end, Trends Genet. 20 (2004) 260–267.
[52] B. Schafer, M. Hansen, B.F. Lang, Transcription and RNA-processing in fission yeast mitochondria, RNA 11 (2005) 785–795.
[53] C.Y. Kao, L.K. Read, Opposing effects of polyadenylation on the stability of edited and unedited mitochondrial RNAs in *Trypanosoma brucei*, Mol. Cell. Biol. 25 (2005) 1634–1644.
[54] S. Slomovic, D. Laufer, D. Geiger, G. Schuster, Polyadenylation of ribosomal RNA in human cells, Nucleic Acids Res. 34 (2006) 2966–2975.
[55] S. West, N. Gromak, C.J. Norbury, N.J. Proudfoot, Adenylation and exosome-mediated degradation of cotranscriptionally cleaved pre-messenger RNA in human cells, Mol. Cell 21 (2006) 437–443.
[56] R.N. Rainey, J.D. Glavin, H.W. Chen, S.W. French, M.A. Teitell, C.M. Koehler, A new function in translocation for the mitochondrial i-AAA protease Yme1: import of polynucleotide phosphorylase into the intermembrane space, Mol. Cell. Biol. 26 (2006) 8488–8497.
[57] J.E. Kwak, M. Wickens, A family of poly(U) polymerases, RNA 13 (2007) 860–867.
[58] O.S. Rissland, A. Mikulasova, C.J. Norbury, Efficient RNA polyuridylation by noncanonical poly(A) polymerases, Mol. Cell. Biol. 27 (2007) 3612–3624.
[59] A.L. Stevenson, C.J. Norbury, The Cid1 family of non-canonical poly(A) polymerases, Yeast 23 (2006) 991–1000.
[60] B. Shen, H.M. Goodman, Uridine addition after microRNA-directed cleavage, Science 306 (2004) 997.
[61] G. Martin, W. Keller, Sequence motifs that distinguish ATP(CTP): tRNA nucleotidyl transferases from eubacterial poly(A) polymerases, RNA 10 (2004) 899–906.
[62] H. Betat, C. Rammelt, G. Martin, M. Morl, Exchange of regions between bacterial poly(A) polymerase and the CCA-adding enzyme generates altered specificities, Mol. Cell 15 (2004) 389–398.
[63] M. Lynch, B. Koskella, S. Schaack, Mutation pressure and the evolution of organelle genomic architecture, Science 311 (2006) 1727–1730.
[64] D. Ojala, J. Montoya, G. Attardi, tRNA punctuation model of RNA processing in human mitochondria, Nature 290 (1981) 470–474.
[65] P. Bralley, G.H. Jones, cDNA cloning confirms the polyadenylation of RNA decay intermediates in *Streptomyces coelicolor*, Microbiology 148 (2002) 1421–1425.
[66] B.K. Mohanty, S.R. Kushner, Polynucleotide phosphorylase, RNase II and RNase E play different roles in the in vivo modulation of polyadenylation in *Escherichia coli*, Mol. Microbiol. 36 (2000) 982–994.
[67] R. Tomecki, A. Dmochowska, K. Gewartowski, A. Dziembowski, P.P. Stepien, Identification of a novel human nuclear-encoded mitochondrial poly(A) polymerase, Nucleic Acids Res. 32 (2004) 6001–6014.
[68] T. Nagaike, T. Suzuki, T. Katoh, T. Ueda, Human mitochondrial mRNAs are stabilized with polyadenylation regulated by mitochondria-specific poly(A) polymerase and polynucleotide phosphorylase, J. Biol. Chem. 280 (2005) 19721–31927.
[69] J. Houseley, D. Tollervey, Yeast Trf5p is a nuclear poly(A) polymerase, EMBO Rep. 7 (2006) 205–211.
[70] L. Haracska, R.E. Johnson, L. Prakash, S. Prakash, Trf4 and Trf5 proteins of *Saccharomyces cerevisiae* exhibit poly(A) RNA polymerase activity but no DNA polymerase activity, Mol. Cell. Biol. 25 (2005) 10183–10189.
[71] D.E. Egecioglu, A.K. Henras, G.F. Chanfreau, Contributions of Trf4p- and Trf5p-dependent polyadenylation to the processing and degradative functions of the yeast nuclear exosome, RNA 12 (2006) 26–32.
[72] L. Wang, C.R. Eckmann, L.C. Kadyk, M. Wickens, J. Kimble, A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans*, Nature 419 (2002) 312–316.
[73] J.E. Kwak, L. Wang, S. Ballantyne, J. Kimble, M. Wickens, Mammalian GLD-2 homologs are poly(A) polymerases, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4407–4412.
[74] M. Edmonds, A history of poly A sequences: from formation to factors to function, Prog. Nucleic Acid Res. Mol. Biol. 71 (2002) 285–389.

Please cite this article as: S. Slomovic, et al., Polynucleotide phosphorylase and the archaeal exosome as poly(A)-polymerases, Biochim. Biophys. Acta (2008), doi:10.1016/j.bbagrm.2007.12.004