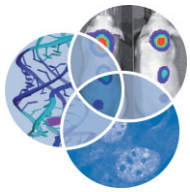
Advanced Review



Exonucleases and endonucleases involved in polyadenylation-assisted RNA decay

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RNA polyadenylation occurs in most forms of life, excluding a small number of biological systems. This posttranscriptional modification undertakes two roles, both of which influence the stability of the polyadenylated transcript. One is associated with the mature 3′ ends of nucleus-encoded mRNAs in eukaryotic cells and is important for nuclear exit, translatability, and longevity. The second form of RNA polyadenylation assumes an almost opposite role; it is termed 'transient' and serves to mediate the degradation of RNA. Poly(A)-assisted RNA decay pathways were once thought to occur only in prokaryotes/organelles but are now known to be a common phenomenon, present in bacteria, organelles, archaea, and the nucleus and cytoplasm of eukaryotic cells, regardless of the fact that in some of these systems, stable polyadenylation exists as well. This article will summarize the current knowledge of polyadenylation and degradation factors involved in poly(A)-assisted RNA decay in the domains of life, focusing mainly on that which occurs in prokaryotes and organelles. In addition, it will offer an evolutionary view of the development of RNA polyadenylation and degradation and the cellular machinery that is involved. *WIREs RNA* 2011 2 106–123 DOI: 10.1002/wrna.45

POLYADENYLATION AND DEGRADATION OF RNA

The Two Faces of RNA Polyadenylation

Polyadenylation of RNA is an almost ubiquitous post-transcriptional modification, absent in only a limited number of life forms. The poly(A) extensions produced in this process can be placed in two categories: transient and stable. In a snapshot taken several years back, the former type was known to occur in bacteria, archaea, and organelles, such as plant mitochondria and chloroplasts. In these systems, polyadenylation assists in the degradation of RNA and is, accordingly, termed 'transient'.[1–3] Transient poly(A) can also play a role in quality control, such as the degradation of defective tRNA.[4] The stable form of poly(A) is produced in the nucleus of eukaryotic cells wherein long poly(A) tails are synthesized at the mature 3′ ends of most nucleus-encoded mRNAs. These poly(A) extensions are important for proper translation initiation, mRNA longevity, and, at least in some cases, nuclear export.[5,6] In bacteria and most organelles, stable poly(A) tails are not present and often, in their place, 3′ stem-loop structures contribute to transcript stability. In fact, stable and transient polyadenylations were once thought to occur only separately but in recent years, systems in which both forms coexist, such as animal and trypanosome mitochondria, have been revealed.[7,8] Also, in yeast and human nuclei a mechanism that employs transient polyadenylation to mark certain RNA substrates for degradation by the exosome complex was discovered.[9–12] Recent studies have shown that almost the entire eukaryotic nuclear genome is transcribed, and unstable, cryptic, and antisense RNAs which are produced therein are transiently adenylated and degraded by the nuclear exosome as well (Figure 1).[1,3,9,11,13–18] The exact mode by which transient and stable poly(A) tails that coexist in the same cellular compartment are differentiated from one another is not fully understood in some cases. Today, it is clear that poly(A)-assisted RNA decay is a widespread process, extending across the biological world much farther than once thought.

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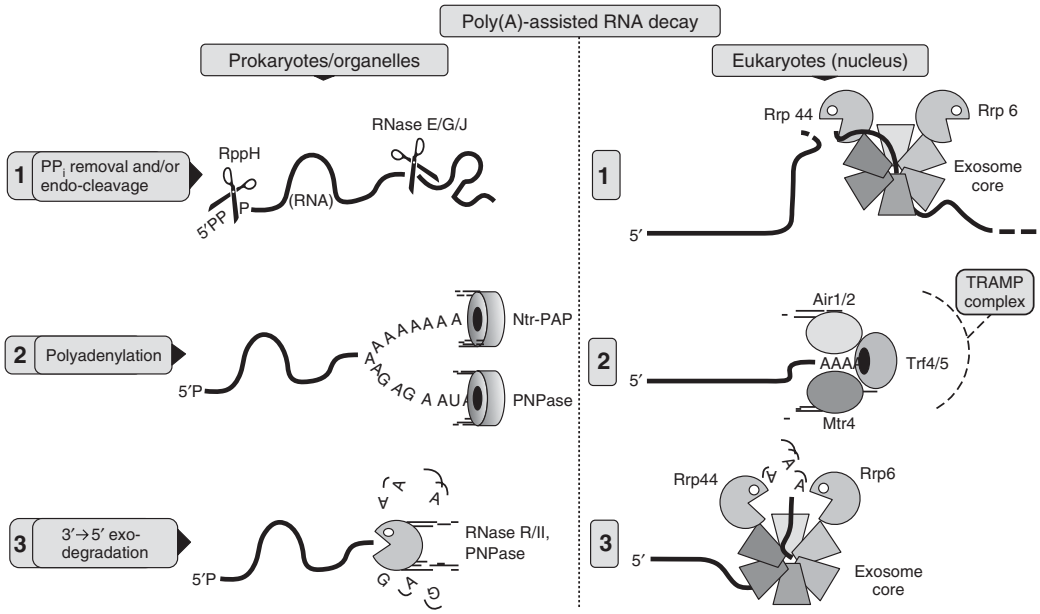

FIGURE 1 | Poly(A)-assisted RNA decay occurs in prokaryotes/organelles (left) and the nuclei and cytoplasm of eukaryotic cells (right). Generally, the process can be divided into three steps, as presented in the figure: (1) endonucleolytic cleavage (not always obligatory), (2) adenylation, and (3) 3′ → 5′ exonucleolytic digestion. In *Escherichia coli*, removal of PPi from the 5′ end by RppH usually precedes endo-cleavage by RNase E. RNase J has been implicated in a similar, RNase E-like function in *Bacillus subtilis*. In the nuclei of eukaryotic cells, endo-cleavage by the PIN domain of Rrp44 can prepare the substrate for the second step—adenylation by Trf4/5 of the TRAMP complex (Air1/2, Trf4/5, Mtr4). In *E. coli*, polyadenylation can be performed by Ntr-PAP, producing homopolymeric tails or by PNPase, producing heteropolymeric poly(A)-rich tails, which are assumed to fulfill a similar function. (In hyperthermophilic and several methanogenic archaea, heteropolymeric tails are believed to be synthesized by the archaeal exosome.) The 3′ → 5′ exonucleolytic degradation step is carried out by PNPase and RNase II/R in bacteria and organelles. The 3′ → 5′ stage that occurs in the nuclei of eukaryotic cells is fulfilled by the exosome (particularly, the two catalytic subunits associated with it, Rrp44 and Rrp6). Note that 5′ → 3′ degradation events are not included in this depiction.

POLYADENYLATION-ASSISTED RNA DEGRADATION

Poly(A)-assisted RNA degradation was first discovered in *E. coli* and its molecular mechanism has been extensively studied in this bacterium. Research in *E. coli* led to the disclosure of similar pathways that occur in other biological systems such as chloroplasts, plant and human mitochondria, certain archaea, and the nucleus and cytoplasm of yeast and human cells.[9,18,19] These pathways, however, may vary from one another in certain aspects, and in some cases the full mechanisms have yet to be revealed. The poly(A)-assisted RNA decay pathway that occurs in *E. coli* is often used (and is applied here) to present a general model that depicts this process, as it contains various features common to other systems. This 'classic' sequence commences with the endonucleolytic cleavage of the transcript, followed by poly(A) or poly(A)-rich 3′ extension, and ends with the exonucleolytic 3′ → 5′ degradation of the tagged RNA fragment[2,20] (Figure 1). Transient poly(A) tails found at the native 3′ ends of RNA molecules indicate that the initial endonucleolytic cleavage is not always required.[1,2]

In relation to nucleotide composition, transient poly(A) tails include two types: homopolymeric (composed of adenosine residues alone) or heteropolymeric/poly(A)-rich (mostly adenosines but other nucleotides are incorporated as well).[19] Although similar functionality is usually assumed for both types of tails, this remains to be fully demonstrated in some recently discovered pathways. In *E. coli*, the endonuclease, RNase E, is believed to carry out the initial transcript cleavage, and the polyadenylation activity is mainly provided by a nucleotidyl transferase-type poly(A)-polymerase (Ntr-PAP), which produces homopolymeric poly(A) tails. Polynucleotide phosphorylase (PNPase) is jointly responsible for this activity, but to a lesser extent, and produces heteropolymeric extensions.[21] The function of exonucleolytic degradation is shared by PNPase, RNase II, and RNase R.[2,20,22] Owing to structural limitations, neither PNPase nor RNase II/R can fully degrade RNA substrates and thus, leave behind them a wake of short oligonucleotides. These fragments (and those produced by mechanisms other than poly(A)-assisted RNA decay) are degraded by an oligoribonuclease which, in *E. coli*, is encoded by the essential *orn*

gene.[4] *Orn* has close homologs in many organisms, including higher eukaryotes.

Pathways similar to those in *E. coli* were found in other organisms, and this usually began with the detection of nonabundant, truncated, adenylated RNA fragments. Not only do such adenylated degradation intermediates serve as a telltale sign of the presence of poly(A)-assisted RNA decay but the nucleotide composition of the tail offers an initial hint toward the identity of the polyadenylating enzyme in the studied organism, as well.[19] This article will describe several major ribonucleases, particularly those that take part in poly(A)-assisted RNA decay processes (albeit, not necessarily exclusive to such pathways), concentrating mainly on bacteria, archaea, and organelles. The enzymes involved in similar processes that occur in the nucleus (and cytoplasm) of eukaryotic cells have been extensively described in recent reviews and will be addressed here only briefly. The article will commence with a description of the main enzymes, followed by that of poly(A)-assisted RNA degradation pathways that occur in different prokaryotes and organelles and will end in a scenario that is offered to describe the evolution of polyadenylation.

EXORIBONUCLEASES

Ribonuclease R and Ribonuclease II (RNase R/II): 3′ → 5′ Hydrolytic Degradation of RNA

The exoribonucleases, RNase R and RNase II, belong to an extensive enzyme family found in bacteria, the majority of archaea, and eukaryotes. These enzymes play major roles in RNA quality control, maturation, and turnover. RNase R/II processively digests single-stranded (ss) RNA from the 3′ end, using a hydrolytic mechanism that releases nucleotide monophosphates. Members of the RNase II family are composed of several domains including two cold-shock domains at the *N*-terminal end, followed by a catalytic domain and an S1 RNA-binding domain. In *E. coli*, both RNase II and RNase R are Present; the latter, acting preferentially in the stationary phase of bacterial growth as well as in stress conditions. Hydrolytic activity by these enzymes reaches 90% of RNA degradation in logarithmic growing cells.[23] *In vitro* assays have shown that RNase R has the innate ability to unwind secondary structures of RNA substrates. RNase II is less efficient in this aspect and is strongly impeded by folded RNA.[22,24–27] The *E. coli* RNase II has been shown to stall a few nucleotides downstream of secondary structures; remaining bound to the substrate and thereby actually protecting some RNAs from further degradation.[28] Crystallographic analysis of the RNase II structure revealed that the RNA substrate is threaded through a central channel and directed to the catalytic site located at the bottom, where one Mg^{++} ion is present, coordinated by aspartates.[29] Mutagenetic studies suggest the involvement of two metal ions in the activation of a water molecule for hydrolysis of the terminal phosphodiester bond, as the inactivating mutation lies at a putative metal-coordinating residue, $D_{209}N$.[29,30] In *E. coli*, this mutation resulted in inactivation but did not affect RNA binding. Also, the corresponding mutation in RNase R and yeast Rrp44 caused inactivation.[30] Crystallographic studies also revealed that the active site of RNase II bears substantial organizational resemblance to that of the endoribonuclease, RNase H, which could mean that the two proteins share similarities in catalytic chemistry as well.

RNase R/II homologs are known to be present in most bacteria (including mycoplasmas) and plant and algae chloroplasts, as well as in plant and yeast mitochondria (Table 1). However, no homologs are known to localize to human mitochondria and none have been found to be encoded in the genomes of certain evaluated archaea (see below). In the nucleus and cytoplasm of eukaryote cells, RNase R/II homologs are included as subunits (Rrp44) in the exosome complex (a major, multi-subunit, 3′ → 5′ RNA processing and degradation machine, described below).

RNase PH: A Bacterial Phosphorylase

RNase PH (EC 2.7.7.56) is a bacterial exoribonuclease with Pi-dependent 3′ → 5′ activity. Its homologs are single-domain proteins, distributed among the three primary life kingdoms. In bacteria, six RNase PH polypeptides form a homohexameric ring.[31] The archaeal and eukaryotic exosome complexes form a similar structure with their RNase PH-like subunits[31,32] as does the homotrimeric PNPase complex (Figure 3). This will be discussed in depth below. In *E. coli*, The most characterized function associated with this enzyme is the trimming of tRNA precursors at their 3′ ends.[33]

Polynucleotide Phosphorylase (PNPase): Phosphorolytic Degradation and Polyadenylation of RNA in Bacteria and Organelles

PNPase (EC 2.7.7.8) was first discovered by Grunberg-Manago and Ochoa, more than 50 years ago,[34] and characterized later by Littauer and Kornberg while investigating the mechanism of ribonucleotide incorporation into nucleic acids, in *E. coli*.[35] This enzyme

TABLE 1 | Exos, endos, and polyadenylation in bacteria, archaea, and organelles

		RNase R/II	PNPase	RNase E/G	RNase J	archaea exosome	Poly(A) (composition)	Poly(A) (type)
Bacteria	*E.coli*	+	+	+	−		homo/hetero	unstable
	Bacillus	+	+	−	+		homo/hetero	unstable
	Synechocystis	+	+	+	+		hetero	unstable
	Mycoplasma	+	−	−	+		—	—
Archaea	Halophytes	+	−	−	+	−	—	—
	Hyperthermophiles	−	−	−	+	+	hetero	unstable
	Mathenogens	+/−	−	−	+	+/−	hetero/(–)	unstable/(−)
Mitochondria	Higher plants	+	+	−	−?		homo	unstable
	Yeast	+	−	−	−?		—	—
	Trypanosomes	+	−	−	−?		homo	stable/unstable
	Animals	−	+(IMS)	−	−?		homo	stable/unstable
Chloroplasts	Higher plants	+	+	+	+		homo/hetero	unstable
	Chlamydomonas	+	+	−	+		homo/hetero	unstable

The presence of the major exo- and endoribonucleases in bacteria, archaea, and organelles is summarized. The presence of polyadenylation in each system and its nucleotide composition (homo- or heteropolymeric) and nature (stable or unstable) are included as well.

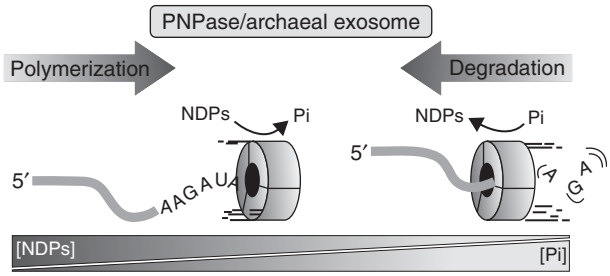

FIGURE 2 | The 'zipper' model of phosphorolytic polymerization and degradation. PNPase and the archaeal exosome are bidirectional; they can synthesize heteropolymeric tails and phosphorolytically degrade RNA from 3′ → 5′, as well. These two activities are modulated by Pi and NDP concentrations, which are influenced by one another and by the enzyme's activity: ↑Pi/↓NDP = degradation. ↑NDP/↓Pi = polymerization. (Reprinted with permission from Ref 19. Copyright 2008 Elsevier.)

was the first to be identified that could catalyze the formation of polynucleotides from ribonucleotides. As a phosphorylase, a mixture of ribonucleotide diphosphates (NDPs) serves the polymerization reaction of this enzyme which results in a random copolymer.

An interesting aspect of PNPase is its bidirectional functionality: in addition to 5′ → 3′ polymerization, PNPase also catalyzes processive 3′ → 5′ phosphorolysis (Figure 2).[35–37] In *E. coli*, PNPase is mostly active in the latter function—degradation and 3′ end processing of RNA substrates—although it contributes a substantial degree of polymerization activity, yielding heteropolymeric tails.[21,38] There, part of the PNPase population is associated with the degradosome—a high molecular weight RNA degradation machine that includes the endoribonuclease, RNase E, an RNA helicase, enolase, and possibly other proteins (Figure 4).[39–44] In addition, the *E. coli* PNPase was found to be associated with RhlB RNA helicase.[45] In spinach chloroplasts, cyanobacteria, and gram-positive bacteria, PNPase is suggested to be the major polyadenylating enzyme.[3,46,47] In an overlapping pathway screen to reveal genes that display coordinated expression as a consequence of terminal differentiation and senescence of melanoma cells, the human PNPase was identified.[48–50] In human cells, PNPase is directed to the mitochondria by an N-terminus transit peptide, which is removed after translocation. Therefore, it may be termed 'hmtPNPase'. The nuclear genome of plants such as *Arabidopsis* encode two PNPases; one is directed to mitochondria and the other to chloroplasts.[51] Unlike in plants, wherein the PNPase enzymes are located in the matrixes of their corresponding organelles and involved in mtRNA and cpRNA metabolism, hmtPNPase is mostly, if not entirely, located in the mitochondrial intermembrane space (IMS).[52–54] Consequentially, it may not play a central or direct role in the processing and degradation of mtRNA to the degree that it does in those systems. Nevertheless, like the bacterial and plant organellar enzymes, biochemical assays have shown that the hmtPNPase is phosphorolytically active and down-expression via RNAi resulted in mitochondrial dysfunction and mtRNA processing and adenylation defects.[52,55,56] Therefore, the role that PNPase plays in human cells and the manner by which it influences mitochondrial integrity remain to be fully deciphered.

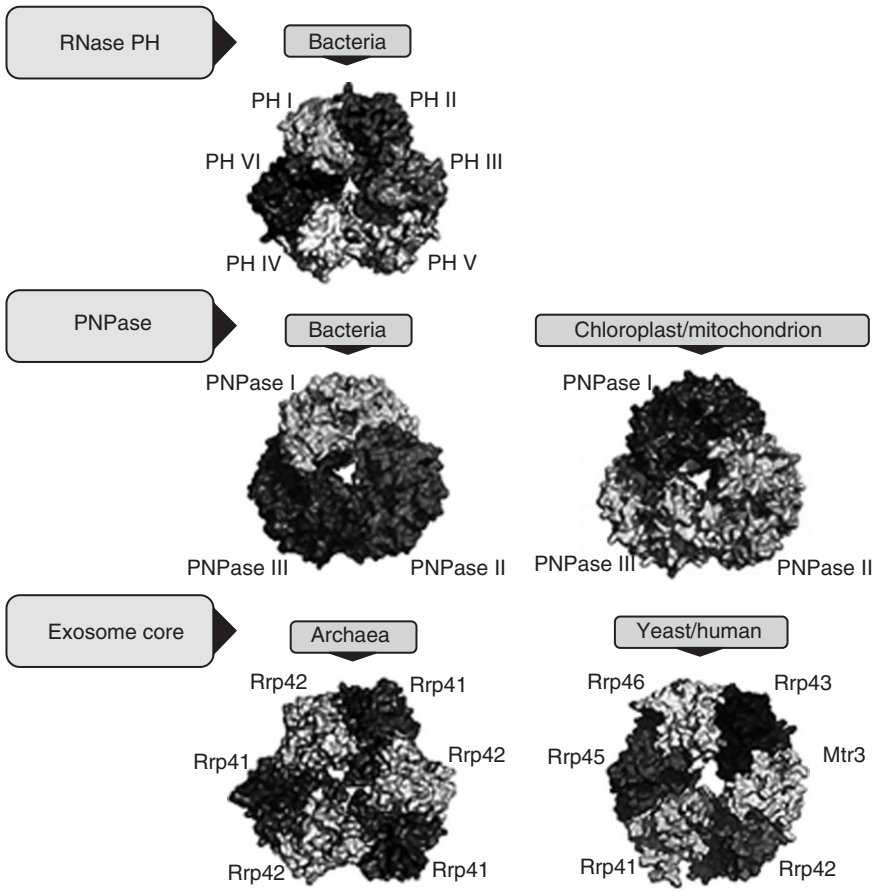

FIGURE 3 | RNase PH, the bacterial and organellar PNPases, and the archaeal and eukaryotic exosome cores share a similar ring-like structure. Phylogenetic analysis and structural comparison lead to an evolutionary model wherein a single-domain phosphorolytic primordial enzyme developed into these enzymes and complex subunits. Ultimately, a common ring-like structure, employing a central channel to lead substrate RNA to the catalytic site within, evolved. The yeast and human exosome cores apparently lost their phosphorolytic capabilities and rely on association with hydrolytically active enzymes, Rrp44 and Rrp6 (not shown), in order to digest RNA. A cap structure (not shown) provides RNA binding and selective properties. (Reprinted with permission from Ref 19. Copyright 2008 Elsevier.)

The amino acid sequences of bacterial PNPases and those encoded in the nuclear genomes of plants and animals display a high level of homology.[40,57–61] The protein is composed of five domains, of which two are related to RNase PH, likewise termed the 'phosphorolytic' or 'RNase PH-like (PH)' core domains. Between them lies an α-helix domain. In the bacterium *Streptomyces antibioticus* the first core domain is engaged in the synthesis of the 'magic spot' nucleotide ppGppp[59], and mutational analysis of the *E. coli* PNPase disclosed that the second core domain contains a phosphorolytically active site.[62] The other two domains are homologous to the KH and S1 domains that characterize RNA-binding proteins. The latter were found to be responsible for the high poly(A)-binding affinity of the enzyme.[61] The three-dimensional structure of the *S. antibioticus* PNPase was determined via X-ray crystallography and was found to be a homotrimeric complex.[40,59,63] Its PH domains form a hexamer that surrounds a central channel that can accommodate a single-stranded RNA molecule. This structure resembles the hexamer that RNase PH forms, as mentioned above.

The Eukaryotic and Archaeal Exosomes

Additional complexes that share structural similarities with the RNase PH hexamer and the PNPase homotrimer and are involved (not exclusively, though) in poly(A)-assisted RNA decay are the eukaryotic and archaeal exosomes (Figure 3). The eukaryotic exosome multi-enzyme complex, comprised of 10–11 different protein subunits, is centrally involved in RNA degradation and processing in both the cytoplasm and nucleus of eukaryotic cells.[64–70] Its core is composed of three heterodimers of six different proteins: Rrp41-Rrp45, Rrp46-Rrp43, and Mtr3-Rrp42.[71–73] A cap structure, composed of Rrp4,

Csl4 and Rrp40, provides KH or S1 RNA binding capabilities.[73] The core of the human and yeast exosomes lacks exonucleolytic activity. This activity is provided by two associated proteins, Rrp6 (PM/Scl-100) and Rrp44 (Dis3).[73,74]

No PNPase exists in the archaeal domain. However, several hyperthermophilic and methanogenic members contain an element termed the 'archaeal exosome'. As mentioned earlier, the core of this complex also forms a hexameric ring, via three interchanging copies of two RNase PH homologs, Rrp41 and Rrp42. A cap, that contributes the KH/S1 domains and, along with the core, completes the full nine-subunit structure, is composed of three copies of Csl4, Rrp4, or a mixture of both. The archaeal exosome is believed to be responsible for both the synthesis of transient poly(A)-rich tails and the degradation of RNA in these organisms (Figure 2).[68,75–79] This conclusion is based on evidence showing that the purified complex presents bidirectional, PNPase-like phosphorolytic activity.[78] This is further strengthened by the fact that heteropolymeric tails are present only in archaea that contain the archaeal exosome, and species that lack this complex lack all forms of polyadenylation.[78,80] Interestingly, in the genomes of exosome-positive archaea, the genes that encode the complex subunits are organized in a single operon.[81]

A Common Origin of the Ring-Shaped Structure of RNase PH-Like Complexes

Despite varying levels of complexity, alignment of the RNase PH, PNPase, and exosome structures (eukaryotic and archaeal) clearly points to striking structural similarities constituted by a ring-shaped body formed by PH-like polypeptides (Figure 3).[73] The fact that the PNPase and RNase PH complexes are both present in bacteria would seems to suggest that one had derived from the other. However, phylogenetic analysis shows a closer relation between the second core domain of PNPase and RNase PH than the PNPase cores between themselves.[61] This would mean that RNase PH and PNPase most likely evolved from a common single-domain ancestor.[57,61] The same enzyme probably provided the base for the archaeal and eukaryotic exosome subunits. Interestingly, though, similar structure and functionality are achieved in bacteria and organelles by the repetitions of a single protein with five domains (PNPase), whereas in archaea the same is achieved by the repetitions of three to four different proteins, each with a single domain.

Surprisingly, unlike RNase PH, PNPase, and the archaeal exosome, the human and yeast exosomes are devoid of phosphorolytic activity.[73,74] Likewise, the amino acids required for this capability, located in the Rrp41 subunit, are not conserved. Contrarily, the Rrp41 subunit of the Arabidopsis exosome was reported to have phosphorolytic degradation and polymerization activity and accordingly, the amino acid residues required for such phosphorolytic activity are preserved in its sequence.[82] This demonstrates that there is no single exosome model that can fully represent exosome function or activity in all eukaryotes. Although the yeast and human exosomes apparently lost their phosphorolytic capacity, in its place, Rrp44 (Dis3), a $3' \rightarrow 5'$ hydrolytic exosome subunit belonging to the RNase R/II family, and Rrp6 (PM/Scl-100), a second $3' \rightarrow 5'$ hydrolytic (RNase D homolog) subunit, provide exonucleolytic degradation activity. The Rrp44 subunit contains an additional region, the PIN domain, which enables endonucleolytic activity as well.[83–85] Therefore, the human and yeast exosomes are active both as hydrolytic exonucleases and endonuclrase; functionally resembling the *E. coli* degradosome to some extent (which includes exonucleolytic (PNPase) and endonucleolytic (RNase E) activity). Recent crystallographic work helped to understand why the human/yeast exosomes retained the ring-like subunit arrangement despite their lack of phosphorolytic activity: the circular shape allows RNA to be threaded through the central channel toward the active site within Rrp44, where the RNA is digested.[86]

Together, these observations imply that the bacterial/organellar PNPases and the archaeal and eukaryotic exosomes constitute a ring-shaped machine of common origin, which has evolved to phosphorolytically degrade and polymerize RNA in prokaryotes and organelles, or hydrolytically degrade RNA in eukaryotes. Similar features and structure and widespread presence suggest that the ancestral base of this machine was most likely present prior to the separation of bacteria, archaea, and eukaryotes.

Rrp 6 (PM/Scl-100): a Hydrolytic $3' \rightarrow 5'$ Exoribonuclease Associated with the Eukaryotic Exosome

In yeast cells, besides Rrp44, an additional hydrolytic exonuclease, Rrp6, associates with the exosome and contributes $3' \rightarrow 5'$ RNA degradation and processing abilities (e.g. processing of the 5.8S rRNA precursor).[87] In yeast, this RNase D homolog (a bacterial $3' \rightarrow 5'$ exonuclease) associates solely with the nuclear exosome and likewise, is not detected in the cytoplasm.[18,88] Human cells, PM/Scl-100 (the human Rrp6 orthologue) was found to associate

with both the nuclear and cytoplasmic exosome complexes, although its role in the cytoplasm is not fully clear.[89] Rrp6 fulfills a crucial role in the joint activity of the nuclear exosome and TRAMP complex (described below) during the poly(A)-assisted degradation of noncoding, antisense, and aberrant mRNAs (Figure 1). In yeast, a long extension at the N-terminus of Rrp6 enables interaction with Rrp47, an RNA-binding protein.[88,90,91] Exosome interaction with Rrp47 and a second co-factor, MPP6, is essential for recognition of certain RNA elements.[92,93]

As stated, although PM/Scl-100 is known to exist in the cytoplasm of human cells, its role in this compartment is still not entirely clear. In a series of RNAi-mediated knockdown experiments, PM/Scl-100 was reported to be nonessential for the stability of the remaining exosome subunits.[94] Nevertheless, it was shown to be required to maintain normal levels of three different tested mRNA reporters, including a wt β-globin RNA, one with an AU-rich (ARE) instability element, and one with a premature termination codon (PTC).[94] These results suggest that PM/Scl-100 is involved in cytoplasmic mRNA degradation but it remains unclear whether this activity is always entwined with that of the full exosome complex or if it can be exosome independent.

ENDORIBONUCLEASES

Ribonuclease E/G: An Extensively Studied Endoribonuclease Involved in RNA Degradation in *E. coli*

More than three decades ago, RNase E was identified in bacteria and much later, its presence in chloroplasts was also confirmed.[43,47,95,96] In *E. coli*, in addition to its involvement in RNA decay, RNase E mediates the processing of certain rRNAs and tRNAs.[97–99] RNase G is a second endoribonuclease found in *E. coli* and other related bacteria. Although it is homologous to RNase E, it lacks the *C*-terminal domain, a region of the *E. coli* RNase E that serves as a scaffold for interactions with the other proteins that comprise the degradosome complex (Figure 4).[43] Furthermore, unlike RNase E, RNase G is not essential in *E. coli*.[96] Proteins that are highly homologous to RNase E are encoded in the nuclear genomes of many higher plant species but possess a long *N*-terminal extension that includes a chloroplast transit peptide and, like RNase G, they lack the *C*-terminal domain (degradosome scaffold) found in the *E. coli* RNase E (Figure 4). Actually, in plant chloroplasts, *Synechocystis*, and the large majority of other bacteria, the type of degradosome complex found in *E. coli* is not present. In *B. subtilis* and the green alga, *Chlamydomonas reinhardtii*, there are no RNase E/G homologs at all.[100–102]

In *E. coli* and *Arabidopsis*, with some exceptions,[103] RNase E displays significantly higher activity on substrates bearing a monophosphorylated versus triphosphorylated 5′ end, the latter of which is characteristic of primary rather than processed transcripts. A recent study identified an *E. coli* RNA pyrophosphohydrolase, RppH, as responsible for removing the pyrophosphate from the 5′ ends of triphosphorylated RNA and thereby, providing an ideal substrate for RNase E endonucleolytic cleavage (Figure 1).[104] Crystallographic studies of the catalytic domain, located at the *N*-terminal half of RNase E, disclosed a 260-kDa homo-tetrameric structure. This tetramer is organized as a dimer-of-dimers with the arrangement of the domains within each dimer resulting in a shape that resembles the handles and blades of an open pair of scissors. An intradomain zink ion-link coordination site exists at the scissor junction, mediated by a pair of cysteine residues in the conserved CPxCxGxG motif.[105] Solving the crystal structure of RNase E provided explanations as to the discriminative and high activity directed toward RNA substrates harboring a monophosphorylated nucleotide at their 5′ end. A 5′ end phosphate-'sensing' domain undergoes conformational change upon binding a 5′ monophosphate RNA molecule, which enables the catalytically active site to cleave the substrate. This model has been termed, the 'mouse and trap' mechanism.[44,63,105]

Ribonuclease Y: A Recently Discovered Endoribonuclease in *B. Subtilis*

RNase Y is a recently identified endoribonuclease that seems to be important for the initial step of RNA degradation in *B. subtilis* and many other related bacteria. The enzyme was characterized in the cleavage reaction of *S*-adenosylmethionine-dependent riboswitches in *B. subtilis*, and bulk RNA life time was more than doubled upon its depletion.[106] Its role in poly(A)-assisted RNA decay has yet to be established.

ENDO/EXO-RIBONCLEASES

Ribonuclease J: An Endo- and Exonuclease All in One

RNase J1 (and the related J2) was first described in *B. subtilis*, in 2005.[107] As mentioned earlier, in this bacterium RNase E is absent; however, various lines of evidence suggest that RNase J is a functional homolog of RNase E in bacteria and organelles in which the latter is not present and therefore,

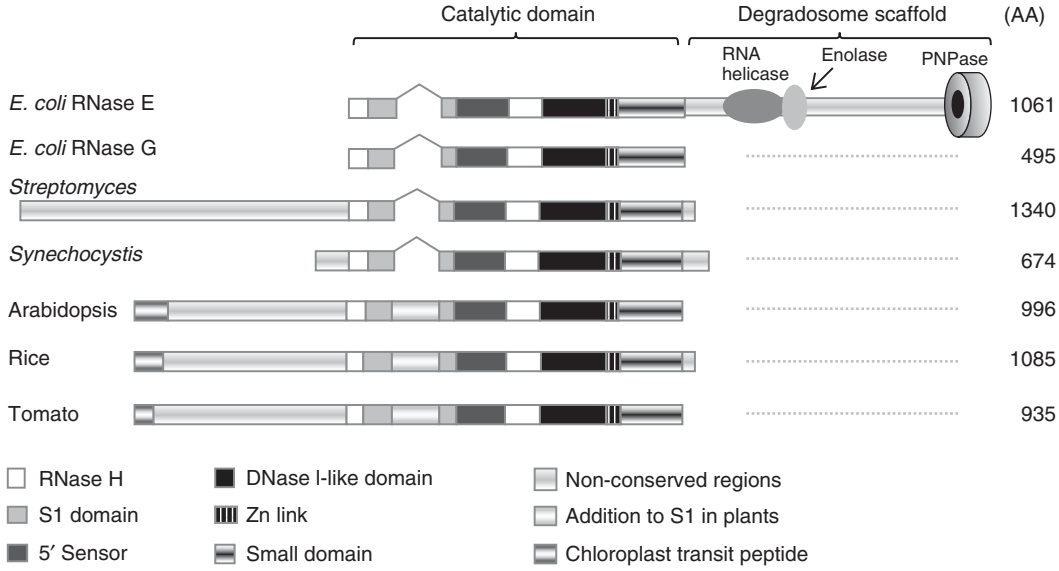

FIGURE 4 | RNase E/G proteins. RNase E, first discovered in *E. coli*, has homologs in many other biological systems, displayed here with different grayscale patterns representing the domain types. As evident, amino acid alignment reveals a high level of homology shared between the RNase E/G-like enzymes. The *E. coli* RNase E contains a *C*-terminal domain which is not present in RNase G or the other RNase E homologs. This domain is the scaffold for the degradosome complex, which includes an RNA helicase, enolase, and part of the PNPase population. In plants, RNase E bears an *N*-terminal chloroplast target peptide and a stretch of ~120 amino acids within the S1 domain, which is not present in any bacterial sequence.

could provide the cleavage activity that occurs at the initial stage of poly(A)-assisted RNA decay (Figure 1). Some studies of RNase J, all carried out in bacteria, focused on its role in rRNA maturation,[108] while others investigated its involvement in mRNA and noncoding RNA metabolism.[109–111] RNase J contains a metallo-β-lactamase domain akin to other RNA metabolism factors, is active as a dimer, and is likely zinc dependent. Another member of the metallo-β-lactamase domain family, for example, is the cleavage and polyadenylation specific factor (CPSF). This endoribonuclease cleaves precursor mRNAs encoded in the nuclear genome, prior to the polymerization of the long, stable poly(A) tail.[112]

Remarkably, RNase J wields both $5' \rightarrow 3'$ exonucleolytic and endonucleolytic activities, which have been rationalized on the basis of its crystal structure.[111,113] As of now, it is the only known prokaryotic/organellar protein capable of exoribonucleolytic $5' \rightarrow 3'$ activity. One perspective is that RNase J's endonucleolytic activity provides substrates for its exonucleolytic activity; bypassing $5'$ end substrate triphosphorylation, which might impede exonucleolytic degradation as in the case of RNase E. This is supported by studies of the RNase J of *B. subtilis* and the thermophilic bacteria, *Thermus thermophilus*, that show that the exonucleolytic activity is stimulated upon dephosphorylation of $5'$ end triphosphates into a monophosphate. Therefore, three terminal phosphates would promote endonucleolytic activity while a single phosphate or OH group would lead to exonucleolytic digestion.[113] In methanogenic archaea, of those that lack the archaeal exosome, RNase J is the only enzyme known to degrade RNA. Especially in such a case, both the exo- and endonucleolytic activities would be required in order to efficiently degrade RNA molecules.[80]

RNase J, but not RNase E, was identified in the genome of the green algae, *C. reinhardtii*, suggesting that only the former is present in its chloroplast (Table 1). However, the presence of RNase J is not restricted to organisms or organelles that lack RNase E, as a proteomic study showed that higher plant chloroplasts possess both RNase E and J (Table1). Likewise, in this study, RNase J was found to contain an *N*-terminal chloroplast transit peptide. Another specific example of coexisting genes that encode RNase E and J is the cyanobacteria, *Synechocystis*.

Rrp 44 Provides Both Endo- and Exonucleolytic Activity to the Eukaryotic Exosome

Rrp44 (or Dis3) is a hydrolytic $3' \rightarrow 5'$ exoribonuclease associated with the yeast exosome in both the nucleus and cytoplasm and is the only catalytic exosome subunit in the cytoplasm of yeast cells. An RNase R/II domain is that which provides the hydrolytic $3' \rightarrow 5'$ exonucleolytic activity. In addition, Rrp44 contains an *N*-terminal PIN domain which enables

endonucleolytic activity as well (Figure 1).[83–85] As mentioned earlier, structural analysis of the yeast exosome disclosed a mechanism by which the ring structure of the catalytically inactive core channels the substrate RNA to the catalytic site, found within Rrp44.[86] Substrate selection and categorization by the core and the cap is most likely an essential stage that determines whether the RNA will be degraded or processed and to what extent. There is a known human Rrp44 homolog, hDis3; however, it does not closely associate with the human exosome. Recently, an additional Rrp44 homolog was identified in human cells and termed, hDis3L1 (human Dis3-like 1). hDis3L1 is most likely the true functional ortholog of Rrp44 in human cells as, in immunoprecipitation assays, it was found to be strongly associated with the human cytoplasmic exosome[114] Furthermore, RNAi-mediated silencing of hDis3L1 resulted in the accumulation of adenylated rRNA fragments in the cytoplasm of human cells, suggesting that whether in an exosome-dependent or independent manner, it is involved in poly(A)-assisted RNA decay in this cellular location.[114]

The Distribution of Ribonucleases in Different Organisms and Organelles

After identifying and discussing the properties of important ribonucleases, particularly those related to poly(A)-assisted RNA decay, in the coming section, we present their distribution between various organisms and organelles. Comparison of the distribution of these enzymes based on current knowledge can provide evolutionary insight and understanding of the underlying mechanism of poly(A)-assisted RNA decay as its presence in most biological systems suggests that in those in which it is absent, it was eliminated during evolutionary progression. Indeed, the pathways found in archaea and cyanobacteria, for example, may be reminiscent of early evolutionary stages of poly(A)-assisted RNA decay, while others, such as those found in *E. coli* and eukaryotic nuclei, may represent advanced stages of the development of this mechanism.

BACTERIA

E. coli: Where Poly(A)-Assisted RNA Degradation and Central Enzymes, Including the Degradosome, Were First Explored

The object of most of the studies on RNA degradation (and many other aspects of pioneering research in molecular biology) and accordingly, where most of the ribonucleases were discovered, was the gram-negative bacterium, *E. coli*. In comparison with other prokaryotes, *E. coli* contains a particularly advanced RNA metabolism system and a large number of ribonucleases. In this system, the exoribonucleases, RNase II, RNase R, PNPase, RNase PH, and oligoribonuclease were first characterized. Of the endonucleases involved in RNA degradation, RNase E was the first discovered and the most thoroughly explored. The discovery and analyses of these enzymes in *E. coli* paved the way for finding their homologs in other bacteria, organelles, and eukaryotic cells (Table 1). Of notable difference is RNase J, which is not present in *E. coli* and was only recently found in *B. subtillis*. RNase J, however, is now known to exist in many biological systems including various bacteria, archaea, and higher plants (Table 1). Therefore, the recent discovery of RNase J is an example of a long delay in revealing an important enzyme simply because it is not present in the well-studied bacteria, *E. coli*.[97]

Another important *E. coli* finding related to RNA degradation is the degradosome complex, which has been the subject of many studies that concentrated on defining its structure and biological function.[43] Contrary to the individual enzymes described above, which were discovered in *E. coli* and later found to be widely dispersed throughout the biological world (excluding RNase J), the degradosome complex, as it appears in this bacterium, is not a common phenomenon and is known to exist only in certain evolutionarily close *E. coli* relatives (Figure 4). Again, this points toward a relatively well-developed RNA degradation mechanism in these bacteria. This is most likely the reason that *E. coli* contains two RNase E-like proteins (RNase E and G), the main difference between them being that RNase G lacks the degradosome scaffold domain. Most other organisms have just one RNase E-like protein, which is actually more closely related to RNase G, as it lacks the degradosome scaffold region (Figure 4).

Bacillus subtilis: The Discovery of RNase J

As a gram-positive bacterium and one that does not encode an RNase E, *B. subtilis* served, among additional purposes, to reveal components related to RNA degradation that do not exist in *E. coli*. As stated, this led to the recent and important discovery of RNase J. In *B. subtilis*, of the exonucleolytic enzymes, there appears to be a single member of the RNase II/R family and PNPase as well. The latter, like in *E. coli*, is responsible for polymerizing heteropolymeric poly(A) tails.[115] In a PNPase minus mutant,

two RNA molecules with heteropolymeric extensions were detected. However, subsequent sequence analysis showed that these 'tails' were related to the genomes of what would appear to be contaminating organisms and, therefore, most likely do not represent true heteropolymeric tailing.[115] Unlike *E. coli*, though, there is no Ntr-PAP and the identity of the poly(A)-polymerase that produces homopolymeric poly(A) extensions in *B. subtilis* is yet to be identified.[115]

Cyanobacteria: PNPase as the Sole Polyadenylating Enzyme and the Presence of Both RNase E and RNase J

Cyanobacteria are oxygenic, photosynthetic prokaryotes that take part in the global fixation of CO_2 during oxygen consumption and are a major constituent of the biomass in the world's oceans.[116] This organism is believed to be closely related to the evolutionary ancestor of the chloroplast that began its endosymbiotic existence about 150 million years ago. The *Synchocystis PCC6803* strain is a well-established study model; a major point of interest being the coexistence of RNase E and J in this organism. This most likely lead to the situation occurs in the chloroplasts of higher plants where similarly both enzymes are present (see below). Unlike *E. coli*, this organism does not encode an Ntr-PAP, leaving PNPase as the sole enzyme responsible for polymerizing transient tails (Table 1).[101] The heteropolymeric extensions that it produces mediate RNA degradation.

Mycoplasma: A Minimalistic RNA Degradation System that Lacks Polyadenylation

The *Mycoplasma* genus is comprised of small-genome parasitic bacteria and as such, minimal mechanistic requirements for RNA processing and degradation can be evaluated by studying these organisms. Mycoplasmas contain one RNase R/II-type enzyme and an RNase J homolog (Table 1). There is no PNPase nor poly(A)-assisted degradation pathway in this organism.[117] Though minimal, an even more limited RNA metabolism system exists in certain methanogenic archaea, wherein no phosphorylase or RNase R/II-like enzymes are present and RNA turnover seems to be carried out by RNase J homologs alone (see below) (Table 1).

Archaea: Three Entirely Different RNA Degradation Mechanisms in One Domain

The distribution of the enzymes that partake in RNA degradation in the archaeal domain is interesting because of the manner in which it follows the division between different archaeal groups (Table 1). This division provides a unique opportunity to study systems in which only phosphorolytic or hydrolytic exonucleolytic $3' \rightarrow 5'$ activities are present. For example, in hyperthermophylic archaea, no RNase R/II-like hydrolytic enzyme is present and the archaeal exosome is believed to be exclusively responsible for both the RNA polyadenylation and exonucleolytic $3' \rightarrow 5'$ degradation activities (for reasons described above).[78] Therefore, in this group, phosphorolytic activity is the force behind both of these processes.

In an interestingly opposite situation, halophilic archaea employ only an RNase R/II enzyme as an exonuclease to metabolize RNA and accordingly, the $3' \rightarrow 5'$ degradation is achieved hydrolytically.[80] Together with mycoplasmas, halophylic archaea are the only known organisms whose RNA does not undergo polyadenylation of any form (Table 1).

The third group consists of the methanogenic archaea and can be divided into two subgroups in terms of RNA degradation. Members of the first subgroup, as do the hyperthermophiles, contain the archaeal exosome and accordingly, their RNA is assumed to be polyadenylated and $3' \rightarrow 5'$ phosphorolytically degraded by this complex, as well.[80] The second subgroup is composed of methanogens that lack both the archaeal exosome and any RNase R/II-like enzymes. Of the enzymes known to be involved in RNA turnover in other systems, the only type that is present in the genomes of these archaea is of the RNase J family.[80] Interestingly, all evaluated archaea were found to contain homologs of RNase J and these enzymes have yet to be experimentally analyzed, as far as their endonucleolytic and/or $5' \rightarrow 3'$ exonucleolytic capacities. It could be hypothesized that in those methanogenic members that do not contain exosomes or an RNase R/II homolog, RNA is exclusively processed and degraded by RNase J. In such cases, both endo- and exonucleolytic activities are expected to be provided by RNase J.

ORGANELLES

Chloroplasts: Akin to Cyanobacteria; Like Father, Like Son

The chloroplasts of higher plants contain an arsenal of RNA processing and degrading enzymes, similar to that of cyanobacteria—most likely a close relative of the chloroplasts' evolutionary ancestor. These enzymes include PNPase, RNase II/R, RNase E, and RNase J (Table 1). While it is possible that some

of these enzymes bear a certain extent of functional redundancy, knocking out some of them results in photosynthetic or embryonic lethality.[47] Therefore, despite a possible overlap, each of these four enzymes is evidently responsible for a unique and essential function. A significant difference is that, unlike *Synechocystis*, wherein PNPase is exclusively responsible for polyadenylation, a noncanonical PAP (ncPAP) is present in the chloroplast of higher plants.[118] Accordingly, both homo- and heteropolymeric tails have been observed when studying chloroplast transcripts in higher plants.

The genome of the green alga, *C. reinhardtii*, does not contain an RNase E homolog so this enzyme is not present in its chloroplast. It is conceivable that RNase J is responsible for the endonucleolytic cleavage events of RNA degradation and/or processing in this organelle. RNase J is most likely responsible for the previously reported $5' \rightarrow 3'$ exonucleolytic activity present in the chloroplast of *Chlamydomonas*.[119,120]

Mitochondria: Four Different Systems

The mitochondrion is believed to have evolved from an α-proteobacterium similar to the gram-negative bacteria, *Rickettsia prowazekii*. Despite the likely common evolutionary ancestor, mitochondria of different organisms show remarkable differences in various aspects of gene organization and expression. One striking difference between plant and animal mitochondria is genome size. While the mitochondrial genome in human cells was reduced during evolution to a minimal size of ~16.5Kb, that of maize, for example, exceeds 500Kb. Concerning RNA processing, degradation, and the enzymes involved, there is great variety when comparing the mitochondria of plants, yeast, animals, and trypanosomes.

Plant Mitochondria

In plant mitochondria, both RNase R and PNPase are present and take part in the $3' \rightarrow 5'$ degradation and processing of plant mtRNA.[121] As of today, no endonuclease of the RNase J or RNase E classes has been identified in this system and it is not known whether $5' \rightarrow 3'$ exonucleolytic degradation occurs during plant mtRNA degradation.[121] The endonucleases, RNase P and RNase Z, are responsible for the generation of the mature $5'$ and $3'$ ends of tRNAs in plant mitochondria and it is interesting to question whether these enzymes are also involved in mtRNA degradation in this organelle. Plant mtRNA decay is mediated by transient adenylation, resembling the general bacterial poly(A)-assisted RNA decay scheme. The tails are homopolymeric, suggesting that they are synthesized by a poly(A)-polymerase rather than by PNPase, although the latter, as mentioned earlier, is present. Recently, poly(U)-rich tails were detected in the mitochondria of the green algae, *C. reinhardtii*.[118] Interestingly, poly(U) tails are involved in the degradation of microRNA in plants and histone mRNA in human cells. Several of the recently characterized ncPAPs (also called, PUPs or TUTases) of *S. Pombe*, humans, and trypanosomes were found to be linked to these events.[122,123] *In vitro* and *in vivo* assays confirmed the uridylation capability of several members of the ncPAP family.[124–126] More analysis is needed in order to characterize the role of uridylation in the mitochondria of *Chlamydomonas* and to determine whether it too assists in RNA decay in this organelle.

Yeast Mitochondria

There is no PNPase homolog encoded in the genomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In their mitochondria, devoid of PNPase, RNA degradation is performed by an RNase R/II-type enzyme that is associated with the RNA helicase, Suv3.[8,127] A significant difference between the yeast mitochondrial system and that of plants and animals is the total lack of polyadenylation (Table 1). In its place, a conserved dodecamer sequence is encoded at the $3'$ end of its transcripts. Similar to their plant counterparts, no homologs of the known endonucleases involved in RNA decay have been identified in yeast mitochondria.

Trypanosome Mitochondria

In terms of RNA metabolism, trypanosome mitochondria are very unique organelles. As in yeast, no PNPase was found in the trypanosome nuclear genome. RNA encoded in the mitochondrial genomes of these parasitic organisms undergoes significant editing, governed by an intricate network of guide RNAs, which alters the transcripts to a great extent, including the deletion and insertion of numerous uridines.[128] Another difference between the posttranscriptional modifications that trypanosome mitochondrial transcripts undergo, when compared to plants and yeast mtRNA, is that a stable poly(A) tail is extended at their $3'$ ends, resembling that which occurs in the nucleus of eukaryotic cells and animal mitochondria (see below). In addition, as in plant mitochondria, transient poly(A) tails are utilized to assist in the degradation of trypanosome mtRNA although the mechanism has not been fully deciphered. In terms of time course, along with human

mitochondria, trypanosome mitochondria represented the first biological system in which truncated, transiently adenylated transcripts were found to coexist with stable mature 3′ polyadenylation.[129] Several ncPAPs, such as kPAP1 and kPAP2, were found to be involved in the adenylation of *T. brucei* mtRNA, the latter of which is a homolog of the human mitochondrial PAP (hmtPAP).[130,131] Another interesting feature of trypanosome mtRNAs is that they can undergo uridylation, a modification that is related to the maturation stage of the editing process of the corresponding transcript.

Human Mitochondria

About 30 years ago, mRNAs encoded in the human mitochondrial genome were reported to undergo stable polyadenylation at their mature 3′ ends, similar to nucleus-encoded mRNAs.[132] These poly(A) tails are synthesized during the cleavage and processing stages of the polycistronic mtRNA and they complete the partially encoded stop codons (e.g. 'UA' or 'U') of a number of mitochondrial mRNAs.[8] There is still controversy concerning their influence upon stability and translatability of the transcripts. Aside from stable polyadenylation, truncated, adenylated transcripts were detected and isolated as well—evidence that human mitochondria remain 'loyal' to their prokaryotic origin and most likely employ transient poly(A) to degrade mtRNA.[7] Indeed, bioinformatic analysis disclosed that the entire mitochondrial transcriptome undergoes transient adenylation at sites within the full length transcript sequences.[7] Therefore, along with its trypanosome counterpart, the human mitochondrion was the first biological system known to employ both stable and transient poly(A), although much research remains to be done in order to fully understand this mechanism. The identities of the enzymes that degrade human mtRNA still remain an open question. As previously stated, the human PNPase bears a transit peptide that directs it to the mitochondrial intermembrane space (IMS) and most, if not all, of this enzyme is localized there while the mtRNA is located in the matrix.[54] Therefore, unless trace amounts of this protein enter the matrix, it most likely does not play a direct role in human mtRNA degradation although, interestingly, its RNAi depletion caused mitochondrial dysfunction.[56] As no RNA is known to be present in the IMS, the biological function of PNPase and the reason for its cellular localization remain an enigma. Another powerful nuclease, Endo G, is localized to the IMS and can be liberated into the cytoplasm and it is possible that PNPase may act similarly. Indeed, there are reports of the involvement of PNPase in degrading nucleus-encoded transcripts in the cytoplasm.[49]

No RNase R/II homologs are known to enter the human mitochondrion and such is the case for any endoribonucleases of the type associated with bacterial RNA degradation. Of the enzymes involved in mtRNA processing, RNase P was recently identified and found to be evolutionarily distinct from the conserved RNase P proteins present in almost all living organisms.[133] The enzyme responsible for extending the stable poly(A) tails in this organelle, hmtPAP, was identified and found to belong to the seven-member family (in human cells) of ncPAPs.[134,135] There is evidence that suggests that this enzyme may be involved in the initial stages of transient adenylation that takes place in human mitochondria as well.[56]

EUKARYOTES

Nucleus

A full description encompassing the RNA degradation processes that occur in the nucleus and cytoplasm of eukaryotic cells is beyond the scope of this article, and therefore, only points related to poly(A)-assisted RNA decay will be presented. Aberrant transcripts can be transiently adenylated by an ncPAP component (Trf4/5) of the TRAMP complex (which includes an RNA helicase and RNA-binding proteins as well) and degraded by the nuclear exosome. Also, it has recently become evident that most of the genome in yeast and human cells is transcribed and the plethora of antisense, cryptic, and noncoding RNAs produced by this 'lax' transcription are also substrates of the joint operation of TRAMP and the exosome.[13,14,17,136] This mechanism bears basic motifs that resemble the poly(A)-assisted RNA decay pathways first discovered in prokaryotes/organelles (Figure 1).[9,18] Aside from this, polyuridilation has been reported to mediate the degradation of histone mRNAs during specific cell cycle stages.[11,122]

CYTOPLASM

In the cytoplasm of eukaryotic cells, the general mechanism of mRNA decay begins with the shortening of the long, stable poly(A)-tail located at the 3′ end, followed by decapping and finally, exonucleolytic $5' \rightarrow 3'$ degradation by Xrn1 and $3' \rightarrow 5'$ degradation by the exosome.[9] Of relevance to the main topic of this review, a recent study has provided evidence that, besides this well-characterized RNA degradation mechanism, RNA can undergo poly(A)-assisted

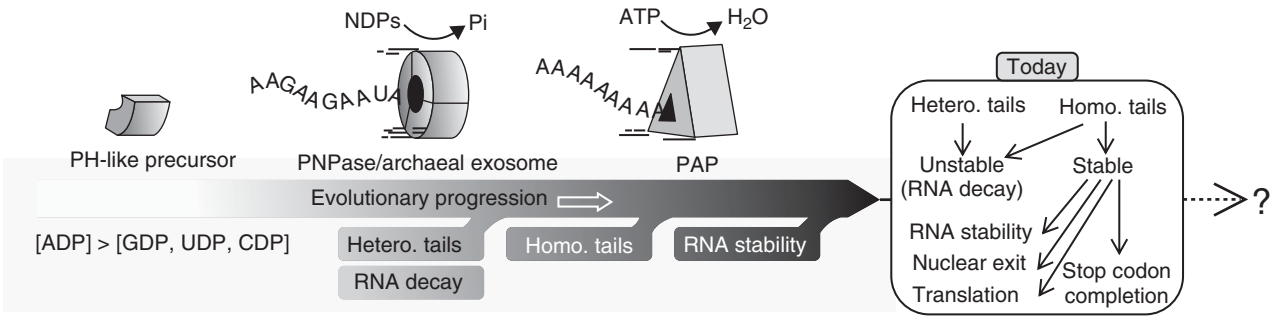

FIGURE 5 | Suggested scenario for the evolution of polyadenylation. When comparing the various roles and nucleotide compositions of poly(A) tails in different systems, as well as the structures and activities of the polyadenylating and degrading exoribonucleases, the following scenario can be offered: A single-domain PH-like ancestor enzyme developed into the PNPase and archaeal exosome complexes which produced heteropolymeric extensions—poly(A)-rich in nature, because despite the non-specific polymerization properties of these enzymes, adenosine triphosphate (ATP) was the cell's 'energy currency' and therefore, present at a relatively high concentration. The function of these transient (unstable) tails was to assist in RNA decay, which the same complexes carried out. Later, enzymes specific to ATP emerged and the unstable homopolymeric poly(A) tails that they produced assisted in RNA decay as well. At the next stage, cellular mechanisms adopted new roles for the homopolymeric tails which required their stability. In some cases the stable tails influenced translation initiation and longevity of the tailed transcript (nucleus) and in others (animal mitochondrion) stable poly(A) tails helped to complete partially encoded stop codons. Today, tails can be categorized as hetero- or homopolymeric, wherein the former is unstable and promotes RNA decay (although additional function cannot be ruled out). The latter can be both unstable (transient) and stable, depending on the system/process and in some cases, stable poly(A) tails can encourage RNA stability. In summary, the reason that nucleus-encoded mRNAs bear homopolymeric stable adenosine tails and that adenosines dominate transient poly(A) extensions is linked to the reason that 'A' was chosen as life's 'energy coin'.

RNA decay within the cytoplasm of human cells as well. Adenylated rRNA fragments were shown to accumulate upon RNAi-mediated knock down of the Rrp44 (hDis3-L1) subunit of the exosome and that of Xrn1.[114] Together, this represents another instance, not only of poly(A)-assisted RNA decay, but also of coexisting transient and stable polyadenylation as well.

CONCLUSION

Wherefore Art Thou Poly(A)?

Aside from a number of exceptions—mycoplasmas, halophytic and certain methanogenic archaea, and yeast mitochondria—poly(A)-assisted RNA decay occurs in all life systems, from bacteria through archaea and organelles to the nucleus and cytoplasm of eukaryotic cells. This would suggest a common evolutionary origin of this mechanism. Accordingly, the polymerizing and endo-/exonucleolytic enzymes share many functional and structural similarities. Nonetheless, there are several surprising differences that were likely introduced during the evolutionary development of each of the systems, such as the eukaryotic loss of the exosome's phosphorolytic capabilities. We present a scenario depicting the evolution of this process and reveal a connection between the identity of the cell's 'energy coin' and poly(A) tails as they appear today.

The almost ubiquitous presence of poly(A)-assisted RNA decay, the similarities observed when comparing this process in numerous systems, and the heteropolymeric nature of the tails produced by PNPase and the archaeal exosome can be combined to suggest the following hypothesis: The ancestral purpose that polyadenylation served was to mediate RNA degradation and the first enzyme to produce these tails was the evolutionary precursor of PNPase and the archaeal exosome (Figure 5). Fulfilling its role as the cellular 'energy currency', adenosine was the nucleotide present at the highest concentration; hence, although the enzyme was capable of incorporating all nucleotide types, it actually produced poly(A)-rich, heteropolymeric tails. Much later, the poly(A) polymerases arose, already specific to the highly abundant ATP, and produced homopolymeric, degradation-assisting poly(A) tails (Figure 5). Together with these new enzymes, it could be speculated that the function of homopolymeric polyadenylation adopted additional faces that required tail stability (e.g., to direct translation initiation and to stabilize RNA). These new roles were implemented in the nucleus of eukaryotic cells in the form of stable poly(A) tails at the mature 3′ ends of transcripts, although the previous, ancestral role, continued to exist (Figure 5).

When viewing the chain of events from this perspective, an interesting conclusion may come to mind: The reason that mRNAs encoded in the nuclear genomes of eukaryotes are decorated with long homopolymeric adenosine tails and that adenosines

are the main component in transient, RNA decay-assisting tails is strongly linked to the very reason that adenosine was 'chosen' to be the 'energy coin of life' in the first place. When that question is answered, we will know why poly(A) tails are, in fact, poly(A).

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