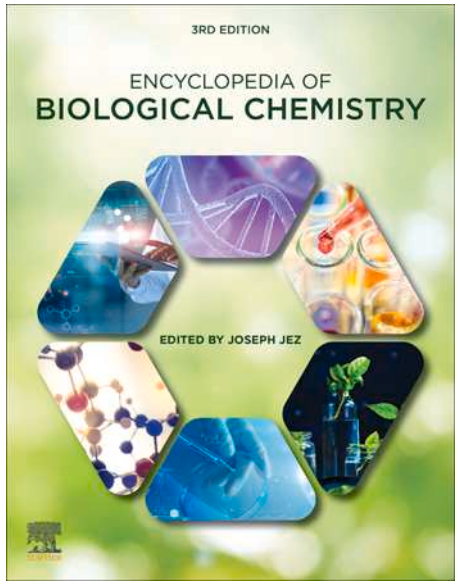

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Binding Proteins | RNA-Binding Proteins in Bacterial and Mitochondrial RNA Decay

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Glossary

DEAD-box RNA helicase The largest RNA helicase sub-family of superfamily 2 (SF2) helicases. The name "DEAD box" derives from the conserved amino acid sequence Asp-Glu-Ala-Asp (D-E-A-D) within the two RecA-like domains in the helicase core of this group of helicases. Coupled with ATP binding and hydrolysis, DEAD-box RNA helicases catalyze RNA structural rearrangements, including secondary structure disruption, strand separation, and RNA-protein dissociation.

Endoribonuclease An enzyme that cleaves the phosphodiester bonds between ribonucleotides in the middle of an RNA chain.

Exoribonuclease An enzyme that binds at the 5′ or 3′ end of an RNA chain and cleaves the phosphodiester bonds between ribonucleotides to remove terminal ribonucleotides from either the 5′ end, called 5′ – 3′ exoribonuclease (or 5′-exoribonuclease), or the 3′ end, termed 3′ – 5′ exoribonuclease (or 3′-exoribonuclease).

Mitochondrial RNA granules (MRGs) Subcompartments of fluid condensate positioned adjacent to the mitochondrial membrane in the matrix that contain newly synthesized RNA, RNA-processing proteins, and mitoribosome assembly factors.

NanoRNAs Oligoribonucleotides of 2–5 nucleotides in length that are degraded by specific ribonucleases, such as ORN in *E. coli* and Rexo2 in human.

Polyadenylation A post-transcriptional modification process in which a poly(A) tail is added to an RNA transcript. This long poly(A) tail consists of multiple adenine nucleotides at the 3′ end of mRNA, which may influence its maturation, stability, translation and decay.

Post-transcriptional regulation A set of biological processes executed at the RNA level after an RNA molecule has been transcribed. These processes – including RNA splicing, RNA maturation, control of RNA stability, RNA interference, RNA modification and RNA decay – can be regulated to control gene expression.

Processive enzymes Enzymes that remain attached to their substrate without releasing it as they perform multiple rounds of catalysis.

RNA decay A conserved process prevalent across all kingdoms of life by which ribonucleic acid (RNA) molecules are enzymatically degraded. RNA decay is well regulated, serving to control the quality and levels of cellular RNA molecules, and it plays a crucial role as a regulatory mechanism for gene expression.

RNA helicase An enzyme that remodels RNA conformation or protein-RNA interactions. RNA helicases play crucial roles in nearly all aspects of RNA metabolism, such as transcription, RNA splicing, RNA editing, ribosome biogenesis, translation, RNA transport, and RNA degradation.

Introduction

A large group of RNA-binding proteins are enzymes that manipulate RNA, not only binding it, but also processing it via unwinding, refolding, cleaving and modifying actions. These RNA enzymes are often conserved among bacteria and eukaryotes. One cellular process that is heavily dependent on RNA enzymes is decay of messenger RNA (mRNA), which plays a vital role in regulating post-transcriptional gene expression and RNA quality control (Garneau *et al.*, 2007; Schoenberg and Maquat, 2012). Altering the half-life of mRNA or interfering with RNA decay pathways can alter protein expression levels or result in defective protein molecules, leading to various physiological changes and pathological conditions (Eberhardt *et al.*, 2007; Rigby and Rehwinkel, 2015). Therefore, it is important to investigate RNA decay, particularly to identify the working mechanisms of RNA-binding enzymes and to elucidate their roles in RNA decay pathways and how they are regulated.

Similar enzymes and pathways are responsible for RNA decay among bacteria and the mitochondria of eukaryotic cells. This is likely because the first eukaryotic cell formed more than one billion years ago by a cell engulfing an α-proteobacterium, with this latter subsequently evolving into the mitochondrion of eukaryotic cells (Timmis *et al.*, 2004; Vanvalen and Maiorana, 1980). Thus, mitochondrial DNA has the same circular form as bacterial genomes, and multiple copies are distributed throughout the bacterial cytoplasm or mitochondrial matrix, respectively (Burger and Lang, 2003). The mRNA molecules transcribed from these circular DNA genomes are processed into mature mRNAs, which are subsequently degraded by various cooperating enzymes, including endoribonucleases, exoribonucleases and RNA helicases. In this review, we focus on comparing the similar functions, pathways, and structures of the major RNA enzymes that participate in mRNA decay in bacteria and the mitochondria of eukaryotic cells.

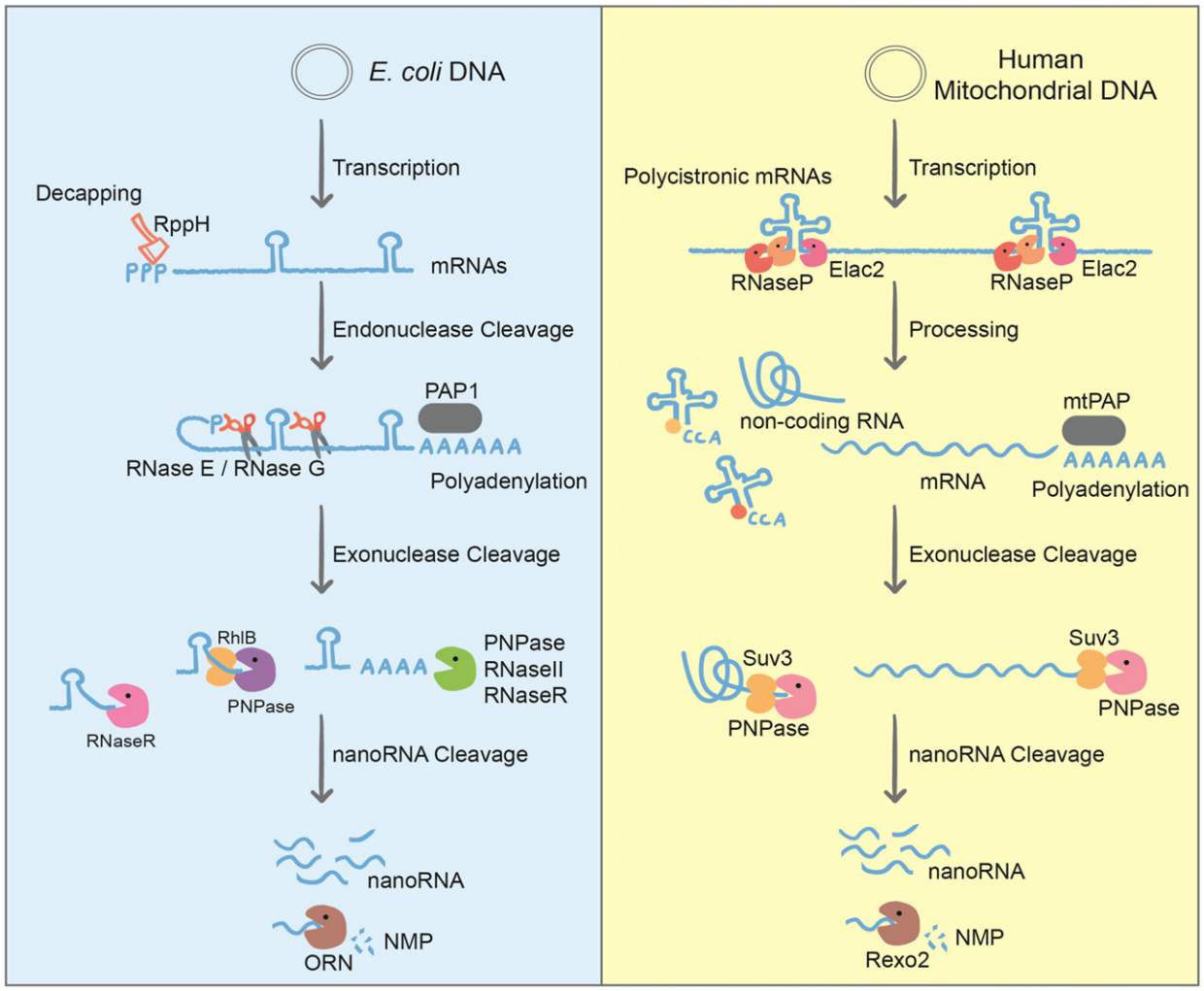

Fig. 1 RNA decay pathways in *E. coli* and the human mitochondrion. *E. coli* mRNAs are degraded by RNA-degrading enzymes, including endoribonucleases (RNase E and RNase G), exoribonucleases (RNase II, RNase R, PNPase and ORN), and the helicase RhlB. The human mitochondrial genome is first transcribed as a large polycistronic RNA. These RNAs are processed by various endoribonucleases, including RNase P and Elac2, to generate single RNA molecules that are ultimately degraded primarily by the mitochondrial degradosome, the PNPase-Suv3 complex, and Rexo2. PolyA polymerase in *E. coli* (PAP1) and human mitochondria (mtPAP) add poly(A) tails to mRNA and regulate the stability and half-life of mRNAs.

RNA-Binding Enzymes in Bacterial mRNA Decay

Prokaryotic organisms have to adapt precisely and rapidly to swiftly changing environmental conditions, and a prompt response can best be achieved through post-transcriptional regulation whereby mRNA levels can be altered within a few seconds (Coburn and Mackie, 1999; Shenhar *et al.*, 2009). Bacterial mRNA half-lives may vary from a few seconds to more than half an hour in the Gram-negative bacterium *Escherichia coli* or in Gram-positive *Bacillus subtilis* (Esquerre *et al.*, 2014; Hambraeus *et al.*, 2003). Generally, mRNA decay in prokaryotic cells is initiated by pyrophosphohydrolase (RppH) removing the 5′-end pyrophosphate (Deana *et al.*, 2008). The 5′-monophosphate mRNA is then cleaved by endoribonucleases at one or multiple internal sites, mostly by RNase E in *E. coli* or RNase Y in *B. subtilis*, respectively (**Fig. 1**; Durand *et al.*, 2012; Jain, 2002). In contrast to RNase E that is essential for *E. coli* growth, RNase Y is not essential in *B. subtilis*, but its deletion negatively impacts the amounts of a number of mRNAs and results in strong phenotypes (Ali and Gowrishankar, 2020; Figaro *et al.*, 2013; Lehnik-Habrink *et al.*, 2011b). Both enzymes are attached to the bacterial membrane, either via a membrane-binding helix in RNase E or a trans-membrane domain in RNase Y (Khemici *et al.*, 2008; Lehnik-Habrink *et al.*, 2011a). The membrane localization of both enzymes is crucial for correct bacterial cellular functions, as RNase E dissociation from the membrane results in a global slowdown of mRNA decay and RNase Y dissociation is lethal in *B. subtilis* (Hadjeras *et al.*, 2019; Lehnik-Habrink *et al.*, 2011a).

RNase E in *E. coli* can initiate mRNA decay by either 5′ end-dependent or direct entry pathways (Hui *et al.*, 2014). In the 5′ end-dependent pathway, RNase E recognizes and interacts with the 5′-monophosphate generated by RppH, and consequently starts

searching for a cleavage site along single-stranded regions of mRNA (Celesnik *et al.*, 2007; Jiang *et al.*, 2000; Mackie, 1998). A similar scenario could be true of RNase Y in *B. subtilis* as it is sensitive to the removal of the 5′-triphosphate group, but the underlying mechanism remains unclear (Shahbabian *et al.*, 2009). Alternatively, the C-terminus RNA-binding domain of RNase E can recognize structured RNA motifs to induce the direct entry pathway (Bandyra *et al.*, 2018; Clarke *et al.*, 2014). Although RNase E in *E. coli* and RNase Y in *B. subtilis* represent the major endoribonucleases initiating mRNA decay in those bacteria, other endoribonucleases such as RNase G, RNase III and RNase J1 can also perform the first endonucleolytic cleavage step (Durand *et al.*, 2012; Lee *et al.*, 2002).

Both RNase E and RNase Y act as a scaffold for the assembly of a multimeric complex called the RNA degradosome (Kaberdin *et al.*, 1998; Lehnik-Habrink *et al.*, 2011a, 2010). The composition of the degradosome varies among bacteria, but it typically includes endoribonucleases, exoribonucleases, glycolytic enzymes and RNA helicases to ensure that the correct RNAs are processed or degraded (Ait-Bara and Carpousis, 2015). The component proteins of the RNA degradosome and the interactions among them have been studied most extensively in *E. coli*, involving a $3' - 5'$ exoribonuclease polynucleotide phosphorylase (PNPase), a DEAD-box RNA helicase RhlB, and the glycolytic enzyme enolase, all of which bind to the carboxy-terminal half of RNase E (Carpousis *et al.*, 1994; Py *et al.*, 1996; Vanzo *et al.*, 1998). The RNA degradosome of bacteria exhibits some plasticity in that it can interact with RNase R (exoribonuclease), polyA polymerase (PAP1), Hfq (RNA chaperon) or CsdA (helicase) (Carabetta *et al.*, 2010; Khemici *et al.*, 2004; Prud'homme-Genereux *et al.*, 2004; Raynal and Carpousis, 1999). It has been observed that only small fractions of degradosome component proteins are associated in the RNA degradosome, with most of them being dissociated from the degradosome in cytoplasm. For example, PNPase forms a complex with the RNA helicase RhlB independently of the RNA degradosome, and this PNPase-RhlB complex, referred to as the "minimal RNA degradosome", plays a vital role in cysteine homeostasis and regulating cysB RNA degradation (Lin and Lin-Chao, 2005; Tseng *et al.*, 2015).

After initial endonucleolytic cleavage of mRNA, a group of $3' - 5'$ exoribonucleases continue to degrade RNA fragments by removing one nucleotide at a time from the 3′ end of RNA chains (Hui *et al.*, 2014). Four 3′-exoribonucleases participate in mRNA decay in *E. coli*, including PNPase, RNase R, RNase II and oligoribonuclease (ORN) (Anderson and Dunman, 2009). Similarly, four 3′-exoribonucleases are engaged in mRNA turnover in *B. subtilis*, namely PNPase, RNase PH, RNase R and YhaM (Oussenko *et al.*, 2005). *E. coli* RNase II and RNase R are typical members of the RNase II (or RNR) family of ribonucleases that all bear a conserved RNB domain possessing $3' - 5'$ exoribonuclease activity and auxiliary RNA-binding domains at the N- and C-terminal regions, including cold shock domains (CSD1 and CSD2), S1 and K/R-rich domains (**Fig. 2**; Reis *et al.*, 2013). However, RNase R is quite unique among 3′-exoribonucleases in that it can degrade duplex RNA with a 3′-overhang, suggesting that RNase R possesses

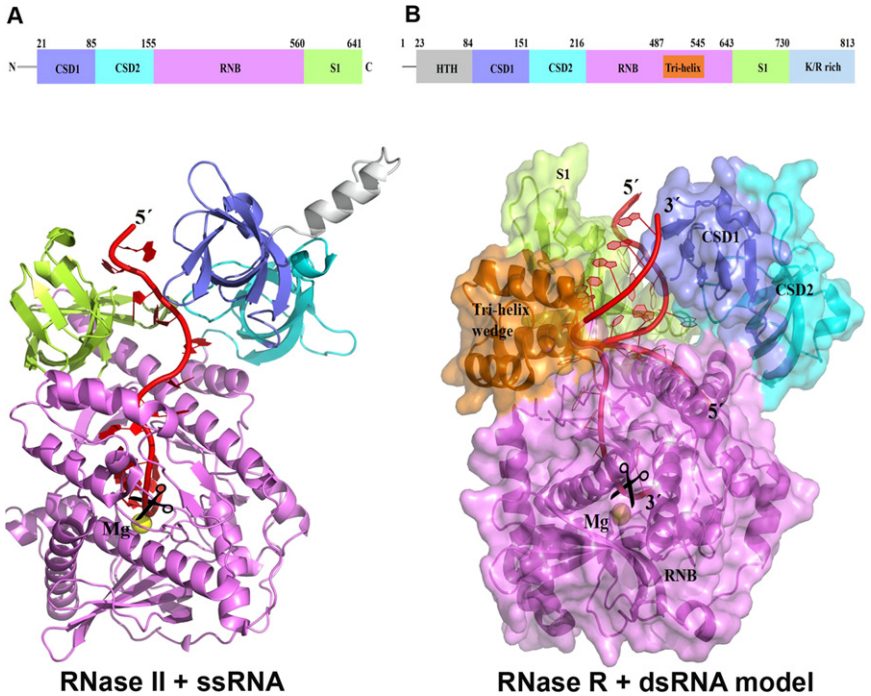

Fig. 2 Crystal structures of *E. coli* RNase II and RNase R. (A) Overall domain organization of RNase II and the crystal structure of RNase II-ssRNA complex (PDB: 2IX1). The 3′ end of ssRNA is threaded through the RNA-binding channel formed by the RNA-binding S1, CSD1 and CSD2 domains into the narrow channel of the RNB domain. The yellow sphere represents the magnesium ion located in the exoribonucease active site. (B) Overall domain organization of RNase R and the crystal structure of HTH and K/R-rich domain-truncated RNase R (PDB: 5XGU) bound with a modeled duplex RNA with a 3′ overhang. This structure indicates that the 3′ overhang of duplex RNA enters all the way into the active site from the narrow RNA-binding channel of the RNB domain and that the duplex RNA is unwound upon reaching the tri-helix wedge region in the RNB domain. The cleavage site in the RNA is marked by a scissors symbol.

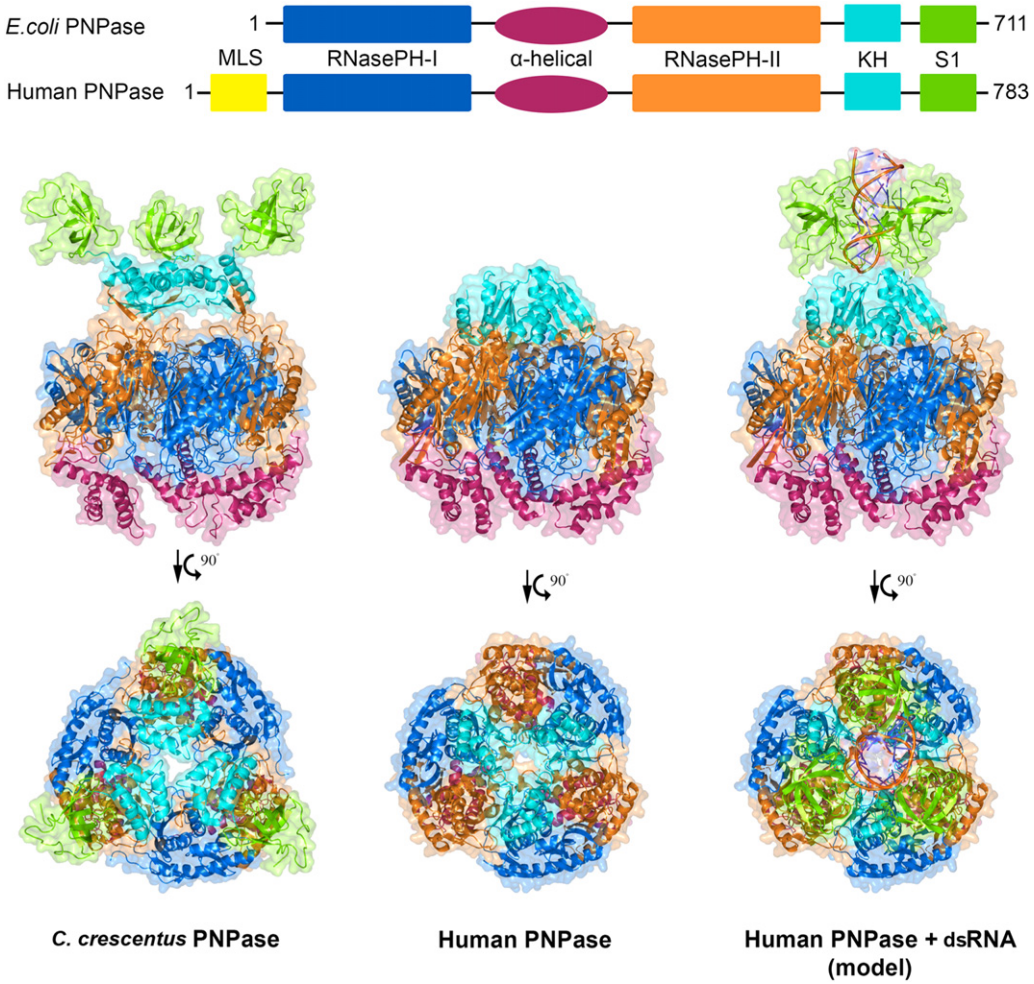

Fig. 3 Crystal structures of bacterial and human PNPase. Bacterial PNPase (PDB: 4AIM; *Caulobacter crescentus*) and human PNPase (PDB: 3UIK) share a similar domain organization and overall trimeric ring-like structure, comprising a central narrow RNA-binding channel formed by the three KH domains and six RNase PH domains. The 3′-end of ssRNA is threaded into the narrow central channel and degraded in one of the active sites in the RNase PH-II domain. In human PNPase, the S1 domain is disordered and flexible, and it is likely responsible for double-stranded RNA interactions for mitochondrial RNA import (see the model in the right panel) or protein-protein interactions (with Suv3 helicase) for RNA degradation.

both RNA degrading and unwinding activities (Awano *et al.*, 2007; Chu *et al.*, 2017). In contrast, RNase II only cleaves single-stranded RNA (ssRNA), but it stalls upon reaching stable stem-loop RNA structures (Spickler and Mackie, 2000). Crystal structures of RNase II bound to ssRNA reveal that the CSD1, CSD2, and S1 domains all adopt a nucleic acid-binding OB fold with a five-stranded β-barrel to form an RNA-binding channel (Frazao *et al.*, 2006). The 3′ end of a single-stranded RNA is threaded through this channel into the active site containing a magnesium ion buried deep in the RNB domain (**Fig. 2**). Since the narrow channel can only accommodate single-stranded but not double-stranded RNA, RNase II solely degrades linear RNA and it dissociates from it a few nucleotides downstream of structured RNA (Spickler and Mackie, 2000). In comparison to RNase II, the crystal structure of RNase R reveals an exclusive "tri-helix wedge" region in the RNB domain that may bind RNA more tightly to induce RNA unwinding. The 3′ overhang RNA is threaded into the active site for RNA hydrolysis in the RNB domain, which provides the driving force for duplex RNA unwinding upon reaching the wedge region (Chu *et al.*, 2017). Thus, RNase R can simultaneously and processively unwind and degrade structured RNA to generate final degradation products of ~4 nucleotides.

PNPase is considered to be the major exoribonuclease participating in mRNA turnover in *E. coli* (Lin-Chao *et al.*, 2007). PNPase is a trimeric enzyme that consists of six RNase PH domains and three RNA-binding S1 and KH domains assembled into a ring-like structure with a central channel for RNA binding (**Fig. 3**; Lin *et al.*, 2012). The domain organization and architecture of PNPase is similar to the core of the eukaryotic RNA degrading complex, termed the "exosome", responsible for degrading mRNA from the 3′ to 5′ end in the cytosol and nucleus (**Fig. 4**; Makino *et al.*, 2013). Bacterial PNPase interacts with the DEAD-box helicases RhlB (in *E. coli*) or CshA (in *B. subtilis*), which unwind duplex RNA and promote the exoribonuclease activity of PNPase in degrading structured RNA (Lin and Lin-Chao, 2005). Trimeric PNPase associates with dimeric RhlB to form a 3:2 complex that is independent of degradosome formation. RhlB and CshA use ATP to catalyze the unwinding of short RNA duplexes by non-processive

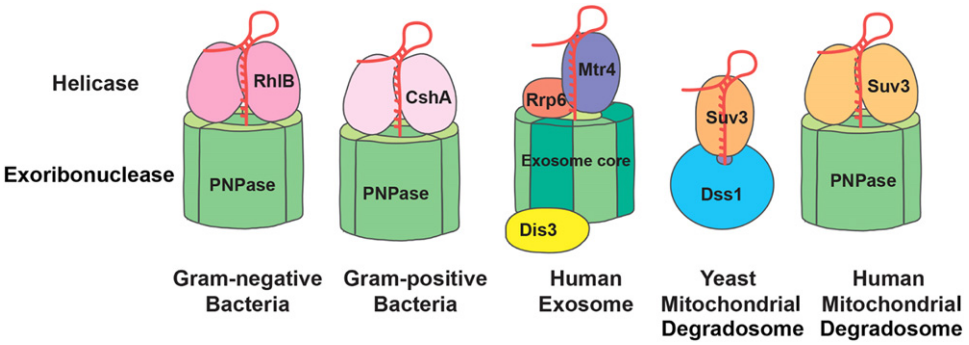

Fig. 4 A conserved exoribonuclease-helicase complex from bacteria to human responsible for RNA decay. The minimal RNA degradosome of bacteria consisting of PNPase and a DEAD-box helicase (RhlB or CshA) shares a similar overall architecture to the eukaryotic exosome core and the human mitochondrial degradosome. PNPase solely degrades ssRNA due to its narrow RNA-binding channel prohibiting entry of structured RNA. A dimeric ATP-dependent helicase, such as RhlB, CshA or Suv3, works cooperatively with PNPase to unwind and ultimately degrade structured RNA.

local strand separation and they facilitate PNPase-mediated degradation of structured RNA (Cordin *et al.*, 2006). This small RNA-degrading machine comprising PNPase and its interacting helicase can thus efficiently unwind and degrade bulk mRNA molecules in bacteria.

PAP1-mediated polyadenylation at the 3′ end of mRNAs in *E. coli* regulates mRNA decay by enabling PNPase and RNase R to proceed through secondary structures for efficient RNA degradation (Cheng and Deutscher, 2005; Raynal *et al.*, 1996; Xu and Cohen, 1995). In contrast, RNase II appears to play a protective role by eliminating the poly(A) tail of polyadenylated mRNAs that can inhibit mRNA degradation by PNPase and RNase R (Marujo *et al.*, 2000). Moreover, the chaperon Hfq can bind to the poly(A) tails of mRNAs and inhibit RNA degradation by $3' - 5'$ exoribonucleases (Regnier and Hajnsdorf, 2009).

RNA decay in *E. coli* is completed by ORN, which is an evolutionarily conserved 3′–5′ DEDDh-family exonuclease that preferentially cleaves small oligonucleotides (nanoRNAs). Knockdown of ORN in *E. coli* leads to an accumulation of nanoRNAs and a dramatic shift in transcription start sites for a significant proportion of promoters, indicating that these nanoRNAs may serve as primers for transcriptional initiation and thus impact gene expression (Goldman *et al.*, 2011). Deletion of ORN from *E. coli*, but not other exoribonucleases, is lethal, evidencing its essential role in cellular metabolic pathways (Ghosh and Deutscher, 1999; Zhang *et al.*, 1998). Hence, ORN is indispensable in RNA decay pathways for recycling short RNA fragments into nucleotide monophosphates to refill the cellular pool of RNA precursors (Datta and Niyogi, 1975; Ghosh and Deutscher, 1999).

RNA-Binding Enzymes in Mitochondrial mRNA Decay

Mitochondria are the power plants of eukaryotic cells required for energy production to maintain cellular homeostasis. Proper mitochondrial function depends on accurately tuned mitochondrial gene expression, whereas any dysfunctions may lead to pathological conditions, including aging, inflammation and a wide spectrum of diseases (Kotrys and Szczesny, 2020). The human mitochondrial genome is a circular double-stranded DNA of approximately 16,569 base pairs that encodes 13 proteins, 2 rRNAs and 22 tRNAs. Both strands of mitochondrial DNA (mtDNA) are transcribed into large polycistronic mitochondrial RNA chains (mtRNA) via a single promoter that is located, respectively, in the heavy and light chains of mtDNA (Taanman, 1999). These polycistronic mtRNAs are cleaved and modified to generate mature tRNAs, rRNAs, and mRNAs. Although transcription levels from the identical promoter are the same for both strands, the half-life of each respective mRNA differs, indicating that the level of each mitochondrial mRNA is regulated at the post-transcriptional level, including via a well-controlled RNA degradation mechanism (Duborjal *et al.*, 2002).

Newly transcribed RNAs are localized in dynamic protein structures in close proximity to nucleoids, termed mitochondrial RNA granules (MRGs) (Antonicka and Shoubridge, 2015; Iborra *et al.*, 2004). MRGs contain many factors necessary for RNA processing, RNA maturation, RNA decay, mitochondrial ribosome assembly and translation (Antonicka and Shoubridge, 2015; Borowski *et al.*, 2013; Jourdain *et al.*, 2015, 2013; Lee *et al.*, 2013; Wilson *et al.*, 2014). As MRGs are dynamic structures, their ribonucleoprotein composition may change in response to progression of RNA processing, translation and decay. Based on the tRNA punctuation model, processing of long polycistronic mtRNAs starts at MRGs by excising tRNAs from the 5′ and 3′ ends through RNase P- and Elac2-mediated endonucleolytic cleavage, respectively, with this process generating most of the mitochondrial tRNAs, mRNAs and rRNAs (Holzmann *et al.*, 2008; Ojala *et al.*, 1981; Takaku *et al.*, 2003). Some other proteins have been shown to participate in tRNA excision, such as GRSF1 (G-rich sequence factor 1), which interacts with RNase P and is required for correct tRNA processing (Jourdain *et al.*, 2013). In contrast, PTCD1 directly interacts with Elac2 and affects 3′ end processing of tRNAs (Sanchez *et al.*, 2011). Although the tRNA punctuation model may reflect how most mitochondrial mRNAs are generated, some mRNAs are not flanked by any tRNAs, such as those of ND5-Cyt B and ATP6/8-COIII. Consequently, other factors have been

identified that participate in mtRNA processing, including PTCD2 that was shown to affect the steady state levels of mature cytb and ND5 mRNAs in a mouse model (Xu *et al.*, 2008). Moreover, depletion of the helicase Suv3 results in an accumulation of inappropriately sized transcripts, indicating that it too plays a role in mitochondrial RNA processing (Szczesny *et al.*, 2010). Additionally, FASKD5 has been reported as playing an essential part in the processing of transcripts not flanked by tRNAs (Antonicka and Shoubridge, 2015).

The cleaved mitochondrial tRNAs and rRNAs are further modified by enzymes to generate mature tRNAs and rRNAs, whereas poly(A) tails of ~50 adenylates are added to mRNAs by mitochondrial poly(A) polymerase (mtPAP) in human mitochondria (Chang and Tong, 2012). Since 7 of the 13 mRNAs have a truncated stop codon of only U or UA, the polyadenylation of mRNA by mtPAP completes the UAA stop codon. Polyadenylation of mitochondrial mRNAs also regulates mRNA stability, but polyA tails have complex and diverse effects on the stability of mitochondrial mRNAs, as knockdown of mtPAP may either increase or decrease the half-lives of mitochondrial mRNAs (Levy and Schuster, 2016).

Degradation of mtRNAs in the mitochondrial matrix is performed primarily by a complex comprising PNPase and Suv3 helicase, termed the mitochondrial degradosome (Borowski *et al.*, 2013; Szczesny *et al.*, 2010). Human PNPase is a multi-functional protein involved not only in RNA decay but also in mitochondrial RNA import (Wang *et al.*, 2010). A small proportion of human PNPase is located in the cytoplasm where it specifically degrades *c-myc* mRNA and a subset of microRNAs, and it regulates cell growth arrest and apoptosis (Das *et al.*, 2010; Sarkar *et al.*, 2003, 2006). As a result, PNPase overexpression is associated with growth inhibition and some pathological changes that occur during aging, such as inflammation (Sarkar and Fisher, 2006; Sarkar *et al.*, 2007). Human PNPase is also located in the mitochondrial inner membrane space where it is involved in importing small structured RNAs with a signature stem-loop motif from the cytosol into the mitochondria, such as RNase P, 5S rRNA and mitochondrial RNA processing (MRP) RNAs (Chen *et al.*, 2006; Wang *et al.*, 2010). PNPase is also located in the mitochondrial matrix where it forms a 3:2 complex with Suv3 for cooperative degradation of mtRNA (Minczuk *et al.*, 2002; Wang *et al.*, 2009). Thus, Suv3, which belongs to the Ski2-like DExH/D-box family of helicases, appears to play a similar role to RhlB and CshA in bacteria (**Fig. 4**). Knockdown studies indicate that the PNPase-Suv3 complex may be involved in removing aberrant truncated RNA species in mammalian mitochondria since truncated polyadenylated mtRNA species were found to accumulate in mammalian cells lacking Suv3 or PNPase (Khidr *et al.*, 2008). Dysfunction of the degradosome-dependent mtRNA decay pathway leads to an accumulation of antisense mtRNAs and mitochondrial dsRNA, and release of that dsRNA into cytoplasm can trigger an innate immune response (Dhir *et al.*, 2018). The human mitochondrial degradosome also prevents the harmful formation of R loops that may interfere with mtDNA maintenance and stability (Silva *et al.*, 2018).

Crystal structures of bacterial and human PNPase reveal a conserved trimeric ring-like architecture, with a central channel formed by six RNase PH domains through which the 3′ end of ssRNA is threaded to interact with the catalytic site in one of the RNase PHII domains (**Fig. 3**; Hardwick *et al.*, 2012; Nurmohamed *et al.*, 2009; Shi *et al.*, 2008). The S1 and KH domains of PNPase are involved in RNA binding, whereas the KH domains form a KH pore that plays the principle role in ssRNA interactions (Lin *et al.*, 2012), and the S1 domains are more flexible and may be involved in interactions with duplex RNA (for RNA import) or interactions with the helicase Suv3 (Golzarroshan *et al.*, 2018). Due to the long and narrow RNA-binding channel, RNA is continuously associated with PNPase during the RNA degradation process, so PNPase degrades RNA in a highly processive manner (Fazala *et al.*, 2015).

In degrading ssRNA, PNPase generates final nanoRNA products of ~4 nucleotides (Lin *et al.*, 2012). These nanoRNAs are further degraded by Rexo2, which acts as a scavenger to generate NMPs for RNA salvage in mitochondria (Bruni *et al.*, 2013; Goldman *et al.*, 2011; Golzarroshan *et al.*, 2018). Rexo2 is a 3′–5′ exoribonuclease in the DEDDh superfamily and shares high sequence identity and enzymatic activity with *E. coli* ORN (Nguyen *et al.*, 2000). Depletion of Rexo2 from HeLa cells affects mitochondrial morphology and cell growth, leading to a substantial decrease in mtDNA, mtRNA and protein levels (Bruni *et al.*, 2013). Rexo2 in mammals is essential for embryonic development, and it exhibits the highest dinucleotide degradation activity, which if not achieved can erroneously initiate dinucleotide-mediated priming of mitochondrial transcription, as demonstrated in Rexo2-knockout mice (Nicholls *et al.*, 2019). Rexo2 depletion in mitochondria also results in a massive accumulation of short RNA species, confirming its roles in scavenging the nanoRNAs produced by the degradosome and clearing the short RNAs generated from RNA processing pathways (Szewczyk *et al.*, 2020). The crystal structure of Rexo2 reveals a similar structure to that of *E. coli* ORN, with both enzymes sharing a homodimeric structure and a classical DEDDh active site binding two magnesium ions in each protomer (**Fig. 5**; Chu *et al.*, 2019). Rexo2 solely interacts with the last two 3′-end nucleotide bases of ssRNA via hydrophobic and $\pi - \pi$ stacking interactions in the DEDDh active site pocket (Chu *et al.*, 2019; Nicholls *et al.*, 2019; Szewczyk *et al.*, 2020). Due to the shallowness of the RNA-binding pocket and these non-sequence-specific interactions, Rexo2 preferentially binds to and degrades short oligonucleotides, particularly dinucleotides, without sequence specificity.

Cooperative RNA Unwinding and Degradation by RNA Degradosomes

In terms of RNA degrading pathways, the degradosome complex is conserved from bacteria to human, exhibiting similar domain arrangements and assembly (**Fig. 4**). The degradosomes of various organisms also share a similar architecture to the eukaryotic exosome that is responsible for degrading mRNA from the 3′ to 5′ end in the cytosol and nucleus upon removal of the 3′-end poly

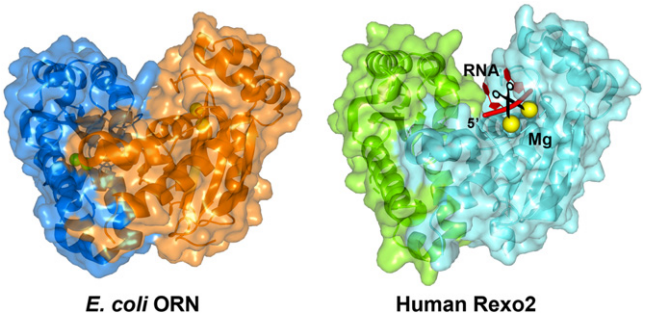

Fig. 5 Crystal structures of the nanoRNA-degrading exoribonucleases *E. coli* ORN (PDB: 2IGI) and human Rexo2 (PDB: 6J7Z). Rexo2, similar to ORN, forms a homodimer and interacts primarily with the last two 3′-end nucleobases of RNA substrates via hydrophobic and $\pi - \pi$ stacking interactions, explaining its preference for binding and degrading short oligonucleotides without sequence specificity. The cleavage site in the RNA is marked by a scissors symbol.

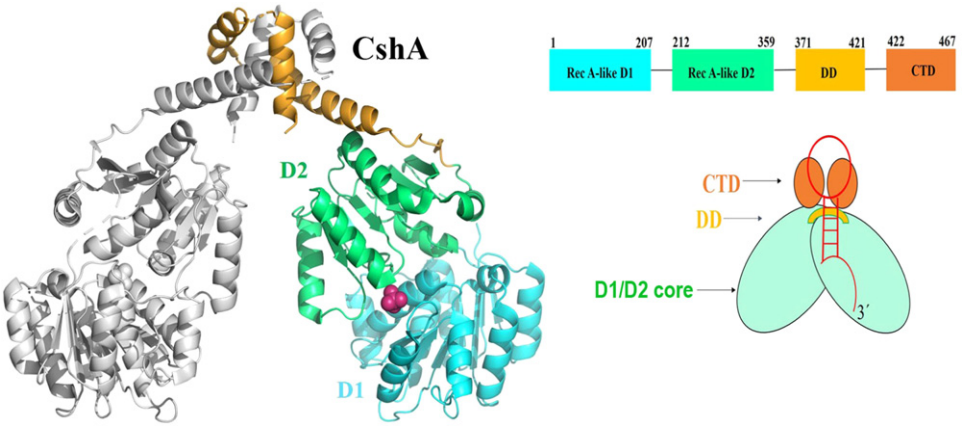

Fig. 6 Crystal structure and domain organization of CshA helicase participating in RNA decay. The crystal structure of the CTD-truncated CshA helicase (PDB: 5IVL) from *GeoBacillus stearothermophilus* reveals a homodimeric V-shaped structure, with the D1-D2 helicase core domains located in the two arms. A sulfate ion displayed in the CPK model is bound in the ATP-binding site of the helicase core domain. A structural model of CshA dimer binding to a single stem-loop RNA is displayed in the right panel, showing that the CTD is important for RNA binding. DD; dimerization domain, CTD; C-terminal domain.

(A) tail of mRNA by deadenylation nucleases (Lin-Chao *et al.*, 2007). PNPase is conserved in almost all species, but is absent among yeast, so RNA processing and decay in yeast mitochondria is performed by a yeast mitochondrial degradosome consisting of Dss1, an RNase II family exoribonuclease, and Suv3 helicase (Dziembowski *et al.*, 2003). In human mitochondria, similar to the bacterial minimal RNA degradosome, PNPase and Suv3 form a pentameric complex for cooperative RNA unwinding and degradation.

Intriguingly, all PNPase-interacting helicases are dimeric enzymes, including RhlB, CshA and Suv3, each of which is an ATP-dependent helicase containing classical RecA-like 1 (D1) and RecA-like 2 (D2) helicase core domains for ATP hydrolysis and RNA remodeling, as well as accessory domains at the N- or C-terminus that participate in RNA interactions or protein-protein interactions (Russell *et al.*, 2013). The crystal structure of CshA from *GeoBacillus stearothermophilus* reveals that it forms a V-shaped dimer via its dimerization domain (DD), with the D1 and D2 helicase core domains of each protomer being located in the two arms (**Fig. 6**; Huen *et al.*, 2017). Unlike most monomeric DEAD-box helicases whose RNA-binding activity is typically greatly enhanced by binding of ATP, CshA binds RNA independently of ATP or ADP. As a result, RNA substrates can be associated with CshA while the D1/D2 core cycles through ATP hydrolysis, resulting in continuous unwinding of the RNA substrate. Thus, dimeric CshA is an ideal partner for the processive activity of PNPase to cooperatively unwind and degrade RNA (**Fig. 7**). This scenario likely explains why a dimeric helicase is preserved in the RNA degradosome for efficient RNA turnover in prokaryotes and eukaryotes. The crystal structure of the 1:1 yeast Dss1: Suv3 complex shows an ssRNA fragment bound in Dss1 (Razew *et al.*, 2018), revealing how Dss1 interacts with Suv3 (**Fig. 7**). However, it remains unknown how the two protomers of dimeric helicases cooperate in RNA unwinding, and how the unwound RNAs are passed from the helicase to PNPase for degradation in bacterial and mammalian mitochondrial degradosomes.

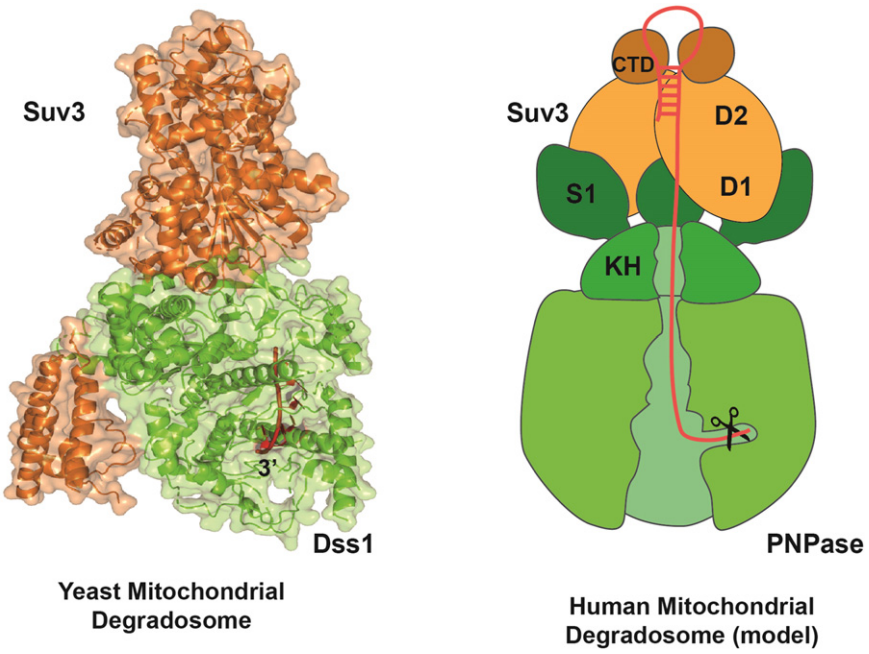

Fig. 7 Crystal structure of the yeast mitochondrial degradosome and a structure model of the human mitochondrial degradosome. The crystal structure of the yeast mitochondrial degradosome (PDB: 6F4A) consists of a monomeric Suv3 helicase in complex with the exorobironuclease Dss1 bound with ssRNA. The human mitochondrial degradosome consists of a dimeric Suv3 helicase (in orange-brown) and a trimeric PNPase (in green). A duplex RNA is unwound by the Suv3 dimer, and the unwound 3′ overhang is threaded into the narrow RNA-binding channel of PNPase for degradation. It remains unclear how Suv3 interacts with PNPase for cooperative RNA unwinding and degradation. The RNA cleavage site in PNPase is marked by a scissors symbol.

Closing Remarks

In summary, it appears that most of the RNA degrading enzymes bind and cleave RNA without sequence specificity, but they exhibit a preference for degrading RNAs of specific structure or length. The ssRNA-specific degrading enzymes usually form narrow RNA-binding channels via assembly of multiple RNA-binding domains or repeated protein subunits, such as the narrow channels observed in RNase II family enzymes and PNPase. These narrow channels prohibit binding of structured RNAs and ensure efficient and processive degradation solely of ssRNAs. Most exoribonucleases alone cannot degrade structured RNA, instead requiring helicases to promote their activities in degrading such RNA species. Bacterial minimal RNA degradosomes and mitochondrial degradosomes consisting of PNPase and its interacting helicase represent examples of such exoribonuclease-helicase complexes responsible for cooperatively unwinding and degrading structured RNA. Many gaps remain in our understanding of RNA-degrading enzymes that warrant further investigation, such as how these degrading enzymes function cooperatively, how they are regulated, and how the modification of RNA substrates affects degradation by these RNA enzymes to fine-tune mRNA half-life.

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