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MINIREVIEW

Trans-acting regulators of ribonuclease activity

Jaejin Lee, Minho Lee*, and Kangseok Lee*

Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

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RNA metabolism needs to be tightly regulated in response to changes in cellular physiology. Ribonucleases (RNases) play an essential role in almost all aspects of RNA metabolism, including processing, degradation, and recycling of RNA molecules. Thus, living systems have evolved to regulate RNase activity at multiple levels, including transcription, post-transcription, post-translation, and cellular localization. In addition, various *trans*-acting regulators of RNase activity have been discovered in recent years. This review focuses on the physiological roles and underlying mechanisms of *trans*-acting regulators of RNase activity.

Keywords: RNase regulator, RNase stability, post-translational regulation of RNase, *trans*-acting regulator, proteolysis

Introduction

Ribonucleases (RNases) play a crucial role in processing and maturation reactions that convert RNA precursors into functional forms, as well as in degrading pathways of mRNA turnover (Green, 1994; Irie, 1997; Dyer and Rosenberg, 2006; Ulferts and Ziebuhr, 2011; Bechhofer and Deutscher, 2019). Over the last half century, many RNases have been discovered and characterized in both prokaryotes and eukaryotes; therefore, our current knowledge of their function and structure has dramatically expanded. They are well conserved not only in prokaryotes (e.g., bacteria) but also in eukaryotes (e.g., fungi, animals, and plants) and even in viruses (Green, 1994; Irie, 1997; Dyer and Rosenberg, 2006; Ulferts and Ziebuhr, 2011; Bechhofer and Deutscher, 2019).

RNases can be classified into two groups: endoribonucleases that cleave RNA molecules internally and exoribonucleases that degrade RNA by removing nucleotides from either the 5′ end or the 3′ end (Fig. 1). These enzymes can be further classified based on additional characteristics, including catalytic mechanism, hydrolysis or phosphorolysis, substrate specificity, action on single-stranded RNA (ssRNA)/double-stranded RNA (dsRNA)/DNA, sequence specificity, mode of action, and processive or distributive manner of cleavage reaction. In addition, recent studies have shown 5′-terminal modifications incorporated at the transcriptional level in *Escherichia coli* (Celesnik *et al.*, 2007; Richards *et al.*, 2012; Cahová *et al.*, 2015; Luciano *et al.*, 2017, 2019; Baek *et al.*, 2019). These 5′ modifications protect mRNAs from endoribonucleases that cleave in a 5′-end-dependent manner (e.g., RNase E) (Celesnik *et al.*, 2007; Richards *et al.*, 2012; Cahová *et al.*, 2015; Luciano *et al.*, 2017, 2019; Baek *et al.*, 2019).

As RNases play an important role in RNA processing and degradation, their activities must be properly regulated. The mechanisms that modulate RNase activity have been identified at various levels, including transcription, post-transcription, post-translation, and cellular localization.

Post-transcriptional autoregulatory feedback is known to be an effective mechanism regulating gene expression. Several endoribonucleases of *E. coli*, including RNase E, RNase III, and polynucleotide phosphorylase (PNPase), are subject to direct autoregulation by cleaving their own transcripts to maintain appropriate cellular levels of these enzymes (Jain and Belasco, 1995; Matsunaga *et al.*, 1996a, 1996b; Sousa *et al.*, 2001; Carzaniga *et al.*, 2009).

In addition, the expression of several RNases is indirectly regulated by other RNases. For example, the precursor M1 RNA encoding the subunit of RNase P is processed at the 3′ end by RNase E (Sim *et al.*, 2002). The cleavage of *pnp* mRNA occurs in the stem-loop of 5′ UTR by RNase III, followed by autodigestion by PNPase (Robert-Le Meur and Portier, 1992; Jarrige *et al.*, 2001). Following this, the remaining *pnp* mRNA is degraded by RNase E (Carzaniga *et al.*, 2009). In the case of *rnb* mRNA encoding RNase II, the mRNA is degraded by PNPase in conjunction with RNase E, thus limiting RNase II expression (Zilhão *et al.*, 1995, 1996). More recently, it has been revealed that RNase III cleaves in the coding region of *rng* mRNA, thus controlling RNase G expression (Song *et al.*, 2014). These findings suggest that primary transcripts encoding RNases can be regulated not only by autoregulation but also by diverse combinations of other RNase actions.

Cellular localization also contributes to the regulation of RNase activity in *E. coli*. RNases can act easily only when their substrates are accessible. Therefore, if RNases are compartmentalized into periplasm or membrane or are secreted into the extracellular space, their potential substrates that exist in the cytoplasm can avoid being degraded by not being accessible. For example, RNase I, which degrades RNA molecules

*For correspondence. (K. Lee) E-mail: kangseok@cau.ac.kr; Tel.: +82-2-820-5241; Fax: +82-2-825-5206 / (M. Lee) E-mail: w3110@naver.com

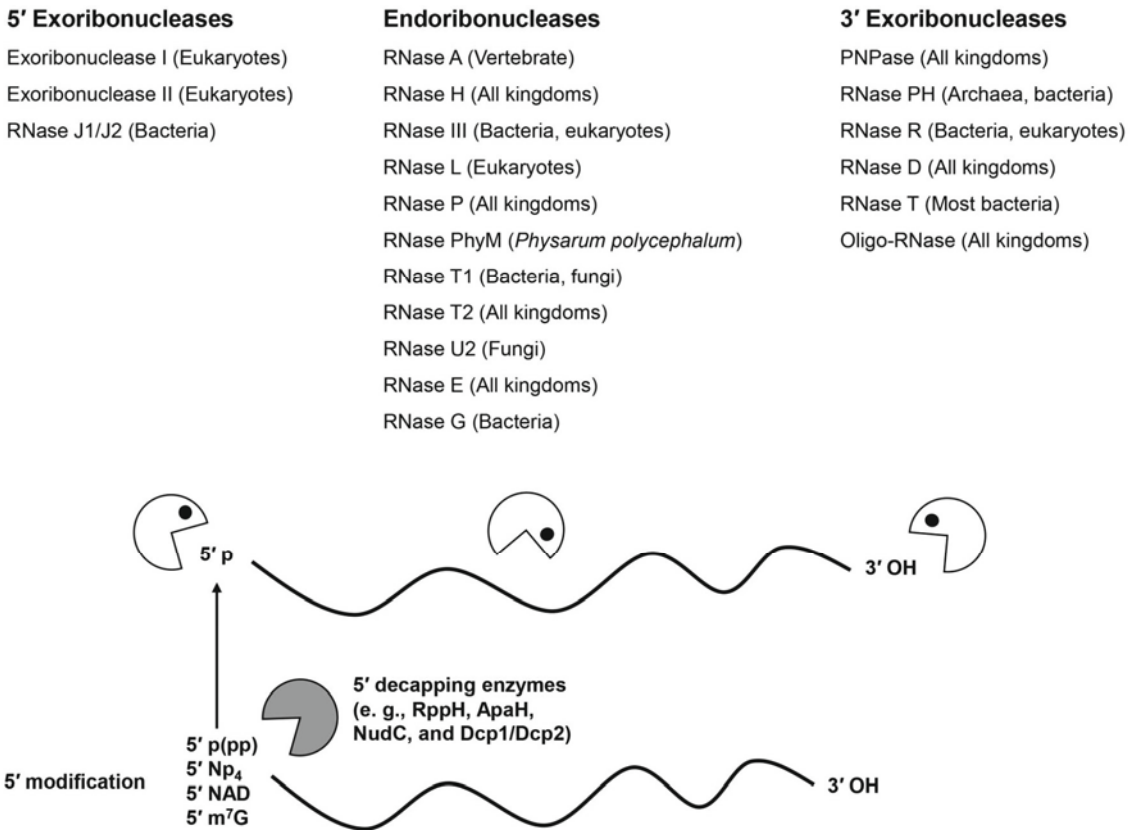

Fig. 1. Schematic diagram of the major types of RNases. Representatives of endoribonucleases and 5′ to 3′ and 3′ to 5′ exoribonucleases are listed on the top of the illustration showing their cleavage characteristics. In addition, 5′ modifications and enzymes that cleave them are shown.

regardless of specificity, is mainly localized into the periplasmic space, thus avoiding interaction with potential substrates. However, when the membrane is damaged by spheroplasting, osmotic stress, or chemicals, RNase I can enter the cytoplasm, leading to excessive RNA degradation and promoting cell death (Neu and Heppel, 1964b, 1965; Abrell, 1971; Lambert and Smith, 1976).

On the other hand, RNase E in *E. coli* and RNase Y in *Bacillus subtilis* are inner membrane-bound proteins (Liou *et al.*, 2001; Hunt *et al.*, 2006; Khemici *et al.*, 2008). These enzymes are components of degradosomes, which are multiprotein complexes involved in RNA metabolism (Kaberdin *et al.*, 2011; Górna *et al.*, 2012; Cho, 2017). The disruption of the transmembrane domain of these enzymes leads to growth retardation or cell death (Khemici *et al.*, 2008; Lehnik-Habrink *et al.*, 2011).

In eukaryotes, post-translational modifications, such as phosphorylation, affect the cellular location of Drosha, a member of the RNase III family, which cleaves the primary microRNA (pri-miRNA) to release a short hairpin microRNA (pre-miRNA) (Lee *et al.*, 2003a; Denli *et al.*, 2004; Gregory *et al.*, 2004; Han *et al.*, 2004). Phosphorylation of Drosha is mediated by two protein kinases: p38 mitogen-activated protein kinase (MAPK) and glycogen synthase kinase 3 beta (GSK3β) within the N-terminal domain of Drosha. However, these two protein kinases are involved in opposing processes (Tang *et al.*, 2011; Yang *et al.*, 2015).

Among a variety of processes and mechanisms regulating RNase activity, various *trans*-acting regulators that stimulate or inhibit RNase activity by direct interaction have been recently identified (Table 1).

In *E. coli*, the *trans*-acting RNase regulators include RNase E regulators, the regulator of ribonuclease activity A and B (RraA and RraB), ribosomal protein L4, RNA chaperone Hfq, amidase C (AmiC), two viral proteins, RNase III regulators YmdB and T7 protein kinase (T7PK), RNase R regulator peptidyl-lysine N-acetyltransferase (Pka). In addition, Dip (degradosome interacting protein) of giant phage and exoribonuclease II were identified as regulators that inhibit RNase E activity in *Pseudomonas aeruginosa* and cyanobacterium *Anabaena*, respectively. The barstar protein against the extracellular RNase barnase was also well characterized in *Bacillus amyloliquefaciens*. (Hartley, 1988, 1989). Regulators generally occlude substrate binding and/or catalytic centers or lead to conformational changes to efficiently modulate the catalytic activity via post-translational modification. Although the mechanism of action of these regulators are similar, they are not significantly similar from an evolutionary point of view (Fig. 2).

On the other hand, phosphorylation-based RNase regulators can occasionally regulate multiple RNases. Protein kinase of T7 bacteriophage (T7PK) phosphorylates both RNase E and RNase III, but the results are entirely different. This is because the former causes decreased RNase E activity by pho-

Table 1. ***Trans*-acting RNase regulators and their properties**

Ribonuclease regulator	MW (kDa)	Target ribonuclease	Mechanism of action	Physiological aspect	K_D (M)	References
Bacterial RNase regulator						
RraA	17	EcRNase E	inhibits the catalytic activity by occluding RNA binding domain	globally regulates the RNA abundance	3.2×10^{-10}	Lee *et al.* (2003b) Gao *et al.* (2006) Górna *et al.* (2010)
RraB	15	EcRNase E	inhibits the catalytic activity by occluding RhlB binding domain	globally regulates the RNA abundance, but shows distinct transcript profiles compared to that of RraA	2.82×10^{-7}	Gaó *et al.* (2006)
L4	22	EcRNase E	inhibits the catalytic activity by occluding RhlB and PNPase binding site	selectively stabilizes stress-responsive and some other transcripts	ND	Singh *et al.* (2009)
T7 protein kinase*	41	EcRNase E EcRNase III	inhibits RNase E activity by phosphorylation of CTH stimulates RNase III activity by enhancing product release	stabilizes mRNAs that are synthesized by T7 RNAP facilitates the processing of the early polycistronic mRNA of phage T7	ND	Mayer and Schweiger (1983) Marchand *et al.* (2001) Gone *et al.* (2016)
Srd*	29	EcRNase E	stimulates the catalytic activity by binding to the catalytic domain	contributes to the growth of T4 phage	ND	Qi *et al.* (2015)
Dip*	31	PaRNase E EcRNase E	inhibits the catalytic activity by occluding RNA binding domain	stabilizes cellular RNA *in vivo*	ND	Van den Bossche *et al.* (2016)
AnaRNase II	87	AnaRNase E	stimulates the catalytic activity by binding to catalytic region	ND	2.77×10^{-6}	Zhou *et al.* (2020)
YmdB	18	EcRNase III	inhibits the dimerization by binding to the catalytic domain	may help to cope with the cold shock stress	6.1×10^{-10}	Kim *et al.* (2008) Paudyal *et al.* (2015)
Barstar	10	Barnase	inhibits the catalytic activity by blocking the active site	protects intracellular RNAs from Barnase until it is secreted	10^{-14}	Hartley (1988, 1989, 1993) Guillet *et al.* (1993)
Pka	97	EcRNase II EcRNase R	inhibits substrate binding of RNase II stimulates binding of tmRNA-SmpB to RNase R for proteolysis	may contribute to the cell's response to stress conditions	ND	Liang *et al.* (2011) Song *et al.* (2016)
AmiC	45	EcRNase E	stimulates the multimerization of RNase E	selectively affects the specific activity of RNase E on the length of RNA substrates	ND	Moore *et al.* (2021)
Mammlian RNase regulator						
RI	49	RNase A superfamily	inhibits the catalytic activity by blocking active site	protects the cell from invading ribonucleases and regulates the activity of intracellular ribonucleases	10^{-15}	Lee and Vallee (1993) Rutkoski and Raines (2008)
TRBP	40	Dicer	promotes the processing activity by interacting with helicase domain	enhances RNA silencing	2.8×10^{-9}	Chendrimada *et al.* (2005) Haase *et al.* (2005) Macrae *et al.* (2008) Wang *et al.* (2009) Wilson *et al.* (2015)
PACT	39	Dicer	promotes the processing activity by interacting with helicase domain	enhances RNA silencing	ND	Lee *et al.* (2006) Wilson *et al.* (2015)
ADAR1	110	Dicer	increases the cleavage rate of pre-miRNA or pri-siRNA by interacting with helicase domain	generates significantly mature miRNAs or siRNAs	ND	Yang *et al.* (2006) Ota *et al.* (2013)
5Lo	78	Dicer	influences the clevage pattern of pre-miRNA by binding to DSB	may contribute to inflammatory processes in human cells	ND	Rådmark *et al.* (2007) Dincbas-Renqvist *et al.* (2009)
CLIMP-63	63	Dicer	increases the stability and assists transition by interacting with helicase domain	involves in maintaining Dicer levels in human cells	ND	Pepin *et al.* (2012)
HCV core protein*	21	Dicer	inhibits the processing activity	may contribute to the viral infection and pathogenesis of HCV	ND	Chen *et al.*, (2008)
HIV-1 protein R*	14	Dicer	stimulates the proteasomal degradation by recruiting the ubiquitin-ligase complex	enhances the infectivity of HIV-1 in macrophage	ND	Klockow *et al.*, (2013)
ERK	ND	Dicer	inhibits the catalytic acitivity during the oocyte-to-embryo by the phosphorylation of RNase IIIb and dsRBD	contributes to transition of oocyte to embryo in *C. elegans*	ND	Drake *et al.* (2014)

Table 1. Continued

Ribonuclease regulator	MW (kDa)	Target ribonuclease	Mechanism of action	Physiological aspect	K_D (M)	References
Mammlian RNase regulator						
Ubc9	18	Dicer	inhibits the processing activity by SUMOlyation	promotes the pathophysiologic phenotype of macrophage	ND	Gross *et al.* (2014)
p38 MAPK	41	Drosha	export Drosha to cytoplasm for subsequent degradation by phosphorylation	promotes cell death under H_2O_2 stress in human cells	ND	Yang *et al.* (2015)
p300, CBP, GCN5	264, 101, 93	Drosha	increases the protein stability by acetylation	may involve in Drosha homeostasis in human cells	ND	Tang *et al.* (2013)
MDM2	55	Drosha	stimulates the protein degradation by ubiquitination	promotes cell death under energy-deprived conditions in human cells	ND	Ye *et al.* (2015)
Plant RNase regulator						
SLF	44	S-RNase	stimulates the ubiquitination of S-RNase for 26S proteasome-mediated degradation	prevents the self-incompatibility	ND	Williams *et al.* (2015)
Apple RNase inhibitor	63 and/or 56	RNase A ?	Unknown	ND	5×10^{-8}	Kosuge *et al.* (2003)

Abbreviations: *E. coli* RNase E; EcRNase E, *E. coli* RNase III; EcRNase III, *E. coli* RNase II; EcRNase II, *E. coli* RNase R; EcRNase R, *P. aeruginosa* RNase E; PaRNase E, and *Anabaena* RNase E; AnaRNase E.
*Viral proteins that originate from bacteriophage T7, T4, ϕKZ, HCV, and HIV-1, respectively.
ND, not determined.

sphorylation of the C-terminal half (CTH), while the latter lead to the stimulation of RNase III activity by enhancing the substrate release. In the case of phosphorylation of RNase II and RNase R by Pka, the RNases are inactivated through a different mechanism in which the substrate binding affinity of RNase II is inhibited, and the binding of transfer messenger RNA (tmRNA)–SmpB for the degradation of RNase R by HslUV and Lon protease is stimulated.

In mammals, since the inhibitor of the pancreatic superfamily RNase was first inferred in 1952, the characteristics of such RNase inhibitors (RIs) were extensively investigated (Dickson *et al.*, 2005). This cytoplasmic RI strongly interacts with pancreatic RNase A at 1:1 stoichiometry and renders it inactive by steric occlusion of the catalytic site (Lee and Vallee, 1993; Shapiro, 2001; Dickson *et al.*, 2005; Rutkoski and Raines, 2008). Although the structure of this RI is well characterized, its biological roles remain unclear. For Drosha and Dicer, which are RNase III family enzymes, various protein regulators have been discovered (Table 1). Similar to those in bacteria, some regulators influence the activity of RNase III family enzymes by mediating proteolysis or post-translational modifications.

In plants, S-locus F-box protein (SLF) is responsible for the degradation of nonself S-locus ribonuclease (S-RNase) to prevent self-incompatibility (SI) (Williams *et al.*, 2015). Further, apple RIs have been isolated and partially characterized (Kosuge *et al.*, 2003).

In this review, we summarize the features and mechanisms of RNase regulator proteins that have been characterized in bacteria, mammals, and plants. A broad overview of *trans*-acting regulators controlling RNase activity will provide clues on how organisms have evolved to regulate RNases for their physiological needs.

Bacterial RNase regulators

Regulators of RNase E

RNase E is a multifunctional endoribonuclease that occupies an irreplaceable position in the control of gene expression, including in processing of ribosomal RNA (rRNA), transfer RNA (tRNA), and turnover of a large portion of messenger RNA (mRNA) (Gegenheimer *et al.*, 1977; Jain *et al.*, 2002; Lee *et al.*, 2002; Li and Deutscher, 2002). It is now generally accepted that RNase E is the initiating enzyme for mRNA decay in *E. coli* (Bandyra and Luisi, 2018). It has been recently found to play a pivotal role in the degradation of rRNA during quality control and starvation (Sulthana *et al.*, 2016).

The essential protein RNase E can be divided into two distinct halves. The N-terminal half (NTH) of RNase E (residues 1-529) contains a catalytic domain consisting of an RNase H domain, S1 RNA-binding domain, and a 5′ monophosphate sensor domain (Fig. 3) (McDowall and Cohen, 1996; Bycroft *et al.*, 1997; Callaghan *et al.*, 2005a). The NTH can be divided into two subdomains connected via a Zn link: a large domain (residues 1–400), Zn-link (residues 401–414), and a small domain (residues 415–529). Among these, the small domain serves as a dimerization interface (Fig. 3) (Callaghan *et al.*, 2005a; Koslover *et al.*, 2008). The CTH of the protein (residues 530–1,061) contains noncatalytic domains,

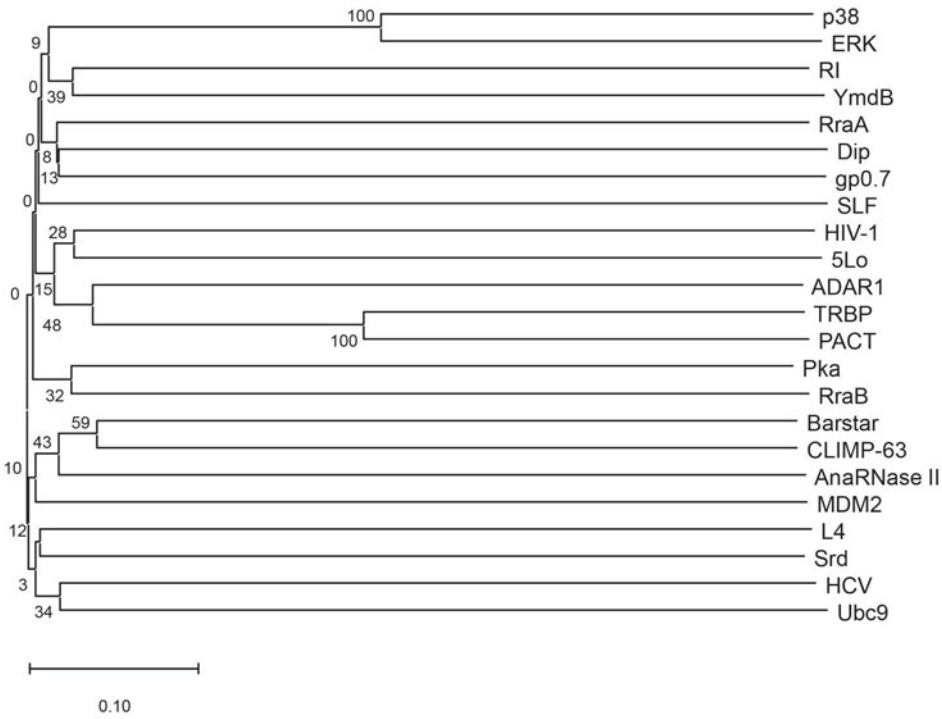

Fig. 2. The phylogenetic tree of RNase regulators. The amino acid sequences of RNase regulators were obtained from the UniProt protein database (https://www.uniprot.org). The tree was constructed using MEGA-X software with the neighbor-joining method. The numbers on branches are shown in percentages from 10,000 bootstrap replicates (Na, 2020).

including the microdomain called segment A required for the interaction between RNase E and the inner cell membrane (Khemici *et al.*, 2008), two arginine-rich regions associated with RNA-binding (McDowall and Cohen, 1996) and a scaffold domain (residues 650–1,061) for binding multiple protein partners. The scaffold protein binds to RNA helicase (RhlB), PNPase, and the glycolytic enzyme enolase that forms the RNA degradosome complex (Fig. 3) (Miczak *et al.*, 1996; Py *et al.*, 1996; Kaberdin *et al.*, 1998). RNase E is a membrane-bound protein. Electron microscopy data show that the N-terminal region (residues 1–602) is sufficient for membrane localization but not for binding to the degradosome component RhlB, as the scaffold domain that binds to RhlB is located in the CTH (Liou *et al.*, 2001). The interaction of the N-terminal catalytic domain with the membrane increases the structural stability and RNA substrate affinity of the RNase (Murashko *et al.*, 2012).

In vivo, the active RNase E exists as a tetramer composed of dimerized dimers. The tetramer constitutes two symmetrical RNA-binding channels, enabling it to cleave the substrate efficiently in the absence of a 5′-monophosphorylated end. Each dimer is stabilized by coordinating Zn^{2+} in a central cluster region called the Zn-link, which constitutes the core of the membrane-associated degradosome complex (Fig. 3B) (Callaghan *et al.*, 2005a).

In *E. coli*, a paralog of RNase E, RNase G, which is highly similar to the N-terminal catalytic domain of RNase E, is present (Li *et al.*, 1999; Wachi *et al.*, 1999; Tock *et al.*, 2000; Lee *et al.*, 2019b).

Regulator of ribonuclease activity A: Among various types of RNase E regulators, the regulator of ribonuclease activity A (RraA) is a small protein of 17 kDa that has been extensively studied as a *trans*-acting regulator of RNase E in *E. coli*. It was originally annotated as a methyltransferase enzyme involved in menaquinone biosynthesis on the basis of sequence analysis. However, studies by Lee *et al.* (2003b) and Monzingo *et al.* (2003) showed that it lacks both structural and functional ability of working as a methyltransferase. Lee *et al.* (2003b) first identified a novel function of RraA based on genetic screening; in this study, researchers showed that RraA expression affects the stability of *dsbC* mRNA (encoding the disulfide isomerase DsbC), which is known as an RNase E substrate. *In vitro* experimental data showed that RraA physically binds to RNase E and inhibits its activity; however, RraA does not interact with the RNA substrates or interfere with the substrate binding activity of RNase E. Although the C-terminal domain of RNase E is required for the high-affinity interaction of RraA with RNase E, RraA also weakly inhibits the activity of the NTH of RNase E. Based on the experimental data through mass spectrometry and X-ray structure, RraA biologically exists as a trimer or dimer of trimers (Monzingo *et al.*, 2003; Górna *et al.*, 2010).

Using deletion analyses and surface plasmon resonance, researchers have identified three sites at which RraA binds to RNase E, which include two alternative RNA-binding domains (RBDs) (Górna *et al.*, 2010). The three sites are RBD, arginine-rich region 2 (AR2), and the helicase binding site (Fig. 4). However, the interactions of RraA with RNase E predominantly occur in the RBD and AR2 and not in the RhlB binding site. According to the proposed model, the negatively charged surface of RraA interacts with positively charged peptides such as AR2 and the C-terminal tail of RhlB, facilitating protein remodeling via the ATPase activity of RhlB in the presence of ATP (Górna *et al.*, 2010). This model was supported by previous studies showing that the interactions between RraA and RNase E influence the degradosome com-

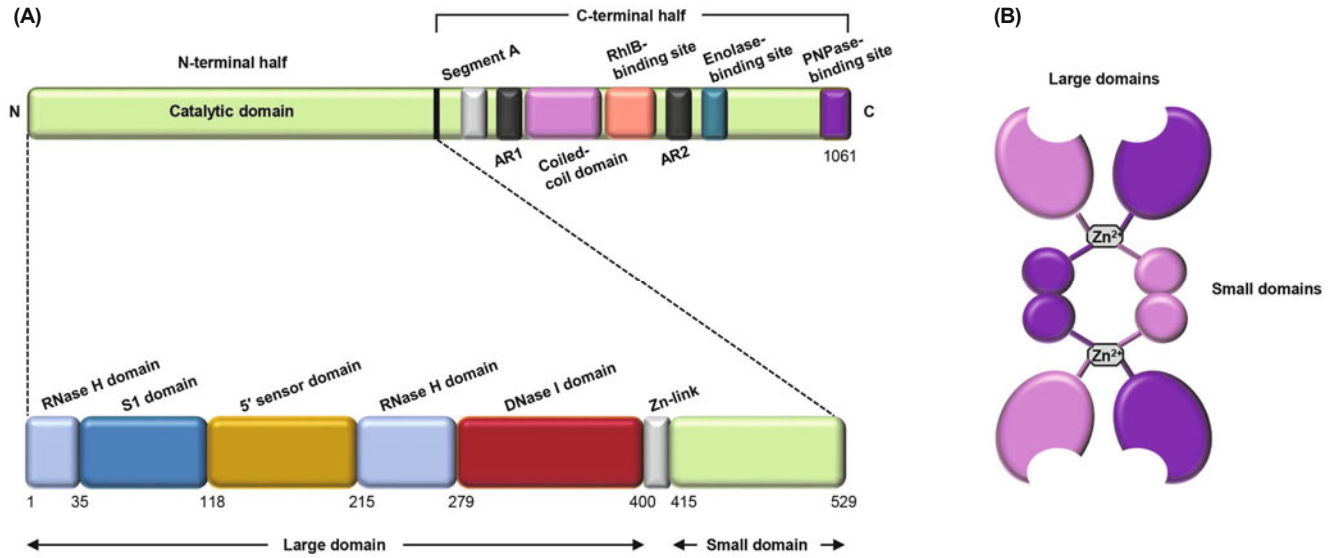

Fig. 3. The Structure of RNase E. (A) The primary structure of RNase E monomer (1,061 amino acid residues). The catalytic domain can be divided into two groups (large domain; residues 1–400 and small domain; residues 415–529). The scaffold domain contains binding sites for multiple interacting proteins: segment A, the membrane-binding domain, residues 565–585; coiled-coil domain, residues 633–712; helicase RhlB, residues 698–752; enolase, residues 823–847; and PNPase, residues 1,021–1,061. The arginine-rich segments (AR1, residues 597–684 and AR2, residues 796–814) probably participate in RNA binding. (B) RNase E exists primarily as a tetramer, composed of a dimer of dimers.

position, which in turn modulates its activity (Lee *et al.*, 2003b; Gao *et al.*, 2006). Recently, it has been reported that RraA can interact with, and modulate the activity of not only RhlB but also another DEAD-box RNA helicase, SrmB (Pietras *et al.*, 2013). An *in vitro* structural analysis has suggested that the negatively charged surface of RraA is commonly used for binding with RhlB and SrmB (Pietras *et al.*, 2013). The models suggest two distinct mechanisms by which RraA might modulate the activity of these and potentially other helicases.

Regulator of ribonuclease activity B: RraB (regulator of ribonuclease activity B), a second regulator of RNase E, is a 15.6 kDa protein that binds the C-terminal domain of RNase E, resulting in the protection of a subset of substrates from the action of RNase E *in vitro* and *in vivo* (Gao *et al.*, 2006). Although RraA homologs are largely distributed in plant and Archaea, as well as γ-proteobacteria, RraB is found only in γ-proteobacteria, suggesting that this inhibitor has a distinct role in regulating RNA degradation (Monzingo *et al.*, 2003; Yeom *et al.*, 2008a). The CTH of RNase E is required for the inhibitory effect of RraB, which exerts differential effects on the global abundance of mRNA through degradosome composition remodeling (Gao *et al.*, 2006). In a study on RraB structure, the protein eluted as a homodimer in size exclusion chromatography, and its crystal structure was determined (Shen *et al.*, 2013). The K_D value of RraB to full-length RNase E is 2.82×10^{-7} M (Gao *et al.*, 2006), while it is 2.6×10^{-6} M for the complex formation between RraA and full-length RNase E (Lee *et al.*, 2003b). However, Górna *et al.* (2010) showed that the inhibition constant of RraA with the CTH of RNase E is in the nanomolar range and demonstrated that such weak binding constants resulted from the nonspecific amine coupling, which may occlude some of the potential interaction sites.

In addition, several studies have shown that the orthologs of RraA, RraB, and RNase E can function in an interspecies manner, indicating that these enzymes appear to be evolutionarily conserved in a distantly related bacterial species (Yeom *et al.*, 2008a, 2008b; Lee *et al.*, 2009).

L4 Ribosomal protein: Although ribosomal proteins (r-proteins) are mainly involved in ribosome assembly and protein translation, certain prokaryotic and eukaryotic r-proteins have extraribosomal functions (Wool, 1996; Aseev and Boni, 2011). For example, L4, which is a component of the 50S subunit, affects RNase E endonucleolytic activity by interacting with the CTH of RNase E in *E. coli* (Singh *et al.*, 2009). The r-protein L4 is known to repress its transcription and translation by two distinct nonoverlapping domains (Lindahl and Zengel, 1979; Yates and Nomura, 1980). However, these functional domains are not necessary for interacting with RNase E. *In vitro* cleavage assays showed that RNase E-mediated cleavage of oligonucleotide BR13, which is derived from RNA I (Lin-Chao and Cohen, 1991), decreased upon L4 binding. However, L4 does not inhibit the activity of an RNase E variant protein containing only the N-terminal region of RNase E, suggesting that the CTH of RNase E is essential for the L4-dependent inhibitory effect. It appears to inhibit RNase E cleavage activity on a specific subset of mRNAs. Microarray and northern blot analyses have revealed that the abundance of several stress-responsive transcripts increased because of the L4-mediated inhibition of RNase E activity (Singh *et al.*, 2009). It has been reported that the free r-proteins including L4 are elevated in response to various stresses (e.g., high temperature or starvation for amino acids, nitrogen, phosphate, or a carbon source) (Kaplan and Apirion, 1975). Thus, the inhibition of RNase E activity might occur due to the accumulation of L4 under the above stress conditions, which, in turn, facilitates adaptation of bacteria to environmental changes. Similar to the properties of RraA

and RraB, L4 contributes to the stabilization of selective groups of transcripts, implying that these proteins do not act as general inhibitors of RNase E. Unlike RraA and RraB, L4-dependent inhibition does not influence the degradosome composition or its remodeling (Lee *et al.*, 2003b; Gao *et al.*, 2006; Singh *et al.*, 2009).

Hfq: RNase E also plays a crucial role in the sRNA-mediated degradation of specific mRNAs (Masse *et al.*, 2003; Pfeiffer *et al.*, 2009), and a specialized RNase E-small regulatory RNA (sRNA)-Hfq complex assembly has been suggested as an alternative degradosome, which is distinct from the major RNA degradosome composed of RNase E, enolase, RhlB, and PNPase (Morita *et al.*, 2005; Aiba, 2007). Hfq binds to the C-terminal region of RNase E complexed with SgrS sRNA under phosphoglucose stress. This complex facilitates the efficient degradation of *ptsG* mRNA by the action of RNase E (Morita *et al.*, 2005; Worrall *et al.*, 2008). Therefore, Hfq may also be considered a protein regulator of RNase E activity that acts by targeting specific sRNA-mRNA bases for rapid RNase E-mediated degradation (Masse *et al.*, 2003; Morita *et al.*, 2005; Prévost *et al.*, 2011). Although the binding of Hfq and RNase E was originally considered to be a direct protein-protein interaction, recent studies have indicated that the interaction appears to be mediated by sRNA (e.g., MicC) (Worrall *et al.*, 2008; Bruce *et al.*, 2018).

AnaRNase II: Cyanobacteria are evolutionarily close to plant chloroplasts (Martin *et al.*, 2002; Jensen and Leister, 2014). Although the RNA metabolism of cyanobacteria has not been well studied, homologs of RNase E are present in cyanobacterial strains (Zhang *et al.*, 2014). In *E. coli*, RNase E can recruit the exoribonuclease RNase II to form an alternative degradosome complex that controls cellular RNA turnover. (Lu and Taghbalout, 2014). Similar degradosome complexes associated with exoribonucleases have been found in other bacteria (Purusharth *et al.*, 2005; Hardwick *et al.*, 2011; Voss *et al.*, 2014); however, their compositions differ among bacterial species (Fig. 4). Recently, it was reported that RNase II of a cyanobacterium, *Anabaena*, (AnaRNase II) is associated

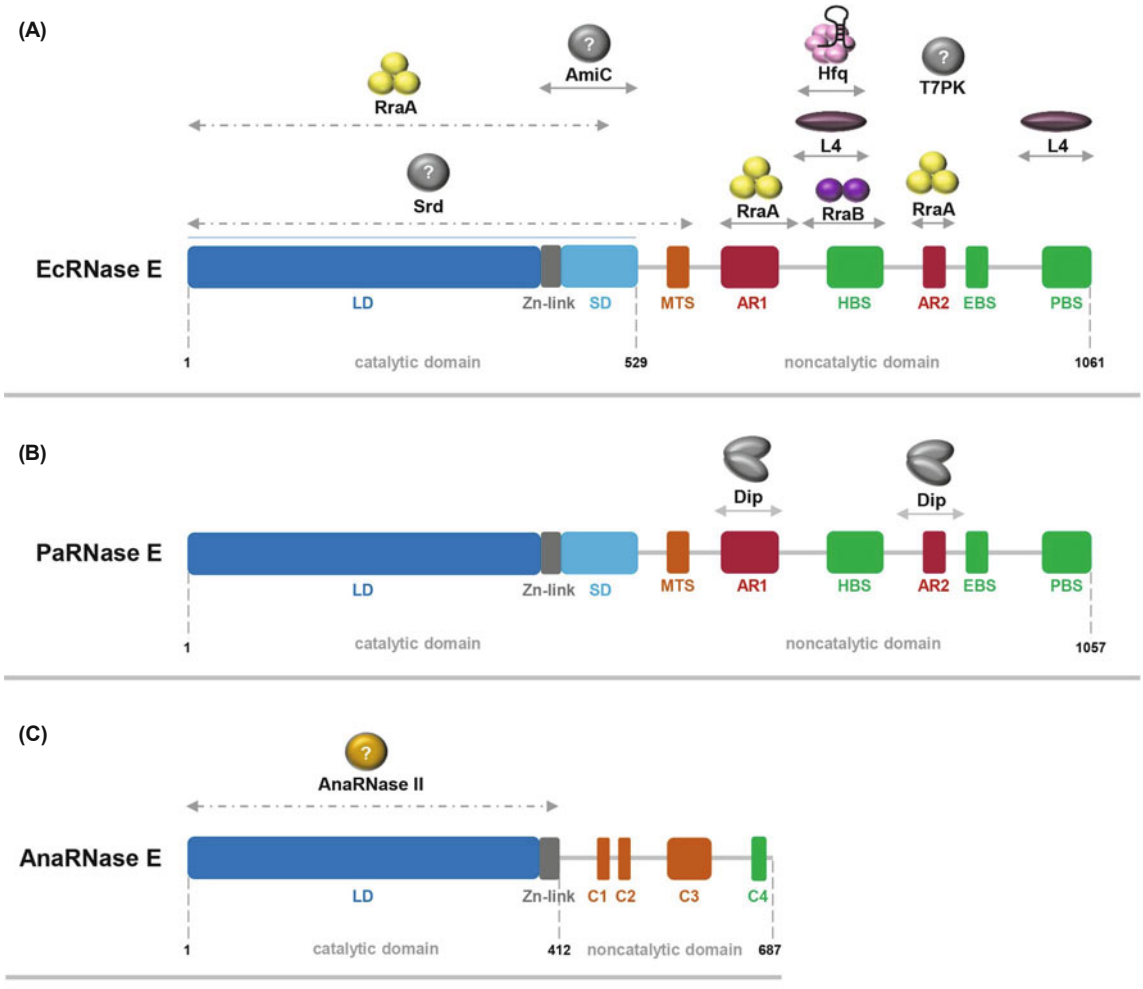

Fig. 4. Schematic diagrams of RNase E homologs and binding sites of regulators. (A) The domain structure and binding partner interaction sites of *E. coli* RNase E. LD, large domain; SD, small domain; MTS, membrane targeting sequence; AR1 and 2, arginine-rich region 1 and 2; HBS, helicase binding site; EBS, enolase binding site; PBS, PNPase binding site. (B) The domain structure and binding partner interaction sites of *P. aeruginosa* RNase E showing the interaction of Dip in AR1 and AR2. (C) *Anabaena* RNase E can interact with AnaRNase II in the catalytic domain. C1–C4 are the subregion conserved across all sequenced cyanobacterial strains. C4 is the PNPase binding site in *Anabaena* RNase E.

with a degradosome, which enhances its RNase E (AnaRNase E) activity (Fig. 4C) (Zhou *et al.*, 2020). Notably, in contrast to other ribonucleases interacting with the CTH of EcRNase E, AnaRNase II binds to the NTH of AnaRNase E, whereas PNPase also binds to the noncatalytic region via a specific nonapeptide of cyanobacterium (Zhang *et al.*, 2014). Mutational analysis of the AnaRNase II domain revealed that two cold-shock domains (CSD) near the N-terminal region and the S1 domain located in the C-terminal region bind to the NTH of AnaRNase E. The interaction of AnaRNase II with AnaRNase E results in significantly faster degradation of the synthetic substrate compared to that of AnaRNase E alone, indicating that the exoribonuclease AnaRNase II and AnaRNase E cooperatively degrade the RNA substrates *in vivo*. Moreover, two ribonucleases are co-localized in the *Anabaena* cytoplasm, which is distinct from the membrane-associated EcRNase II and EcRNase E (Khemici *et al.*, 2008; Lu and Taghbalout, 2013). However, the mechanisms underlying the functions of RNase II in cyanobacteria remain unclear.

Amidase C: In a recent study, Amidase C (AmiC), a bacterial cell wall peptidoglycan hydrolase, has been identified as another positive regulator of RNase E (Moore *et al.*, 2021, In press). AmiC positively regulates the RNase E activity by physically interacting with the C-terminal end of gene encoding the NTH of RNase E, which contains both the Zn-link and small domain. Previous crystallographic studies showed that these regions are required for multimer formation of RNase E, using the RNase E variant, Rne-395, which lacks that segment (Callaghan *et al.*, 2005a, 2005b; Caruthers *et al.*, 2006). The fact that AmiC has no effect on the substrate cleavage activity of Rne-395 supported that the protein is associated with RNase E multimerization (Moore *et al.*, 2021, In press). This study showed that AmiC is able to enhance the multimerization of another RNase E variant, NTH-Rne (residues 1–529) *in vitro*, indicating that it binds to a region between residues 396–529 of the Rne protein. The cleavage activity of RNase E with AmiC is independent of 5′ phosphorylation state of the substrates, but can be dependent of the substrate's length. These findings suggest that AmiC selectively affects the specific activity of RNase E on the length of RNA substrates by enhancing the formation of RNase E multimerization.

Virus-derived regulators: Although many *trans*-acting regulators of RNases originate from the genomes of host organisms, several viral proteins target the RNA degradation machinery. In the case of RNase E, three phage proteins that affect RNase E activity were identified. These include an RNA degradosome activator (Srd) from bacteriophage T4, a phosphorylation-based inhibitor from bacteriophage T7, and Dip of giant phage ϕKZ (Marchand *et al.*, 2001; Qi *et al.*, 2015; Van den Bossche *et al.*, 2016).

It has been shown that expression of a T7 gene, *0.7*, encoding the protein kinase T7PK in *E. coli*, protects mRNAs that are transcribed by T7 RNA polymerase from RNase E cleavage (Marchand *et al.*, 2001). In these cells, the CTH of RNase E and RhlB are heavily phosphorylated by T7PK. It has been proposed that the phosphorylation of RNase E leads to the inactivation of its RBD and/or inhibition of the formation or function of degradosome, resulting in the stabilization of the host and bacteriophage T7 mRNAs.

Upon bacteriophage T4 infection in *E. coli*, the host mRNAs are rapidly degraded, and this process is mediated by RNase E action (Ueno and Yonesaki, 2004; Qi *et al.*, 2015). Srd (Similarity with *rpoD*) is the first positive regulator of RNase E. It physically interacts with the NTH of RNase E and stimulates the catalytic activity of RNase E (Qi *et al.*, 2015). Srd apparently promotes both 5′-end-dependent and –independent cleavage activities of RNase E either directly or indirectly. However, neither is the domain of RNase E associated with Srd protein nor has the mechanism related to the stabilization of host and T4 phage mRNA been identified.

Finally, a giant phage ϕKZ-encoded protein termed gp37/Dip (Degradosome interacting protein) was identified as an RNase E regulator in *P. aeruginosa*. It directly associates with and inhibits the activity of RNase E (Van den Bossche *et al.*, 2016). The predicted molecular weight of the inhibitor is 31.7 kDa, which has no homologous sequence with any protein registered in databases. Based on the results of affinity purification and mass spectrometry, Dip was shown to bind to two RNA binding sites (residues 583–636 and 756–775) in the CTH of RNase E of *P. aeruginosa*, occluding them from the substrates and leading to effective inhibition of the RNA degradation and processing activity of the degradosome (Fig. 4B). Notably, ϕKZ infection leads to more than a 5-fold increase in total RNA regardless of host transcription (Ceyssens *et al.*, 2014), suggesting that Dip globally affects RNA degradation of *P. aeruginosa*. Moreover, the inhibitory effect was confirmed in *E. coli*, indicating that the inhibition activity of Dip to RNase E might be conserved in other bacteria (Van den Bossche *et al.*, 2016). The 2.2 Å crystal structure revealed that this inhibitor protein exists in a novel homodimeric form and has no detectable structural homologs. Like other RNase regulators such as RraB and L4, the inhibitory effect of Dip only occurs in the presence of CTH of RNase E, implying that the protein may not directly influence the RNase E catalytic activity.

The existence of viral protein regulators suggests that the regulation of RNase E activity might be a common strategy of bacteriophages to support efficient phage infection.

Regulators of RNase III

RNase III is an endonuclease that cleaves dsRNA to yield 5′-phosphates and 3′-hydroxyls with two-nucleotide (nt) 3′ overhangs (Crouch, 1974; Regnier and Portier, 1986; Portier *et al.*, 1987). Members of the RNase III family include bacterial RNase III and eukaryotic RNase III (e.g., Rnt1p, Drosha, and Dicer) (Filippov *et al.*, 2000; Blaszczyk *et al.*, 2004; Ji, 2008). Bacterial RNase III functions as a homodimer (Nicholson, 2014; Lim *et al.*, 2015). It cleaves intramolecular duplexes by the formation of a stem-loop structure in ssRNA and occasionally cleaves complementary dsRNA created by binding antisense RNA to mRNA (Court *et al.*, 2013; Lim *et al.*, 2015; Lee *et al.*, 2019a). In general, the endoribonuclease is responsible for the proper function of rRNA and tRNA via regulated processing or maturation pathways (Young and Steitz, 1978; Gurevitz and Apirion, 1983). There are two RNase III regulators identified so far: YmdB that inhibits dimerization of RNase III and T7 protein kinase that stimulates RNase III action by phosphorylation (Mayer and Schweiger,

1983; Kim *et al.*, 2008). However, its activity and expression have been shown to be regulated under various stress conditions (Sim *et al.*, 2010, 2014; Lim and Lee, 2015; Lee *et al.*, 2019a).

YmdB: The first suggested *trans*-acting regulator of RNase III was identified as an inhibitor of RNase III-mediated p10Sa RNA (tmRNA) processing in *E. coli* crude cell extracts (Makarov and Apirion, 1992). Later, it was shown that YmdB, an evolutionarily conserved 18.8 kDa protein, inhibits RNase III activity *in vivo* and *in vitro* by interacting with the region (residues 120–140) required for the dimerization of RNase III monomers without affecting its dsRNA binding domain (dsRBD) activity (Kim *et al.*, 2008). The interaction model using site-directed mutagenesis confirmed that D128 of RNase III and R40 of YmdB are necessary for heterodimer formation (Paudyal *et al.*, 2015). Like RNase III, YmdB also exists as a dimer, indicating that YmdB can diminish RNase III activity effectively by forming a one-to-one complex. Expression of YmdB is transcriptionally elevated during entry into the stationary phase or cold-shock stress (Kim *et al.*, 2008; Kim and Kim, 2017). This elevation is dependent on *rpoS*, which encodes a σ-factor for the regulation of general stress response (Kim *et al.*, 2008). It was observed that increased expression of YmdB during cold shock downregulates RNase III activity. In addition, overexpression of YmdB inhibits biofilm formation by interacting with RpoS, but it is not dependent on RNase III (Kim *et al.*, 2013; Kim and Kim, 2017).

T7 protein kinase: T7PK phosphorylates both RNase III (Mayer and Schweiger, 1983) and RNase E (Marchand *et al.*, 2001), and the consequences are quite different. The former results in the stimulation of RNase III, whereas the latter leads to decreased RNase E activity. Upon infection with bacteriophage T7, RNase III is phosphorylated by a serine/threonine-specific protein kinase, resulting in an approximately 4-fold increase in RNase III activity. This upregulation of RNase III activity by phage T7 facilitates the cleavage of its early polycistronic mRNA, enabling T7 messages to be processed to the functional form (Mayer and Schweiger, 1983; Robertson *et al.*, 1994). Further *in vitro* biochemical analysis showed that S33 and/or S34 residues of RNase III are the targets of phosphorylation, and they enhance product release without altering RNase III catalytic activity (Gone *et al.*, 2016).

Regulators of RNase I

RNase I is an endonuclease that cleaves phosphodiester bonds between any nucleotides in RNA to yield mononucleotides. The enzyme belongs to the T2 superfamily of RNases, whose members are widely spread throughout nature (Irie, 1997; Condon and Putzer, 2002). RNase I is mostly localized to the periplasmic space and the enzyme has a moderate effect on global mRNA degradation (Neu and Heppel, 1964b; Zhu *et al.*, 1990). However, mutant deficient in RNase I was essentially identical to wild-type with respect to their general biological properties (Dürwald and Hoffmann-Berling, 1968). Forms of RNase I with multiple mutations have slightly different catalytic activities and have been referred to as RNase IV (Spahr and Gesteland, 1968), RNase F (Gurevitz *et al.*, 1982), RNase I (Cannistraro and Kennell, 1991), RNase M (Cannistraro and Kennell, 1989), and RNase R (Srivastava *et al.*, 1992). A recent study suggested that intracellular RNase I (~10% of total) is required for generating 2′, 3′-cyclic nucleotides, which regulate biofilm formation in *E. coli* (Fontaine *et al.*, 2018). However, the physiological role of periplasmic RNase I (~90% of total) is still unclear.

In vitro experimental studies have shown that the 30S ribosomal subunit inhibits RNase I activity in *E. coli* and *Salmonella* Typhimurium (Elson, 1958; Neu and Heppel, 1964a; Datta and Burma, 1972). However, neither 16S rRNA nor total ribosomal proteins appear to inhibit RNase I (Datta and Burma, 1972). Further, mutational and hybrid ribosome studies have shown that this inhibitory effect is not because of ribosomal protein, but it is caused by a direct interaction between RNase I and helix 41 (h41) of *E. coli* 16S rRNA as a ribonucleoprotein particle (Kitahara and Miyazaki, 2011).

Regulators of RNase II

RNase II is a processive 3′-5′ exoribonuclease that hydrolyzes single-stranded RNA to produce nucleotide 5′-monophosphates (Arraiano *et al.*, 2013). It has been proposed that the enzyme accounts for ~90% of the total hydrolytic activity of *E. coli* crude extracts (Nossal and Singer, 1968). Although RNase II is mainly responsible for RNA degradation (Donovan and Kushner, 1986), it also contributes to the processing and maturation of RNA molecules (Li and Deutscher, 1996; Mohanty and Kushner, 2003). Both RNase II and RNase R, which are processive and nonspecific exoribonucleases, are members of the RNR family and typically possess two RBDs at each terminal end (Vincent and Deutscher, 2006). However, their catalytic properties are quite dissimilar since RNase II specifically hydrolyzes ssRNA molecules, whereas RNase R is able to digest dsRNA as well (Cheng and Deutscher, 2005). Little is known about the factors affecting RNase II activity, but it has been suggested that an acetyltransferase and deacetylase pair-Pka and CobB-can regulate RNase II (Song *et al.*, 2016).

According to a recent study, Pka is responsible for the acetylation of lysine residues in a large number of proteins that have been identified by mass spectrometry (Christensen *et al.*, 2018). Among these, RNase II and RNase R were studied individually as targets of acetylation, which influences the stability of RNase R and the substrate-binding activity of RNase II in *E. coli* (Liang *et al.*, 2011; Song *et al.*, 2016).

Regulation of RNase II activity has been shown to occur via acetylation by Pka and deacetylation by CobB at residue L501 within the catalytic center. The acetylation of RNase II affects its substrate-binding activity by partially blocking the RNA channel, thus reducing its catalytic activity; however, it does not influence protein stability (Song *et al.*, 2016). Additionally, recent proteomics analysis revealed that L31, L68, L107, and L501 residues of RNase II are acetylated (Colak *et al.*, 2013; Weinert *et al.*, 2013; Zhang *et al.*, 2013). It is assumed that these are located in the N-terminal RBD (S1 domain), which is responsible for a clamp-like arrangement with a C-terminal RBD to accommodate only ssRNA (Zuo *et al.*, 2006). Under starvation conditions, the level of RNase II is decreased (Cairrão *et al.*, 2001) and its acetylation is elevated (Song *et al.*, 2016), leading to the inhibition of RNase II activity. It has been suggested that the absence

of RNase II leads to increased degradation of rRNAs, enabling other exoribonucleases to bind with rRNA to initiate degradation (Basturea *et al.*, 2011). Presumably, the inhibition of RNase II by acetylation in concert with reduced protein amounts during limited nutrient conditions may allow rRNA to degrade for recycling nucleotides.

Regulators of RNase R

RNase R is a member of the RNR family along with RNase II. It cleaves ssRNA in the 3′ to 5′ direction, and it is widely distributed in different organisms (Arraiano *et al.*, 2013). Unlike RNase II, RNase R can digest structured RNA due to tight interaction between the catalytic domain and RNA; moreover, the RBD in the C-terminal region contributes to this function (Vincent and Deutscher, 2009a, 2009b). RNase R participates in the degradation of defective rRNA fragments during quality control processes and starvation (Zundel *et al.*, 2009; Basturea *et al.*, 2011). It is also involved in removing mRNA during *trans*-translation (Richards *et al.*, 2006; Liang and Deutscher, 2013; Domingues *et al.*, 2015). RNase R abundance increases due to its stabilization under stress conditions such as cold shock, stationary phase, and starvation (Cairrão *et al.*, 2003; Chen and Deutscher, 2005; Andrade *et al.*, 2006). In exponential phase cells the stability of RNase R is determined by direct interaction with tmRNA and SmpB protein in its C-terminal region (Liang and Deutscher, 2010). A subsequent study revealed that the binding of tmRNA-SmpB to RNase R is much tighter in the exponential phase than in the stationary phase due to the acetylation of K544 by Pka (Liang *et al.*, 2011). Consequently, the interaction of tmRNA-SmpB with RNase R stimulates its proteolysis by HslUV and Lon proteases that bind to the N-terminal region of RNase R (Liang and Deutscher, 2012b). RNase II is regulated by acetylation and deacetylation; however, the deacetylase CobB appears to be not involved in the loss of RNase R acetylation in the stationary phase (Liang and Deutscher, 2012a).

RNase R during the exponential phase is post-translationally modified by Pka. Mutational analysis at position 544 showed that the conversion of K to R stabilized the enzyme, while conversion to A destabilized the enzyme during the exponential phase (Liang *et al.*, 2011). Therefore, a positive charge at this position results in the stabilization of RNase R, whereas no charge leads to destabilization. Based on these data, researchers proposed a model in which the acetylation/deacetylation of this residue affects the binding of tmRNA-SmpB to RNase R (Liang *et al.*, 2011). This model was also supported by the structural analysis of RNase R (Venkataraman *et al.*, 2014). The binding of tmRNA-SmpB stimulates the binding of HslUV and Lon protease to the N-terminal region of RNase R, prompting proteolytic degradation (Liang and Deutscher, 2012b). Under cold shock stress and stationary phase, Pka is not present, leading to increased stability of newly synthesized RNase R. Moreover, the deacetylation process does not involve the acetyl group of RNase R, and thus the primary determinant for increased protein stability is only a decrease in the activity of an acetylating enzyme (Chen and Deutscher, 2010; Liang and Deutscher, 2012a). Similar to RNase II, the main effect of the acetylation of RNase R is likely due to the neutralization of the positive charge (Chen and Deutscher, 2010; Song *et al.*, 2016), implying a conformational change in the RNase structure. Several recent studies have demonstrated that many conserved RNases seem to be acetylated *in vivo* and suggested the possibility that this phenomenon is widely distributed in bacteria (Colak *et al.*, 2013; Weinert *et al.*, 2013; Zhang *et al.*, 2013; Schilling *et al.*, 2015; Koo *et al.*, 2020).

Regulator of barnase

Barnase is a guanyl-preferring extracellular ribonuclease that is synthesized and secreted from various strains of *Bacillus amyloliquefaciens*. Inside the cell, barnase is maintained in an inactive form by complexing with its inhibitor, barstar. The inhibitor occludes the active center of barnase, since intracellular barnase activity may provoke cell death (Hartley, 1988, 1989). Moreover, barstar efficiently inhibits barnase homologs, including those found in *Bacillus intermedius* (Yakovlev *et al.*, 1995) and SaRNases of *Streptomyces aureofaciens* (Sevcik *et al.*, 1998).

Barstar is a small protein with only 89 amino acids, and it tightly forms a one-to-one non-covalent complex with barnase in equimolar proportion ($K_D = \sim 10^{-14}$ M) (Hartley, 1988, 1989, 1993; Buckle *et al.*, 1994). A globular barstar sterically blocks the active site of barnase with the second N-terminal α-helix and the loop adjacent to the first α-helix (Guillet *et al.*, 1993; Buckle *et al.*, 1994). Two *Streptomycete* RNase inhibitors, whose amino acid sequence identities with barstar are rather low (~27%), contain the residues required for complexation with RNase (Krajcikova *et al.*, 1998). Another homolog of *Saccharopolyspora erythraea*, Sti, showed a higher binding affinity to RNase Sa3 than barnase (Krajcikova and Hartley, 2004). Comparative sequence analysis revealed that barstar homologs are widely distributed in prokaryotes such as *Clostridium acetobutylicum* and *Yersinia pestis* (Zhang *et al.*, 2012).

Mammalian ribonuclease regulators

Since the inhibitor of pancreatic superfamily RNase was first discovered in 1952, the characteristics of such an RI were extensively investigated (Dickson *et al.*, 2005). This cytoplasmic RI strongly interacts with its cognate RNases despite the low sequence identity among the bound ribonucleases (Rutkoski and Raines, 2008). Although the structure of RI is well characterized, its biological roles remain unclear. On the other hand, the functional roles of protein regulators of Drosha and Dicer, which are RNase III family enzymes that play an essential role in the biogenesis of microRNAs (miRNAs), are well characterized.

Inhibitor of RNase A superfamily

The RNase A superfamily is a pyrimidine-specific endoribonuclease present in high quantities in the pancreas of many mammals, birds, and some reptiles (Beintema and van der Laan, 1986; Cho *et al.*, 2005). The high degrading activity of RNase A for cytosolic RNA, has led to the development of a protein inhibitor in some eukaryotes to prevent RNase activity before it is secreted. The mammalian RI is a 50 kDa cy-

toplasmic protein that interacts with pancreatic RNase A at 1:1 stoichiometry and renders it inactive by steric occlusion of the catalytic site (Lee and Vallee, 1993; Shapiro, 2001; Dickson *et al.*, 2005). RI possesses a high content of repeated amino acids that are composed of leucine-rich residues (Hofsteenge *et al.*, 1988; Lee *et al.*, 1988). These leucine-rich repeats (LRRs) are evolutionarily conserved proteins that are specifically involved in protein-protein and protein-ligand interactions (Kajava, 1998). RI also consists of a large variety of conserved cysteine residues, which must be reduced to retain its structure and function (Fominaya and Hofsteenge, 1992; Blázquez *et al.*, 1996; Kim *et al.*, 1999).

In humans, RNase A superfamily proteins are broadly divided into two subgroups. One is canonical RNases 1-8, and the other is non-canonical RNases 9-13 (Lu *et al.*, 2018). RI can sufficiently inhibit many canonical RNases with the highest binding affinity of any protein-protein interactions (K_D = ~10^{-15} M) (Rutkoski and Raines, 2008). These include RNase A, RNase 1, eosinophil-derived neurotoxin (EDN; RNase 2), RNase 4, and human angiogenin (ANG; RNase 5). Crystallographic structure analysis of homologous RI-RNase complexes proposed that RI folds into a horseshoe form by two types of leucine-rich structural motifs and interacts with RNase A by electrostatic interactions (Lee *et al.*, 1989; Kobe and Deisenhofer, 1995; Papageorgiou *et al.*, 1997; Johnson *et al.*, 2007; Lomax *et al.*, 2014). Thus, most of the RNase A residues, which are required for catalytic activity and substrate binding, are efficiently masked by RI.

Regulators of Dicer and Drosha

Dicer is a member of the RNase III family that cleaves dsRNA and precursor microRNA (pre-miRNA) with hairpin structure into short dsRNA fragments called small interfering RNA (siRNA) and miRNA, respectively (Bernstein *et al.*, 2001). These sRNAs with RNA-induced silencing complex (RISC), which is primarily composed of Argonaute protein (Ago), bind to the complementary target mRNA sequence, preventing gene expression by mRNA degradation (Vermeulen *et al.*, 2005) or translation blocking (Zeng *et al.*, 2003). RNA interference (RNAi) affects many biological processes, including host defense against viruses (van Rij and Berezikov, 2009; Song *et al.*, 2011), regulation of developmental timing and differentiation (Nimmo and Slack, 2009), and maintenance of cellular homeostasis (Zhao and Srivastava, 2007; Bartel, 2009; Wahid *et al.*, 2010).

Human Dicer (hDicer) is a 218 kDa multidomain enzyme comprising a DExD/H-box RNA helicase domain, a PAZ (Piwi/Argonaute/Zwille) domain, two RNase III domains (RNase IIIa and RNase IIIb), and two dsRBDs (DUF283 and dsRBD) (Fig. 5) (Bernstein *et al.*, 2001; Blaszczyk *et al.*, 2004; Macrae *et al.*, 2006; Court *et al.*, 2013; Nicholson, 2014). In particular, its C-terminal region containing RNase IIIb and dsRBD is conserved in other RNase III family proteins, including eukaryotic Droshas and the bacterial RNase IIIs (Nicholson, 2014).

To date, various factors regulating the activity of human Dicer by direct protein-protein interaction have been identified (Fig. 5). In particular, two constitutive partners of Dicer, the *trans*-activation response RNA-binding protein (TRBP) and its paralog, protein activator of protein kinase R (PACT), are known to regulate the activity of Dicer (Chendrimada *et al.*, 2005; Haase *et al.*, 2005; Lee *et al.*, 2006; Wilson *et al.*, 2015). Both proteins not only alter substrate recognition by Dicer but also influence cleavage specificity, which in turn generates different-sized iso-miRNAs (Fukunaga *et al.*, 2012; Lee *et al.*, 2013). In particular, TRBP increases the stability of Dicer-substrate complexes and contributes to the assembly of multicomponent enzyme complexes such as RISC and RISC-loading complex (RLC) (Chendrimada *et al.*, 2005; Haase *et al.*, 2005; Macrae *et al.*, 2008; Wang *et al.*, 2009). In addition, adenosine deaminase acting on RNA 1 (ADAR1), which is required for dsRNA editing (adenosine to inosine), has been identified as another Dicer regulator protein (Yang *et al.*, 2006; Ota *et al.*, 2013). ADAR1 was shown to directly interact with DUF283 and helicase domains of Dicer (Fig. 5), resulting in increased cleavage activity of Dicer by inducing conformational changes (Ota *et al.*, 2013). Remarkably, TRBP is also associated with the helicase domain of Dicer

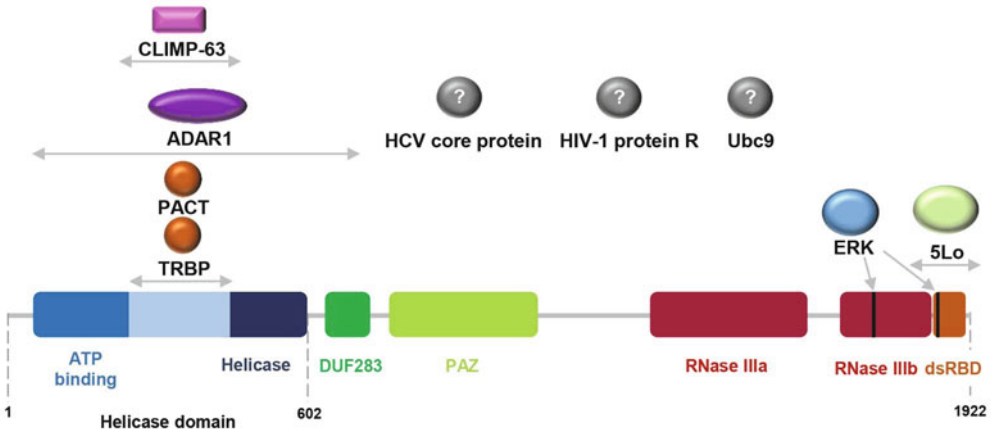

Fig. 5. Schematic diagrams of human Dicer domains and regulator proteins. Dicer contains domains ordered from the N-terminus to the C-terminus as follows: N-terminal ATP-binding and C-terminal helicase domains, a putative RNA-binding domain DUF283, PAZ domain recognizing the 3′ end of siRNA and miRNA precursors, RNase IIIa and RNase IIIb, and a dsRBD.

(Daniels *et al.*, 2009; Wang *et al.*, 2009), indicating that TRBP and ADAR1 partially overlap their interacting domains. Moreover, unlike other interacting proteins associated with the N-terminal domain of Dicer, 5 lipoxygenase (5LO), which is necessary for leukotriene biosynthesis, binds to Dicer C-terminus and may influence cleavage pattern of pre-miRNA by Dicer (Rådmark *et al.*, 2007; Dincbas-Renqvist *et al.*, 2009).

Several viral proteins are also known as inhibitors of Dicer. For instance, the core protein of hepatitis C virus (HCV) inhibits Dicer activity by processing dsRNA into siRNAs by interacting with it (Wang *et al.*, 2006; Chen *et al.*, 2008). Viral protein R (Vpr) of human immunodeficiency virus type 1 (HIV-1) complexes with human Dicer to recruit ubiquitin ligase complexes for subsequent degradation (Klockow *et al.*, 2013).

In addition, post-translational modifications can regulate human Dicer via phosphorylation (Drake *et al.*, 2014) and SUMOylation (Gross *et al.*, 2014). During oogenesis, phosphorylation of Dicer within RNase IIIb and dsRBD domains by extracellular signal-regulated kinase (ERK) is necessary for triggering dicer nuclear localization and inhibiting its function in worm and mammalian cells (Drake *et al.*, 2014). SUMOylation in lysine residues of Dicer affects its ability to process miRNA, resulting in a significant decrease of mature miRNAs in macrophages (Gross *et al.*, 2014).

Post-translational modifications can also affect Drosha function. Drosha and its cofactor Digeorge syndrome critical region 8 (DGCR8) form a complex known as microprocessor, cleaving the pri-miRNA to release a short hairpin (pre-miRNA) (Lee *et al.*, 2003a; Denli *et al.*, 2004; Gregory *et al.*, 2004; Han *et al.*, 2004). Several studies have reported that post-translational modifications can affect Drosha activity by regulating protein translocation or stability. Phosphorylation of Drosha is mediated by two protein kinases: MAPK (Yang *et al.*, 2015) and GSK3β (Tang *et al.*, 2010, 2011). The p38 MAPK-mediated phosphorylation reduces the binding of Drosha to DGCR8 and enhances its nuclear export, thereby degraded by cysteine protease (Yang *et al.*, 2015). On the contrary, its phosphorylation by GSK3β induces translocation from the cytoplasm to the nucleus (Tang *et al.*, 2011); thus, two protein kinases are involved in opposing processes. However, GSK3β does not bind directly to Drosha, but it is mediated in an RNA-dependent manner.

In addition, the N-terminal domain of Drosha is acetylated at various lysine residues by at least three acetyltransferases, and the acetylation reaction inhibits its degradation by ubiquitination (Tang *et al.*, 2013). On the contrary, ubiquitination of the N-terminal domain of Drosha by MDM2, which is known for the primary E3 ubiquitin ligase, allows it to degrade through the mTOR signaling pathway (Ye *et al.*, 2015). Competition between lysine acetylation and ubiquitination of Drosha within the N-terminal domain regulates its cellular concentration and consequently modulates global miRNA levels.

Plant ribonuclease regulators

Self-incompatibility (SI) is a prezygotic reproductive barrier that prevents inbreeding in many types of angiosperms (Takayama and Isogai, 2005). This phenomenon is tightly regulated by two linked genes on the S-locus encoding S-locus F-box protein (SLF) and S-locus ribonuclease (S-RNase) (McClure *et al.*, 1989; Lai *et al.*, 2002). SLF is a component of the E3 ubiquitin ligase complex called the SCF^{SLF} complex and specifically interacts with non-self S-RNase (Lai *et al.*, 2002; Qiao *et al.*, 2004; Hua and Kao, 2006). Several studies have revealed that the SCF^{SLF} complex is composed of SLF protein, Skp1-like protein (SSK1), SBP1 RING-finger protein, and Cullin (Sims and Ordanic, 2001; Zhao *et al.*, 2010; Li *et al.*, 2014). SBP1, which is an E3 ubiquitin ligase, binds directly to the N-terminus of S-RNase and ubiquitinates it (Kerscher *et al.*, 2006; Hua and Kao, 2008). Moreover, SSK1 was shown to interact with the N-terminus of Cullin and the C-terminal of SLF in the SCF complex, and this interaction plays an essential role in the degradation of non-self S-RNase (Zhao *et al.*, 2010). This SCF^{SLF} complex is considered to be a general S-RNase inhibitor and has been extensively distributed in Solanaceae, Plantaginaceae, and Rosaceae (Williams *et al.*, 2015).

A protein inhibiting RNase A activity in *Malus domestica* was found and partially characterized (Kosuge *et al.*, 2003). *In vitro* measurement of RNase inhibitory activity showed that the inhibition constant against RNase A activity is 5×10^{-8} M, which is weaker than that of mammalian RI and RNase A ($K_D = 4.4 \times 10^{-14}$ M) and that of barnase and barstar ($K_D = 1.3 \times 10^{-14}$ M) (Kosuge *et al.*, 2003).

Conclusion

This review summarizes the roles and underlying mechanisms of *trans*-acting regulators of RNases found in diverse organisms. These regulators modulate the activity of RNases through a variety of pathways. They usually inhibit or stimulate by direct interaction either with the catalytic domain or the substrate-binding domain with various binding affinities in the micromolar to femtomolar range (Table 1). Some viral proteins regulate the specific RNases of the host to process or protect their own RNAs. It is considered one of the survival strategies of viruses to efficiently replicate within the host cell. Furthermore, RNase activity is specifically regulated under certain conditions via post-translational modifications, enabling the cells to rapidly cope with the specific transitions. Remarkably, many RNase regulators are involved in the degradation of RNases. For instance, phosphorylation of bacterial RNase R stimulates tmRNA-SmpB binding and thus recruits proteases for subsequent degradation (Deutscher, 2015). Similarly, phosphorylation of Drosha promotes its nuclear export and degradation by proteases (Yang *et al.*, 2015). Additionally, ubiquitination is considered to be an important process in regulating RNase activity in eukaryotes.

RNase regulators do not appear to have close evolutionary relationships with each other (Fig. 2). It has been reported that bacteria and protozoans express their unique ribonucleases and inhibitor proteins which bear no evolutionary or structural similarity to human RI (Hartley, 1989; Gbenle, 1990).

Considering that RNase activity can be regulated by many different types of *trans*-acting regulators, there will be many

more unexpected findings in this emerging field. Recently, innovative genome modification technologies facilitated characterization of RNase cleavage sites that are modulated by these regulators (Lee *et al.*, 2019b, 2021; Ren *et al.*, 2020). Identification of *trans*-acting regulators of RNase activity and their mode of action will contribute to a better understanding of the various dynamic facets of RNA function in biological processes.

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Conflict of Interest

We have no conflicts of interest to report.

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