T4 Srd accelerates *E. coli* mRNA degradation via stimulating the activity of host RNase E

Dan Qi

Department of Biological Sciences, Graduate School of Science,

Osaka University

2015

Table of Contents

Prologue

Regulation of gene expression is of great importance to organisms and numerous studies have been focused on this issue. Controls of gene transcription and mRNA decay are two critical ways to achieve gene regulation. So far, transcriptional regulation has been much known. However, although many ribonucleases have been identified in both prokaryotes and eukaryotes, study of their effects on the control of mRNA decay is still on its way. Among ribonucleases characterized to date, *E. coli* RNase E is one of the most studied endoribonuclease. RNase E is an essential endoribonuclease involved in decay of bulk mRNAs as well as processing of rRNA and tRNA precursors. Cleavage of mRNA by RNase E is a major way of triggering mRNA decay in *E. coli*. Bacteriophage T4 shuts off gene expression of the host immediately after infection, and quickly starts to express its own genes. In parallel, RNase E rapidly degrades host mRNAs, otherwise stable in uninfected cells, possibly helping to accelerate the shift of gene expression from *E. coli* to T4. However, the mechanism for activation of RNase E is still unknown. In this work, I attempted to gain a new insight in the control of mRNA degradation by RNase E, through study of bacteriophage T4 infection-induced host mRNA degradation and current understanding of T4-induced host mRNA degradation is recorded in this thesis.

Overview

Shift of gene expression after T4 infection

Bacteriophage T4 induces multiple mechanisms converting a host cell into an efficient factory for the production of viral progeny (Mathews 1994). A shift of gene expression from host to T4 is one of the most important. Bacteriophage T4 shuts off gene expression of the host, *Escherichia coli*, immediately after infection, and starts expressing its own genes (Kaempfer *et al.,* 1967a and 1967b; Kennell 1970; Nomura, *et al.,* 1962; Svenson *et al.,* 1976). Multiple mechanisms, including shutoff of host-specific synthesis, degradation of host mRNA and DNA, modification of host apparatuses and so on, are involved in this process. For instance, T4 Alc protein inhibits host transcription right after infection, by specifically terminating the elongation of transcription on cytosine-containing DNA (Kutter *et al*., 1994a and 1994b; Miller *et al.,* 2003a); T4 Ndd is known to be responsible for host nuclear disrupt, shut off of host DNA synthesis delays 8-10 min in *ndd* mutant infected *E. coli* cells (Snustad *et al*., 1976; Kutter *et al*., 1994a); T4 induces rapid host mRNA degradation within 5 minutes after infection, though with unclear mechanism (Ueno *et al.,* 2004). Through these, T4 destroys host's genetic functions and substitutes their own, instead of managing a "peaceful genetic coexistence with their bacterial hosts", and transits host-directed gene expression to phage-directed gene expression immediately after infection and followed by highly regulated and efficient gene expression of itself (Stent 1963; Kutter *et al*., 1994a). However, since multiple events occurs within very short times after T4 infection, and some of them are supposed to be necessary and quite important for efficient T4 propagation but not indispensable for growth, the cessation of gene expression from *E. coli* to T4 is still not fully understood. As Kornberg wrote in 1980, "Despite all that is known about T4 infection, there is still no explanation of why host macromolecular synthesis stops so promptly and completely. This remains one of the major unsolved problems of molecular biology (Kutter *et al*., 1994a).

Several infection-induced host mRNA degradation mechanisms

Phage-induced host mRNA degradation is discovered recent years and its mechanism is still rarely known so far (Ueno *et al.,* 2004). However, its significance on efficient phage propagation should be indispensable. In eukaryotic cells, promoting global mRNA degradation is reported to be one of host shutoff phenotypes to block host gene expression among at least three different viral subfamilies, alphaherpesviruses, gammaherpesviruses and betacoronaviruses (Gaglia, *et al*., 2012). Typical example of alphaherpesviruses is herpes simplex virus 1 (HSV-1). Viral factor Vhs（UL41) RNase (Kwong *et al*., 1988; Read *et al*., 1983), a nuclease of the FEN1 family (Doherty *et al*., 1996), is packaged in virions and released after infection (Suzutani *et al*., 2000; Taddeo *et al*., 2013). Several instances of gammaherpesviruses were characterized: SOX in Kaposi's sarcoma-associated herpesvirus (KSHV) (Glaunsinger *et al*., 2004), BGLF5 in Epstein-Barr virus (EBV) (Rowe *et al*., 2007), and muSOX in murine herpesvirus 68 (MHV68) (Covarrubias *et al*., 2009), which are all alkaline exonuclease homolog, a member of the PD(D/E)XK restriction endonuclease superfamily have been demonstrated to induce host mRNA decay. nsp1, a protein with no known similarity to cellular or viral nucleases from severe acute respiratory syndrome (SARS) coronavirus (SCoV) belong to betacoronavirus family, is reported to induce host mRNA degradation by binding to 40S ribosome (Kamitani *et al*., 2006; Huang *et al*., 2011). Moreover, Gaglia's work showed that factors from viruses mentioned above triggers host mRNA degradation by a primary endonucleolytic cleavage causing shutoff of host gene expression either and a host exonuclease such as Xrn1, an important 5′-3′ exonuclease in human cells, were required in subsequent completion of host mRNA turnover (Gaglia *et al*., 2012).

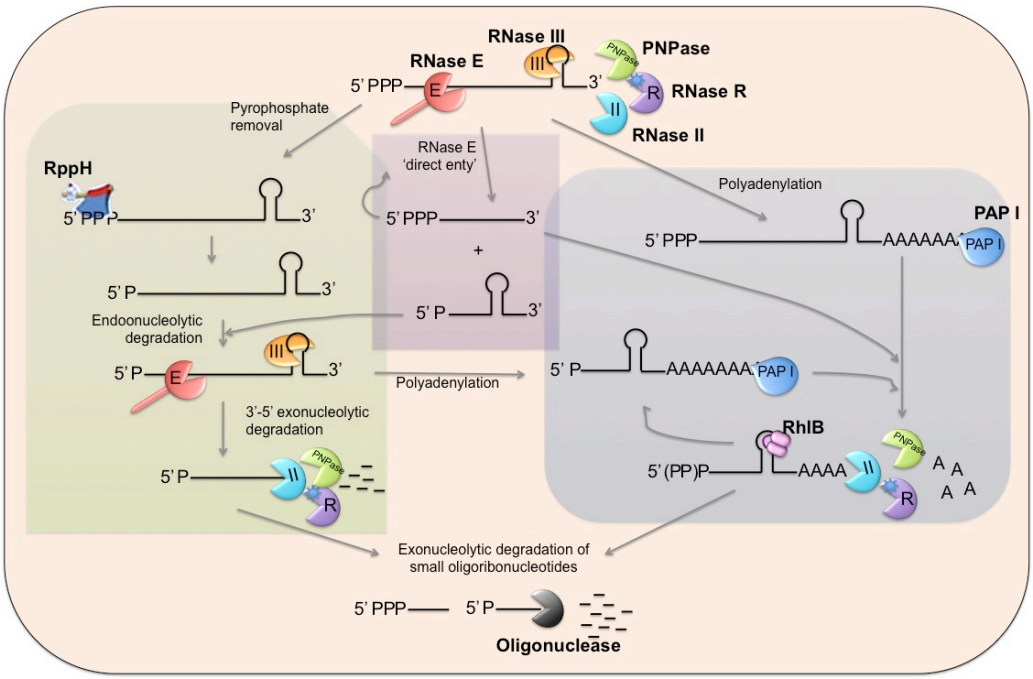
Influence of function defect of viral factors that raises host mRNA turnover on viral replication have been studied for years. Loss of function of viral factors mentioned above usually doesn't stop viral growth, but several evidences have been also obtained that global host mRNA turnover should be required for rapid transition of gene expression from host to viruses and highly efficient viral replication. For

instance, HSV-1 *vhs*-deficient mutant results in low virulence and Vhs plays a role in evasion from non-specific host defence mechanisms during primary infection (Strelow *et al*., 1995; Suzutani *et al*., 2000). Besides, recently Matsuura's group showed that RNA replication of ScoV was much lower when nsp1 was mutated in an *in vitro* RNA replica system (Tanaka *et al*., 2012). Therefore, it maybe a common mechanism in both prokaryote and eukaryote that infection with viral organisms activates the host mRNA degradation machinery for the shift of gene expression from host to virus and thus facilitate efficient viral propagation.

mRNA decay in *E. coli*

In *Escherichia coli*, protein-encoding RNAs have on average overall chemical half-lives of 7-8min (Mohanty *et al*., 1999; Selinger *et al*., 2003), but individual transcripts each have their own characteristic lifetime that can be modulated according to growth stage and stress response with a few mRNAs display half-lives of shorter than 3 min or longer than 20min (Bernstein *et al*., 2002; Nilsson *et al*., 1984; Bandyra, *et al*., 2013). *E. coli* mRNAs are metabolized through a series of endo- and exonucleolytic events; endonucleases cleave mRNAs processively in the 5′-3′ direction, produced RNA fragments are in turn degraded into mononucleotides by several 3′-5′ exonucleases (Apirion 1973; Kushner 2002). Thus, endonucleases play a critical role at the initial and rate-limiting steps of mRNA decay. One of the key enzymes in *E. coli* mRNA decay pathway is RNase E (Carpousis *et al*., 2007 and 2009; Mackie 2013a; Stead *et al*., 2011), which is an essential endoribonuclease in *E. coli* and even in other proteobacteria.

The following picture shows a putative model of *E. coli* mRNA decay pathways.



Schematic Drawing of Model of RNA degradation pathways in *Escherichia coli*

In a majority of cases, decay of transcripts starts with an endoribonucleolytic cleavage by RNase E (middle part in the above figure). It only cleaves single-stranded RNAs with a preference of a monophosphorylated 5'-end, though not in a strict way (Kime *et al*., 2009). Sometimes, before decay starts, 5'-pyrophosphates removal by *E. coli RNA* 5'-Pyrophosphohydrolase (RppH) triggers RNase E-dependent degradation (left part in the above figure). RNase III is another enzyme responsible for the initial endoribonucleolytic cleavage of structured RNAs, which, unlike RNase E, targets double-stranded RNAs (Babitzke, *et al*., 1993). Then the linear transcripts are rapidly degraded by the 3′-5′ exoribonucleases, such as hydrolytic enzymes RNase II, RNase R, or polynucleotide phosphorylase (PNPase). RNase R are close related to RNase II, but unlike RNase II or PNPase, it is efficient against highly structured RNAs (Matos *et al*., 2011). PNPase, in association with other proteins, namely RNA helicases such as *E. coli* RhlB, can also unwind RNA duplexes (Arraiano *et al*., 2010). Also, in a minor of cases, decay begins with an exoribonucleolytic degradation of full-length transcripts (right pathway in the above figure). Poly(A) polymerase (PAP I) adds 3'-terminal polyadenine tail which become sites for processive degradation. Cycles of polyadenylation and exoribonucleolytic digestion can overcome RNA secondary structures (Sarkar 1997). Finally, oligoribo-nuclease degrades small oligoribonucleotides (two to five nucleotides) produced by exoribonucleases (Niyogi *et al*., 1975; Andrade *et al*., 2009; Arraiano *et al*., 2010). For individual transcript, decay pathway may differ under different environmental conditions.

RNase E

RNase E is known as the central player in RNA metabolism in *E. coli* and in many other bacteria, not only through its endonuclease activity, but also through its role in organizing other enzymes of RNA metabolism, including both stable RNA processing and mRNA decay, into a higher-order complex known as the RNA degradosome (Carpousis *et al*., 1994; Py *et al*., 1996; Miczak *et al*., 1996; Mackie 2013a; Babitzke *et al*., 1991; Mudd *et al.* 1990a). To date, RNase E homologs have been found in more than 50 eubacteria, archaebacteria, and plants (Lee *et al*., 2003a). RNase E has been shown to be involved in the maturation of 9S rRNA (Ghora *et al*., 1978), the processing of the 5′-end of 16S rRNA (Li *et al*. 1999; Wachi *et al*. 1999), the 3′-end of tRNAs (Li *et al*., 2002; Ow *et al*., 2002; Ray *et al*., 1981) and tmRNA (Lin-Chao *et al*. 1999), the maturation of the RNA subunit (M1 RNA) of RNase P (Lundberg *et al*., 1995), the degradation of the antisense inhibitor (RNA I) of plasmid colE1 DNA replication (Tomcsányi *et al*., 1985), and the processing of the plasmid T4 gene 32 mRNA(Mudd *et al*.,1988). As such, it is involved in the processing of a large number of nontranslated transcripts. Moreover, Ow and Kushner (2002) reported that the initiation of tRNA maturation by RNase E is essential for cell viability. Meanwhile, RNase E is demonstrated to be primarily responsible for initiating mRNA decay in *E. coli* (Bandyra *et al*., 2013).

Substrate recognition and cleavage sites

The cleavage mechanism of RNase E has been much investigated though not completely known. As a basic feature, it cleaves the phosphodiester backbone of the RNA, generating 3′-OH and 5′-monophosphate termini on the products (Misra *et al*., 1979). RNase E has a strong preference for single-stranded sequences rich in A-U with a purine at the -2 position relative to the scissile bond (Mackie 1992; McDowall *et al*., 1995; Kaberdin *et al*., 2003; Mackie, *et al*., 1993). A typical cleavage site is 5′ -(A/G)N↓AU, in which ↓ denotes the site of cleavage and N represents any ribonucleotide. Potential cleavage sites can be occluded by alternative secondary

structures or by RNA-binding proteins (Mackie *et al*., 1993; Jerome *et al*., 1999; Folichon *et al*., 2003; Vogel *et al*., 2011; Mackie, 2013a).

Cleavage pathways in mRNA decay

The degradation of mRNA by RNase E can occur via two pathways: 5′-monophosphate end-dependent pathway and 5′-end-independent (so-called 'direct entry' or 'internal site entry') pathway (Bouvier *et al*., 2011). The picture below describes putative model of RNase E activities.

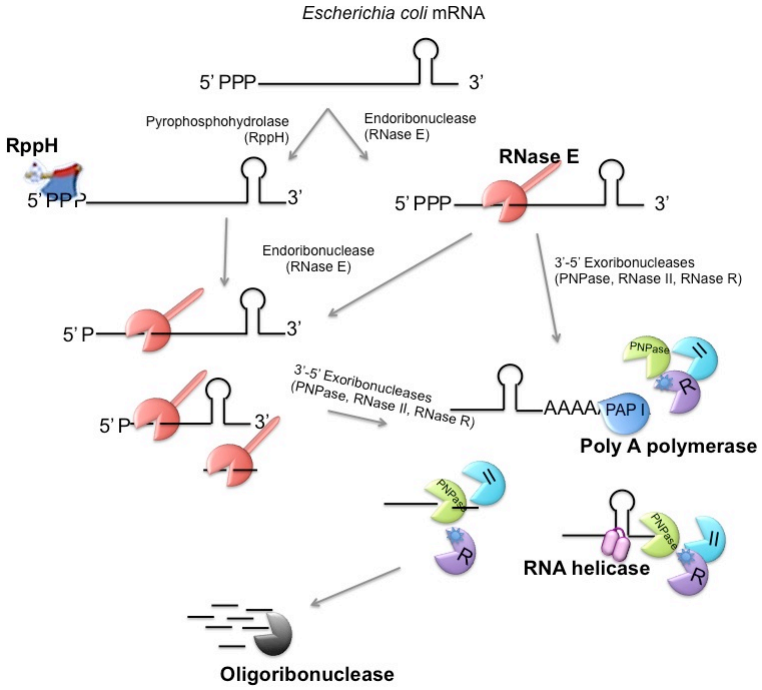
5′-end-dependent

E. coli RNA pyrophos-phohydrolase (RppH) is known to be necessary in the former case (Celesnik *et al*., 2007; Deana *et al.*, 2008; Bouvier *et al*., 2011). RppH removes a pyro-phosphate from 5′-triphosphate end of mRNAs and initiates degradation by RNase E with the preference to 5′-monophosphate. RNase E has a 5′-sensor domain forming a phosphate pocket can easily accommodate a 5′-monophosphorylated residue. This 5′-sensor increases the affinity of RNase E for substrates and enables the enzyme to distinguish between triphosphorylated primary transcripts and RNAs that have already undergone at least one cleavage (Mackie 2013a). Numbers of *E. coli* mRNAs are reported to be the targets of RppH, such as *rpsT*, *yeiP*, *trxB* etc. (Deana *et al.,* 2008; Richards *et al*., 2012).

Internal site entry (direct entry)

In this case, RNase E first triggers degradation of mRNA in internal site regardless of the triphosphate at its 5′-end, generating product with monophosphate, then drastic degradation begins due to the monophosphate substrate preference of RNase E. *E. coli* stable mRNA *ompA* and *lpp* are known as examples for this pathway. So far, two conditions were proposed to promote this pathway. First, a 5′-terminal stem-loops structure inhibits the action of both RppH and RNase E (Mackie 1998; Deara *et al*., 2008). Second, 'naked' mRNA, which becomes exposed when RNA polymerase outpaces the following ribosomes, is highly susceptible to direct entry (Mackie 2013a). In contrast, the substrates for processing are not naked RNAs in isolation but

rather incomplete, undermodified pre-rRNA-ribosomal protein complexes (Mackie 2013a). Though deletion of RNase E is lethal, mutants with 5′-sensor of RNase E defective can be viable (Garrey *et al*., 2009). Recently, Clarke *et al*.'s work proposed that direct entry is a major pathway for the degradation and processing of RNA in *E. coli* and simultaneously binding to multiple unpaired regions by RNase E facilitating the entry attack (Clarke *et al*., 2014).



Schematic Drawing of Model of RNase E-dependent mRNA degradation pathways in *E. coli*

RNA degradosome in E. coli

RNase E is encoded by *rne(=ams)* gene, which is located at 24.58 minutes in the *E. coli* genomic map (Mudd *et al*., 1990b). It is a protein with 1036 amino acids consisting of two domains: N-terminal catalytic half (NTH) (1-529aa) and C-terminal (530-1036aa) scaffold half (CTH). RNase E NTH consists of two RNase H-like domains, S1 domain, 5′-sensor domain (binding to 5′-monophosphate of RNA substrate), DNase I-like domain, Zn-link domain and a small domain (Mackie 2013a). NTH of RNase E is highly conserved in prokaryotes (Marcaida *et al.,* 2006). RNase E can form a multi-enzyme machine of RNA metabolism, known as RNA degradosome

(Tsai *et al*., 2012). The CTH of RNase E, containing a cell membrane-binding region segment A (565-585aa), RNA binding regions, and regions for other macromolecular interactions, acts as the scaffold for the RNA degradosome assembly and recruits its core constituents, which are the exoribonuclease polynucleotide phosphorylase (PNPase), the glycolytic enzyme enolase and the RNA helicase RhlB, a member of the DEAD-box ATP-dependent helicase family (Carpousis 2007; Górna *et al.*, 2012). In addition, a RNA chaperon, *E. coli* Hfq, can also bind to CTH of RNase E contributing to stabilize RNA structure thus participating many cell events, such as sRNA-mediated mRNA decay under certain environmental stress (Valentin-Hansen *et al*., 2004; Guisbert *et al*., 2007; Ikeda *et al*., 2011). The RNase E-based degradosome is suggested to be the primary mechanism for mRNA degradation in *E. coli*, because the degradosome contains all of the proteins necessary to degrade mRNA molecule into small oligonucleotides. However, the significance of RNA degradosome is still under discussion and further investigation, since first, the C-terminal degradosome scaffolding region is not conserved (Kaberdin *et al*. 1998); second, deletion of CTH does affect the activity of RNase E but not essential, RNase E mutants lacking the degradosome scaffolding region exhibit normal mRNA decay (Ow *et al.* 2000); third, but since RNase E mutants lacking the degradosome scaffold domain consistently shows much slower growth than wild-type cells (Ow *et al.* 2000), function of degradosome should be required for unknown pathways in RNA metabolism.

Regulation of RNase E's activity

Accumulated findings suggest that RNase E activity may be regulated by cell physiology, such as the processing of RNAs by RNase E is known to be affected by anaerobiosis during cell growth (Georgellis *et al*., 1993) and also to occur prominently in transcripts that encode proteins involved in energy-generating pathways (Lee *et al*., 2002; Bernstein *et al*., 2002), but little has been known about cellular mechanisms that might modulate the degradation of RNA by RNase E (Lee *et al*., 2003b).

To date, three *E. coli* factors, RraA, RraB and ribosomal protein L4, have been reported to regulate the activity of RNase E. RraA physically interacts with the C-terminal RNA binding sites and inhibits RNase E activity by interrupting the access of

RNase E to RNA substrate (Lee *et al*., 2003b). RraB binds to CTH and interferes with cleavage of target mRNAs (Gao *et al*., 2006). Binding of RraA or RraB differently affects the composition of RNA degradosome (Gao *et al*., 2006; Yeom *et al.,* 2008a and 2008b). L4 interacts with a site outside of the catalytic domain of RNase E to inhibit RNase E cleavage activity *in vitro* and stabilizes mRNAs targeted by RNase E *in vivo* (Singh *et al*., 2009). Mutant of these inhibitors shows normal growth in rich medium suggests that any physiological effect may occur under special growth conditions (Lee *et al*., 2003b). Apart from *E. coli*, a protein kinase of bacteriophage T7 phosphorylates RNase E at its CTH and stabilizes a subset of RNase E substrates (Marchand *et al*., 2001). In contrast, Carpousis' group demonstrated that RhlB, which is a member of DEAD-box RNA helicase and a major component of the degradosome, facilitated RNase E activity for the degradation of several artificial RNAs transcribed by T7 RNA polymerase (Khemici *et al*. 2005), although it is not required for stabilities of endogenous mRNAs (Bernstein *et al*. 2004). Additionally, Görke's group recently reported that *E. coli* RapZ interacted with NTH of RNase E and functioned as an adaptor protein to recruit RNase E for processing of the small RNA GlmZ. The possibility that RapZ directly affects RNase E activity has not been addressed in detail (Göpel *et al*., 2013; Göpel *et al*., 2014). Identification of factors influencing activity of RNase E is expected to be helpful to deeper understanding of RNase E activity regulation.

Doctoral Research —

Bacteriophage T4 Srd Stimulates Activity of *E. coli* RNase E after infection

Abstract

Escherichia coli mRNAs are rapidly degraded immediately after bacteriophage T4 infection and the host RNase E contributes to this process. Here, I found that a factor of T4 phage, Srd, was also involved in T4-induced host mRNA degradation. The rapid decay of *ompA* and *lpp* mRNAs was partially alleviated and a decay intermediate of *lpp* mRNA remarkably accumulated in cells infected with T4 phage lacking *srd*. Exogenous expression of Srd in uninfected cells significantly accelerated the decay of these mRNAs. In addition, *lpp*(T) RNA, which had a sequence identical to the decay intermediate of *lpp* mRNA, was also destabilized by Srd. The destabilization of these RNAs by Srd was not observed in RNase E-defective cells. RNase E cleaves mRNAs at internal sites either directly (RppH-independent) or by 5′-end-dependent mechanism (RppH-dependent). *lpp*(T) RNA, but not *lpp* mRNA, required RppH for Srd-stimulated degradation, suggesting that Srd stimulates both direct and 5′-end-dependent degradation pathways of RNase E cleavage. Furthermore, pull-down and immunoprecipitation analysis demonstrated that Srd physically associated with N-terminal catalytic moiety of RNase E. These results strongly suggest that Srd acts as a stimulator of RNase E via binding to its catalytic domain. Finally, the growth of T4 phage was significantly decreased by the disruption of *srd*. These results strongly suggest that the stimulation of RNase E activity by T4 Srd is required for efficient growth of phage.

Introduction

Bacteriophage T4 shuts off gene expression of the host, *Escherichia coli*, immediately after infection, and quickly starts to express its own genes (Kutter *et al.*, 1994a). Multiple mechanisms, such as modifications of apparatuses for transcription and translation, are involved in this shift of gene expression from *E. coli* to T4 (Carlson *et al.*, 1994). Our previous work revealed that representatively stable *E. coli* mRNAs, *lpp* and *ompA*, were rapidly degraded after T4 infection (T4-induced host mRNA degradation) and RNase E, which is an essential endoribonuclease in *E. coli* (Marcaida *et al.*, 2006), primarily functions in T4-induced host mRNA degradation (Ueno *et al.,* 2004). This rapid degradation of host mRNAs should contribute to quick shift from *E. coli* to T4 metabolism, because it leads to immediate cessation of host gene expression and consequently generation of ribonucleotides and free ribosomes, each of which would stimulate transcription and translation of T4 genes. In fact, the deficiency of RNase E resulted in slow start of T4 gene transcription, reduced the level of transcription (Otsuka *et al.,* 2005) and retarded the growth of T4 (Mudd *et al*., 1990b). In eukaryotic cells, the degradation of host mRNAs after viral infection, such as alphaherpesvirus, gammaherpesvirus, or betacoronavirus, also contributes to the shut-off of host gene expression (Gaglia *et al*., 2012). In these cases, host mRNA degradation is initiated by a viral factor and requires host 5′-3′ exoribonuclease Xrn1. Therefore, it may be a common mechanism in both prokaryote and eukaryote that infection with virus activates the host mRNA degradation machinery for the shift of gene expression from host to virus.

Similar to eukaryotic viruses described above, T4 gene product was suggested to be required for T4-induced host mRNA degradation (Ueno *et al.,* 2004). In this study, I found that host mRNAs were partially stabilized and a decay intermediate was remarkably accumulated in cells infected with $\Delta(39\text{-}56)_6$ mutant lacking consecutive 8 genes, and that the causal gene was *srd* encoding a 29 kDa protein. This gene was named *srd* (similarity with rpoD), since it partly shares sequence similarity with *E. coli* sigma 70 (Mosig *et al*., 1998). They reported that exogenous expression of Srd in *E. coli* cells resulted in extremely slow growth and filamentation of cells. However

the function and the role of Srd in T4-infected cells remained unclear.

RNase E participates in maturation of tRNAs, processing of rRNAs, and turnover of bulk mRNAs in *E. coli* (Mudd *et al.*, 1990a; Mackie 2013a). It consists of two domains, N-terminal (1-529 aa) catalytic half (NTH) and C-terminal (530-1036 aa) scaffold half (CTH), and forms a multi-enzyme complex, called RNA degradosome, involved in degradation of mRNAs (Tsai *et al*., 2012). Here, I demonstrated that T4 Srd associated with the N-terminal catalytic domain of RNase E and stimulated both the 5'-end-dependent and independent mRNA degradation activities of RNase E. Morover, because T4 phage lacking *srd* exhibited lower growth, the degradation of host mRNAs by RNase E activity that Srd stimulates after infection should be required for efficient growth of T4 phage.

Materials and methods

Phages and bacterial strains:

Wild-type bacteriophage T4 is T4D. Δ(*39-56*)$_6$ phage were kindly provided by Prof. H. Takahashi at University of Tokyo. Δ*srd*, Δ*motB.2*, Δ*dda-dda.1*, Δ*dexA.1-dexA.2*, Δ*modA* and Δ*dexA* phages were constructed using the insertion/substitution system (Selick *et al*., 1988). Briefly, a DNA fragment was PCR-amplified using T4 DNA as a template and primers 1 and 2 (Table 1). The amplified fragment was used for second PCR as a primer together with primer 3 using T4 DNA as a template. Consequently, the resulting fragment contained 5′ and 3′ flanking regions of target gene(s) that was deleted. The DNA fragment was inserted into pBSPLO+ and the constructed mutant sequence was transferred to phage genome via homologous recombination between plasmid and T4 DNA.

E. coli K-12 strains (Table 2), MH1 (*sup0 araD139 hsdR* Δ*lacX74 rpsL*) and TY0807 (MH1 *araD*$^+$) (Koga *et al*., 2011) were used as wild types. YT10 (MH1 *zce726*::*Tn10*) was constructed by T4 GT7 phage transduction (Wilson *et al*., 1979) of tetracyclin resistance marker from GW10 (Wachi *et al*., 1997). YT20 (MH1 *zce726*::*Tn10 ams1*) was constructed by GT7 phage transduction of tetracyclin resistance marker together with a temperature sensitive mutation of *ams1* from GW20 (Wachi *et al*., 1997) into YT10. YT007 (MH1 Δ*rppH*::*kan*) was also constructed by GT7 phage transduction of kanamycin resistance marker from JW2798 that was purchased from National BioResource Project (NIG, Japan). The kanamycin resistance cassette of YT007 was removed by yeast Flp recombinase expressed from pCP20 to construct TY1005 (MH1 Δ*rppH*) (Cherepanov *et al.,* 1995). Δ*lpp*::*kan* of JW1667 (NIG, Japan) was transferred into YT10, YT20 or TY1005 to construct TY1001 (MH1 *zce726*::*Tn10* Δ*lpp*::*kan*), TY1002 (MH1 *zce726*::*Tn10 ams1* Δ*lpp*::*kan*) or TY1006 (MH1 Δ*rppH* Δ*lpp*::*kan*), respectively. TY1007 (MH1 *rne-FLAG-cat*) and TY1008 (MH1 *rne598-FLAG-cat*) were also constructed by GT7 phage transduction of *rne-FLAG-cat* or *rne598-FLAG-cat* from TM338 (W3110 *mlc rne-Flag-cat*) or TM529 (W3110 *rne598-Flag-cat*), which was kindly gifted from Prof. H. Aiba at the Nagoya University.

Table 1. Primers used for construction of deletion mutant phages in this study

Phage mutant	Primer 1 (5′-3′)	Primer 2 (5′-3′)	Primer 3 (5′-3′)
Δ*srd*	ACGCGTCGACTCATCGTAAATTC	CGTTTAGAGCATACTCAGGCATCTGCCTGAAAAGAGGATATCCGCTAAATTG	ACGCGTCGACATTGAGGTAGTTG
Δ*motB.2*	TCAACGCCATCTTCCAATCCAT	GACGCTATAACAATACGCCGAGATCTTGGCGGGCATCAAGTAATGTTCCG	GTACCGGAATTCCGATGAATTAGTTTC
Δ*dda-dda.1*	ACGCGTCGACTTATCGTTTTGTTG	GCGATAGTTTACCGAGACAAAGACGGTGTCACCCGTGGTCGTTATGATGTG	ACGCGTCGACGTAAGATGTGAGAA
Δ*dexA.1-dexA.2*	ACGCGTCGACTTATCGTTTTGTTG	GGAAGCTATTCGTGGAAATAACTAAAGAGGTCAAATTCGTGATTTAGCGAGG	ACGCGTCGACCAATTGCAGGGAAT
Δ*modA*	GACTCCCAAGCTTGCGCCTTCTGCTATG	GGATGCATCGGTGCGTGCTTCTATTCGGAAGTAATGATACCAGCTGGAAGTG	ATCTTGCAGATGTTGAACAGT
Δ*dexA*	ACGCGTCGACTTAGTTTAAGGTA	GGGAAGTGGTGAAAAAGCAGCGGTTGGGTCTTGAAGATGCTCCATCAGAGG	ACGCGTCGACAAATTTGAGGAAG

Table 2. The *E. coli* K-12 strains used in this research.

Stain	Genotype	Source
MH1	*sup*^{0} *araD139 hsdR* Δ*lacX74 rpsL*	Kai *et al.*1996
TY0807	MH1 *araD+*	Koga *et al.* 2011
JW1667	BW25113 Δ*lpp*::*kan*	Keio collection
YT10	MH1 *zce726*::*Tn10*	Lab stock
YT20	MH1 *zce726*::*Tn10 ams1*	Lab stock
TY1001	YT10 Δ*lpp*::*kan*	This work
TY1002	YT20 Δ*lpp*::*kan*	This work
YT007	MH1 Δ*rppH*::*kan*	Lab stock
TY1005	MH1 Δ*rppH*	This work
TY1006	MH1 Δ*rppH* Δ*lpp*::*kan*	This work
TY1007	MH1 *rne-Flag-cat*	Lab stock
TY1008	MH1 *rne598-Flag-cat*	Lab stock
TY1009	MH1 *zce726*::*Tn10 ams1 araD+*	Lab stock

Construction of Δ6 phage mutant

A DNA fragment was PCR-amplified using Δ(*39-56*)$_6$ DNA as a template and primers 5′-TCAACGCCATCTTCCAATCCAT and 5′-ATCTTGCAGATGTTGAACAGT Δ(*39-56*)$_6$ DNA was used as a template. Consequently, the resulting fragment was digested with *Eco*RI and *Hind*III. The generated DNA fragment was inserted into *Eco*RI and *Hind*III sites of pBSPLO+ vector and the copied mutant sequence was transferred to phage genome via homologous recombination between plasmid and T4 DNA.

Plasmids:

To clone T4 *srd*, a 785 bp DNA fragment (11039-11803 of GenBank Accession No. NC_000866) was amplified by PCR using T4 DNA as a template and primers 5'-ACGCGTCGACGTAAGATGTGAGAA and 5'-ACGCGTCGACTTATCCTCGGATAAG, and digested with *Sal*I. The resulting fragment was inserted into the *Sal*I site of pBAD18 or pBAD33 (Guzman *et al.,* 1995) to generate pBAD18-*srd* or pBAD33-*srd*, respectively. To construct pQE80L-*srd*-His, a DNA fragment encoding Srd with a His-tag at its C-terminus was amplified by PCR with T4 DNA as a template and the primers, 5'-GACTCCGGAATTCCGGGTAAGATGTGAGAA and 5'-GACCCAAGCTTGGGTTAGTGGTGATGGTGATGATGTCCTCGGATA, digested with *Eco*RI and *Hind*III, and ligated into the corresponding sites of pQE80L (QIAGEN). To generate pBSl*pp*(T), the primer 1, 5'-CTATTACGCCAGCTGGCGAAAAAAAATGGCGCACAATGTGC and the primer 2, 5'-GGCCGATTCATTAATGCAGCTGGC were first phosphorylated using T4 kinase with ATP. The phosphorylated primer 1 and the primer 3, 5'-CGCGCCCAGTACCAGTTTAGTAGCTTCCACACAACATACGAGCCGGAAGC AT were used for PCR *with E. coli* MG1655 DNA as a template. The PCR-amplified fragment was used as a primer for second PCR with the phosphorylated primer 2 and pBluescript II KS (+) as a template. Finally, the resulting fragment was ligated into the *Pvu*II site of pBluescript II KS (+).

Determination of the deleted region in Δ(39-56)$_6$ phage:

Two primers, 5'-TCAACGCCATCTTCCAATCCAT located in *motB.1* and 5′-ATCTTGCAGATGTTGAACAGT located in *modB* were used for PCR. When Δ(*39-56*)$_6$ phage DNA was used as a template, only a prominent band corresponding to 1.2 kb was yielded, although 5.8-kb DNA was amplified with wild-type phage DNA as a template. The 1.2 kb DNA amplified with Δ(*39-56*)$_6$ phage DNA was recovered from the gel and subjected to sequencing. The sequence context was analyzed with Software ApE and the result showed that the nucleotide at 7744 (GenBank Accession No. NC_000866) in the T4 genome is connected with that at 12364 (GenBank Accession No. NC_000866), indicating that Δ(*39-56*)$_6$ lacks 4619 bp sequence.

RNA purification, Northern blot, Primer extension analysis and RT-PCR analysis:

Isolation of total RNA and Northern blot analysis were carried out as described (Kai *et al*., 1996). For Northern blots, radioactive probes for *ompA* and *lpp* were prepared as described (Ueno *et al.,* 2004). An oligo-probe for *srd* was 5′-^{32}P-ACGCGTCGACTTATCCTCGGATAAG. Oligo-probes for detecting *lpp* 5'end and *lpp* 3′ end were 5′-GATTGAGTTAATCTCCATGTAGCG and 5′-TGGCGCACAATGTGCGCCATTTTT, respectively. Primer extension analysis for *lpp* RNA was performed using the antisense primer 5′-^{32}P-GCATTGCGTTCACGTCGTTGCTCA. I performed Northern blot analysis at least two times and similar results were obtained for each experiment. A representative result was shown in each figure.

RT-PCR analysis for *srd* mRNA was carried out as previously described (Otsuka *et al*., 2010) In detail, 2 μg of total RNAs were first incubated at 42°C for 50 min with 80 units of ReverTra Ace reverse transcriptase (TOYOBO), 1 mM of dNTPs, and 2.5 pmol of *srd*‐RT primer (5′-ACGCGTCGACTTATCCTCGGATAAG) in 10 μl of reverse transcription buffer. PCR amplification was performed with 0.2 mM dNTPs, 1 μl of reverse transcription mixture, 10 pmol of sense primer (5′-

CGCATAGCAGAAGGCGCTGAAG) and antisense primer (5′-GCGGATATCCTCTTTTCAGTTT), and 1 unit of KOD Dash (TOYOBO) in 25 μl of PCR Buffer.

Pull-down and Western blot analysis:

TY1007 or TY1008 cells harboring pQE80L-*srd*-His or pQ-orf2-95 encoding His-tagged IscR (Otsuka *et al.* 2010) were grown at 30˚C in 200 ml of LB medium containing 50 μg/ml ampicillin until the OD600 reached 0.6, and treated with 2 mM IPTG for 2 h to induce His-tagged Srd or 20 μM IPTG for 1 h to induce His-tagged IscR. Cells were harvested and suspended in 1.5 ml of lysis buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5 mM DTT, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, and 1X protease inhibitor cocktail). 100 μl of the cell mixture was kept as a whole cell extract. After sonication, the lysate was centrifuged at 20,400 x g for 20 min and separated into the supernatant as a soluble fraction and the pellet as an insoluble fraction. The pellet was suspended with 1.3 ml of TBS buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5 mM DTT). The soluble fraction (1.3 ml) was treated with 20 μg/ml RNase A for 30 min at 25˚C, then 0.6 ml was mixed with 20 μl of Ni-NTA Superflow agarose beads (QIAGEN) or 20 μl of anti-FLAG M2 affinity agarose (Sigma-Aldrich) by end-over-end rotation overnight at 4˚C. The beads were washed four times with 0.6 ml of TBS buffer for anti-FLAG M2 agarose or TBS buffer containing 20 mM imidazole for Ni-NTA agarose, and then 45 μl of sample loading buffer was added to the beads. After boiling the samples, bound proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10% gel for full-length RNase E- FLAG, 12% gel for RNase E(1-598)-FLAG, or 15% gel for Srd-His and His-IscR) and electroblotted onto Immuno-Blot PVDF membrane (Bio-Rad). Western blot analysis was performed as previously described (Koga *et al.* 2011).

Burst size assay:

MH1 cells were grown in M9C medium until the OD600 reached 0.5, and infected

with T4 phage at a multiplicity of infection of 0.1 at 37˚C. At 8 min, the cells were diluted 10^4-fold with fresh M9C and further incubated for 70 min. After the cells were lysed with chloroform, the total number of progeny phages was determined by plating with MH1 as an indicator. The burst size is the ratio of the number of progeny to the number of input phage. Each value indicates the mean and a standard deviation obtained from three independent experiments.

Growth curve and Cell viability measurement

Cells harboring pBAD18 or pBAD18-*srd* were grown overnight in LB medium at 30°C. 50µl of overnight cultures were inoculated into 5 ml fresh LB medium, and cell density was monitored OD at 660nm every 20mins automatically with a Bio-Photo recorder TN-1506 (ADVANTEC). Meanwhile, after addition of 0.15% L-arabinose, 10µl cells were took and diluted by optimal fold followed by plating on LB plates at various times and then incubate at 37°C overnight. Time 0 means the time right before L-arabinose addition.

Results

Confirmation of T4-induced rapid host mRNA degradation

Ueno and Yonesaki's work (2004) found that *E. coli* mRNAs were destabilized immediately after T4 infection and *E. coli* RNase E, which is an essential ribonuclease in *E. coli* RNA metabolism, contributes to T4-induced host mRNA degradation. I first did experiments to confirm this phenomenon again. MH1 cells were grown to log-phase in M9C medium, and then infected with wild type T4 phage (T4D). As seen in Fig 1A, in uninfected cells, half-life of host mRNA *lpp* or *ompA* was 24min or 15min, respectively, which was only 2.5min in T4-infected cells. This again strongly demonstrated that immediately after T4 infection, representative stable host mRNAs were destabilized. Moreover, when RNase E temperature-sensitive cells were infected with T4 phage, half-life of *lpp* or *ompA* was about 21min or 8.2min (Fig 1B), respectively, both were much stabilized, indicating *E. coli* RNase E is a predominant RNase during T4-induced host mRNA degradation. Furthermore, to know whether or not T4 infection causes increased expression of RNase E, I compared the amount of RNase E before and after infection. Cells with Flag-tagged endogenous RNase E harboring plasmid expressing His-tagged E. *coli* IscR were infected with wild type T4, and same amount of cells were analyzed using western blotting. As seen in Fig 1C, amount of RNase E didn't change after T4 infection, His-IscR was detected as loading control.

Identification of a phage factor involved in T4-induced host mRNA degradation

Previous work also found that gene expression is needed in T4-induced host mRNA degradation (Ueno *et al.,* 2004). Thus, T4 may encode factor(s) involved in host mRNA degradation after infection. About two-third of *orf*s in T4 genome have been characterized, in order to identify factor(s) involved, I examined many T4 deletion mutants that lack multiple genes with unknown function in various genomic

regions and found that degradation of host mRNAs was suppressed in Δ(*39-56*)$_6$ mutant. MH1 (wild-type) cells were grown to log phase, total RNAs were extracted from cells at various times after phage infection and examined by northern blot analysis. In wild-type phage-infected cells, representatively stable mRNAs *lpp* and *ompA* were rapidly degraded after infection with the half-lives of 2.4 ± 0.2 and 1.4 ± 0.4 min, respectively (Fig. 3C and 3D). When Δ(*39-56*)$_6$ mutant infected, the half-lives of *lpp* and *ompA* mRNAs were increased to 4.6 ± 0.9 and 3.1 ± 0.3 min, respectively. Additionally, a decay intermediate of *lpp* mRNA was remarkably accumulated in Δ(*39-56*)$_6$-infected cells. This result suggested that a gene lacked in Δ(*39-56*)$_6$ mutant was involved in T4-induced host mRNA degradation. Δ(*39-56*)$_6$ mutant was originally isolated by Homyk and Weil in 1974, but the deletion position in the genome of this phage was not reported clearly. I attempted to determine it by PCR and DNA sequencing, the deleted region is 4619-bp DNA between two 10nt-repeat sequences, TGGTCATCTG, which located separately in gene *motB.2* and *modA* (Fig 2), resulting in lack of whole or a part in 8 ORFs (Fig. 3B). This result is consistent with Mosig's work (Mosig *et al.*, 1998). However the exact deletion position is hard to be further confirmed because of the sequence repetition. The repeat sequence start nucleotides are located at nucleotide position at 7737 and 12356, respectively, in T4 genome.

Since Δ(*39-56*)$_6$ was a randomly obtained mutant (Homyk *et al.,* 1974), there might be unknown mutation also in regions outside of gene *39-56*. To confirm if genes lacking in Δ(*39-56*)$_6$ genome are really correspond to phage-induced mRNA degradation, I first construct Δ6 phage mutant genome of which lacking deleted region that determined from Δ(*39-56*)$_6$ phage using Insertion/Substitution system and checked its effect on T4-induced host mRNA degradation. As seen in Fig 3A, *lpp* mRNA was stabilized and decay intermediate was accumulated similar to that in Δ(*39-56*)$_6$-infected cells. This result strongly suggests that gene(s) lacking in Δ(*39-56*)$_6$ DNA was involved in T4-induced host mRNA degradation.

To further identify the causal gene, I constructed 6 mutants that lacked one or a few of these ORFs and examined the stability of *lpp* mRNA and the accumulation of decay intermediate after T4 infection (Fig. 3C and Fig 4). The deletion mutants except Δ*srd* had little effect on the degradation of host mRNAs. In contrast, *lpp* and

ompA mRNAs were stabilized in Δ*srd*-infected cells and their half-lives were 4.7 ± 0.4 and 3.2 ± 0.4 min, similar to those of Δ(*39-56*)$_6$-infected cells. In addition, the decay intermediate of *lpp* mRNA was highly accumulated in Δ*srd*-infected cells as well as Δ(*39-56*)$_6$-infected cells. This result strongly suggests that T4 gene *srd* is involved in the T4-induced host mRNA degradation.

Effect of Srd on decay of lpp and ompA mRNAs

To examine the effect of Srd on decay of *lpp* and *ompA* mRNAs in uninfected cells, I constructed a plasmid, pBAD18-*srd*, expressing Srd under the control of arabinose-inducible promoter. TY0807 cells harboring pBAD18-*srd* were grown to mid-log phase, and total RNAs were then extracted from cells at 0, 5, 10, or 20 min after addition of arabinose and examined by northern blot analysis. As seen in Fig. 5A, the expression of *srd* mRNA was discernible at 5 min after addition of arabinose and amounts of *lpp* and *ompA* mRNAs reduced in antiparallel with the increase of *srd* mRNA. To further explore whether the reduction of these mRNAs is caused by the increase of decay rate or not, I measured stabilities of *lpp* and *ompA* mRNAs (Fig. 5B and 5C). At 10 min after addition of arabinose, a transcription inhibitor, rifampicin, was added to the culture and total RNAs were extracted at various times. In the absence of arabinose, both *lpp* and *ompA* mRNAs were stable with half-lives of 27.1 ± 1.3 min and 11.7 ± 1.0 min, respectively. In contrast, their half-lives were 12.0 ± 1.6 min for *lpp* and 5.1 ± 1.5 min for *ompA* after addition of arabinose, indicating that their decay rates were increased approximately 2-fold after induction of Srd. Together with the result of Fig. 3C and 3D, it was strongly suggested that Srd destabilized *lpp* and *ompA* mRNAs. Furthermore, I confirmed that Srd stimulated the RNase E-dependent decay of *lpp* and *ompA* mRNAs in RNase E-deficient cells (Fig. 5D and 5E). At 44˚C, the half-lives of *lpp* and *ompA* mRNAs in the presence or absence of *srd* were 1.6 ± 0.2 min and 5.1 ± 0.1 min or 1.5 ± 0.1 and 4.2 ± 0.9 in wild-type cells, respectively, while they were 13.3 ± 1.8 min and 17.7 ± 3.5 or 8.9 ± 1.4 min and 10.5 ± 1.4 min in RNase E temperature-sensitive cells. Finally, I confirmed that Srd stimulated the RNase E-dependent decay of *lpp* and *ompA* after T4 infection. At 44˚C, the half-life of *lpp* mRNA in YT20(*ams1*) cell mutant after infection under the presence and absence of *srd* were 23.8 ± 3.9 min and 25.3 ± 4.8

min. And they are 5.8 ± 1.0 min and 6.4 ± 1.5 min for *ompA* mRNA (Fig. 5F). These results strongly suggest that Srd-accelerated mRNA degradation is RNase E-dependent.

Decay intermediate of lpp mRNA generated by RNase E

The decay intermediate of *lpp* mRNA accumulated in Δ*(39-56)$_6$ or* Δ*srd*-infected cells (Fig. 3C and 3D) would be attributable to cleavage by RNase E, because full-length *lpp* mRNA was considerably stabilized in RNase E-defective cells (Ueno *et al.,* 2004; Fig. 1B). To confirm this possibility, I first examined the effect of RNase E on the decay of *lpp* mRNA in Δ*(39-56)$_6$*-infected cells. Wild type cells or temperature-sensitive RNase E mutant cells were grown at a permissive temperature (30˚C) to mid-log phase and then temperature was shifted to 44˚C, a non-permissive temperature, for 30 min, followed by infection with wild type or Δ*(39-56)$_6$* mutant. RNAs extracted from cells at various times after infection were analyzed by northern blot analysis. Full-length *lpp* mRNA was stabilized and the decay intermediate was hardly detected in RNase E-defective cells infected with Δ*(39-56)$_6$* mutant phage as well as wild-type phage (Fig. 6A). To investigate cleavage site of this decay intermediate, I first designed three primers (see *Material and Methods*) for *lpp* transcript to know where the site is close to. As seen in Fig 6B, decay intermediate of *lpp* mRNA was not detectable when using probe for 5′-end of *lpp* transcript, while detectable when using probes for 3′-end or the middle part of *lpp* transcript. Theses results suggest that the cleavage site should be located close to 5′-end of *lpp* transcript. Then further determination was carried out by primer extension analysis and 5′-terminus of the decay intermediate was 2 nucleotides downstream of the start codon of *lpp* mRNA (AUGA↓AA) (Fig. 6C). RNase E has no canonical target sequence for cleavage, but preferentially cleaves at regions that are single-stranded with AU-rich sequence (Mackie 2013a). These facts clearly support the idea that RNase E generates this decay intermediate.

Effect of Srd on decay of truncated lpp RNA

The above results suggested that the decay intermediate of *lpp* mRNA was processively degraded by RNase E in the presence of Srd. To investigate the effect of Srd on the decay intermediate of *lpp* mRNA, I constructed pBS*lpp*(T), a plasmid expressing truncated *lpp* RNA with sequence identical to that of the decay intermediate of *lpp* mRNA from the *lac* promoter of pBluescript II KS (+) (Fig. 7). First, I examined the decay of *lpp*(T) RNA in the absence of Srd. *lpp*(T) RNA had a half-life as short as 1.4 ± 0.2 min in Δ*lpp*::*kan* cells carrying wild-type RNase E, while it was more stable with the half-life of 9.5 ± 1.2 min in Δ*lpp*::*kan ams1* cells defective in RNase E activity (Fig. 8A and 8B).

The effect of Srd on decay of *lpp*(T) RNA was examined by measuring its amount with or without induction of Srd (Fig. 9A) and found that the amount considerably reduced after induction of Srd for 10 min. Furthermore, I checked the stability of *lpp*(T) RNA. The half-life with or without induction of Srd was 1.3 ± 0.1 or 2.9 ± 0.2 min, respectively (Fig. 9B and 9C), clearly demonstrating that Srd stimulated the degradation of *lpp*(T) RNA. Furthermore, I also checked effect of Srd expression in RNase E temperature sensitive cells (Fig. 9D and 9E). At high temperature (nonpermissive for RNase E), half life of *lpp*(T) RNA was too fast to determine after Srd induction. But its decay was remarkably stabilized with a half life of 8.8 ± 0.2 min in RNase E-deficient cells, additively, a half-life of 8.1 ± 0.5 shows that induction of Srd didn't accelerate degradation of *lpp*(T) RNA in those cells. Finally, I confirmed that Srd stimulated the RNase E-dependent decay of *lpp*(T) after T4 infection (Fig. 9F and 9G). The half-life of *lpp*(T) RNA in the presence or absence of *srd* was 2.9 ± 0.2 min or 7.8 ± 0.3 min, respectively, at 30˚C, while it was 7.2 ± 0.4 min or 9.4 ± 0.2 min at 44˚C. These results indicate that both T4 Srd and RNase E are required for the rapid degradation of *lpp*(T) RNA after T4 infection, and again strongly suggest that Srd stimulates RNase E activity after T4 infection.

Association of Srd and RNase E in vivo

The above results implied that Srd physically associated with RNase E. Hence, I carried out immunoprecipitation and pull-down analysis to examine their interaction *in vivo*. For these experiments, I used TY1007 cells in which the chromosomal *rne*

was replaced with *rne-Flag* encoding FLAG-tagged RNase E. Extracts prepared from TY1007 cells expressing His-tagged Srd or His-tagged IscR as a control were used for immunoprecipitation with FLAG antibody or pull-down with Ni-NTA. Before Immunoprecipitation and pull-down analyses, I checked how much RNase E was solubilized because RNase E is membrane-associated. As seen in Figure 10A (lanes 1-6), the majority of RNase E was present in the soluble fraction under our experimental conditions. In the immunoprecipitation experiment, Srd-His was efficiently recovered together with RNase E-FLAG (lane 7), but His-IscR was not (lane 8). In the reciprocal pull-down experiment, RNase E-FLAG was precipitated together with Srd-His (lane 9), but not with His-IscR (lane 10). These results suggested the physical association of Srd and RNase E *in vivo*.

Dispensability of CTH of RNase E on Srd-stimulated host mRNA degradation

RNase E is composed of NTH and CTH (Callaghan *et al*., 2005; Mackie 2013a). I investigated whether or not CTH was indispensable for T4-induced host mRNA degradation. TY1008 cells expressing FLAG-tagged RNase E lacking CTH were infected with wild type or Δ*srd* phage and total RNAs were extracted at different times for northern blot analysis. In consistent with the previous report (Ueno *et al.,* 2004), CTH-truncated RNase E degraded the full-length *lpp* mRNA more slowly than normal RNase E did and its half-life was 5.0 ± 0.4 min (compare to 2.4 ± 0.2 min in Fig. 3C), when wild-type phage infected (Fig. 11A and 11B). When Δ*srd* phage infected, the full-length *lpp* mRNA was more stabilized with a half-life of 9.2 ± 0.6 min and the decay intermediate was also accumulated. This result was paralleled with the result in Fig. 3C and 3D, suggesting that CTH is not necessary for *E. coli* mRNA degradation stimulated by Srd.

In connection with the above result, I examined the ability of NTH of RNase E in association with Srd. As seen in Fig. 10B, most of RNase E(598)-Flag was present in the soluble fraction (lane 1-6), like an RNase E-Flag. Srd-His was precipitated by FLAG antibody together with RNase E(598)-FLAG (lane 7), but His-IscR was not (lane 8). In the pull-down experiments with Ni-NTA beads, RNase E(598)-FLAG

was recovered in the bound fraction together with Srd-His (lane 9) but hardly with His-IscR (lane 10), suggesting the association of Srd and NTH of RNase E *in vivo*.

Effect of RppH on stimulation of RNase E activity by Srd: Srd stimulates both two activities of RNase E

RNase E have two different cleavage activities, it cleaves mRNAs at internal sites either directly or by 5′-end-dependent mechanism (Bouvier *et al.*, 2011), namely 5′-monophosphate-independent or dependent activities. The latter mechanism requires removal of a pyrophosphate from triphosphate at 5′-end of transcript always by *E. coli* RNA pyrophosphohydrolase, RppH, prior to RNase E cleavage. Hundreds of mRNAs are the targets of 5′-end-dependent mRNA degradation pathway, because disruption of *rppH* resulted in increase of their amounts or stabilities (Deana *et al.*, 2008). On the other hand, many other mRNAs appear to be degraded by RNase E using another mechanism, direct cleavage, independently from RppH (Clarke *et al.*, 2014).

In *E. coli*, full-length *ompA* and *lpp* transcripts have been well-known that their degradation is RppH-independent and through direct entry pathway (Emory *et al.*, 1992; Ow *et al.*, 2003; Deana *et al.*, 2008). Moreover, as seen in Fig 12 A, after T4 infection *lpp* and *ompA* full-lengths in Δ*rppH* cells were degraded as fast as those in wild type cells. Considering with Fig 6 together, 5′-end-independent internal site cleavage activity of RNase E was stimulated by T4 Srd. On the other hand, I examined the effect of RppH on the stability of *lpp*(T) RNA (Fig. 8C and 8D), which is supposed to be plasmid expressed *lpp* decay intermediate. Without infection, the half-life of *lpp*(T) RNA in wild-type or Δ*rppH* cells was 2.8 ± 0.3 or 7.1 ± 0.1 min, respectively. When wild-type phage infected, *lpp*(T) RNA was significantly stabilized in Δ*rppH* cells (6.0 ± 0.6 min), compared with wild-type cells (2.1 ± 0.1 min) (Fig 12 B and C). Considering together, Fig 8C, 8D, 12B and 12C indicated that pyrophosphates removal by RppH is required for degradation of *lpp*(T), which means degradation of *lpp*(T) mainly depends on the 5′-end-dependent cleavage of RNase E. Furthermore, considering together with Fig 10, 5′-end-dependent cleavage activity of RNase E was stimulated by T4 Srd. Taken above together, all these results

suggested T4 Srd could stimulate both two mRNA degradation activities of *E. coli* RNase E after T4 infection.

Effect of Exogenous Srd on E. coli cell growth

I observed the effect of Srd on *E. coli* growth on LB solid medium. Same amount of cells harboring pBAD18 or pBAD18-*srd* were spread on LB plates with or without 0.2% L-Arabinose and incubated at 30, 37 and 42°C overnight (Fig14A). Cells harboring pBAD18-*srd* didn't grow on plates containing 0.2% L-Arabinose, revealing that expressing of exogenous T4 Srd is toxic to *E. coli* cells. To confirm this, I measured growth curve of cells harboring pBAD18 or pBAD18-*srd*. As seen in Fig 14B, after 30min induction of Srd, cell growth starts to pause, cell density increased very slowly. Moreover, cells were spread on solid plates to measure survival rate after induction at time points of 0min, 30min, 1hour, 1.5hour, 2hour and 4hour. Resulting colony forming units were shown in Fig 14C. After about 4hour-induction of T4 Srd, cell viability deduced about 10-fold. Taken together, these results obviously revealed that exogenous expression of T4 Srd is toxic to *E. coli* growth.

Effect of srd on the growth of T4 phage

Finally, I investigated the effect of *srd* on T4 growth. Wild-type or Δ*srd* phage was spread on plates seeded with MH1 cells as an indicator (Fig. 15A). Δ*srd* mutant formed smaller plaques than those of wild type. I also confirmed the stimulatory effect of *srd* on propagation of T4 phage by measuring burst size (Fig. 15B). Consistently with the observation of plaque size, the burst size of Δ*srd* was 24.4 ± 1.9, while that of wild-type was 86.9 ± 4.8. These results indicate that *srd* is required for efficient growth of T4 phage.

Discussion

In this study, I demonstrated that T4 Srd is involved in phage-induced host mRNA degradation, in which *E. coli* RNase E plays a central role. RNase E participates in turnover of bulk mRNAs as well as processing of tRNAs and rRNAs in *E. coli* and many other proteobacteria (Jain *et al*., 2002; Ono *et al*., 1979; Carpousis 2007; Tsai *et al*., 2012). Cleavage of mRNA by RNase E can occur via two different kinds of activities: 5′-monophosphate end-dependent and 5′-end-independent (so-called 'direct entry') (Bouvier *et al*., 2011). Our study showed that plasmid-borne *lpp*(T) RNA was degraded by RNase E in an RppH-dependent fashion, and that Srd stimulated this activity (Fig. 8, 12B and 12C). Moreover, full-length *ompA* and *lpp* mRNA should be degraded by RNase E through direct entry after infection, and Srd could stimulate 5'-end-independent cleavage activity of RNase E (Fig. 6 and 12A). Taken together with the fact that Srd stimulated RppH-dependent *lpp*(T) RNA degradation as described above, I conclude that T4 Srd stimulates both 5'-monophosphate end-dependent and 5'-end-independent mRNA degradation pathways by RNase E.

The NTH of RNase E is necessary and sufficient for cleavage and forms a tetrameric assembly of RNase E (Mackie 2013a). The CTH acts as a scaffold for RNA degradosome assembly and recruits its core constituents: polynucleotide phosphorylase, enolase and RhlB helicase (Carpousis 2007; Górna *et al*., 2012). I demonstrated that T4-induced host mRNA degradation occurred in the absence of CTH (Fig. 11). Furthermore, immunoprecipitation and pull-down experiments showed that Srd was physically associated with NTH of RNase E. Considering that the amount of RNase E did not change after T4 infection (Fig. 1C), Srd should accelerate host mRNA degradation by stimulation of catalytic activity of RNase E. The NTH consists of two RNase H-like domains, S1 domain, 5′-sensor domain, DNase I-like domain, Zn-link domain and a small domain (Mackie 2013a). Further dissection to identify which domain of RNase E interacts with Srd will be needed to understand the stimulatory mechanism by Srd.

To date, three *E. coli* factors, RraA, RraB and ribosomal protein L4, have been reported to regulate the activity of RNase E. RraA physically interacts with the C-

terminal RNA binding sites and inhibits RNase E activity by interrupting the access of RNase E to RNA substrate (Lee *et al*., 2003b). RraB binds to CTH and interferes with cleavage of target mRNAs (Gao *et al*., 2006). Binding of RraA or RraB differently affects the composition of RNA degradosome (Gao *et al*., 2006). L4 interacts with a site outside of the catalytic domain of RNase E to inhibit RNase E cleavage activity in vitro and stabilizes mRNAs targeted by RNase E *in vivo* (Singh *et al*., 2009). Apart from *E. coli*, a protein kinase of bacteriophage T7 phosphorylates RNase E at its CTH and stabilizes a subset of RNase E substrates (Marchand *et al*., 2001). In contrast, Carpousis' group demonstrated that RhlB, which is a member of DEAD-box RNA helicase and a major component of the degradosome, facilitated RNase E activity for the degradation of several artificial RNAs transcribed by T7 RNA polymerase (Khemici *et al*., 2005), although it is not required for stabilities of endogenous mRNAs (Bernstein *et al.* 2004). Additionally, Görke's group recently reported that *E. coli* RapZ interacted with NTH of RNase E and functioned as an adaptor protein to recruit RNase E for processing of the small RNA GlmZ. In this case, it only functions on GlmZ sRNA and the possibility that RapZ directly affect RNase E activity has not been addressed in detail (Göpel *et al.,* 2013; Göpel *et al.,* 2014). T4 Srd is the first example of a viral factor for stimulating RNase E activity. Future investigation on the stimulation mechanism of Srd may give hints to studies on identifying more positive regulators of RNase E acitivities which may thus leads our understandings on control of mRNA degradation to go wider and deeper.

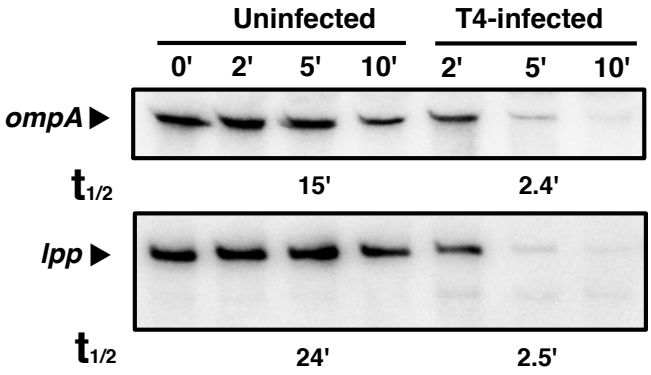
Three different classes of promoters, early, middle, and late, initiate T4 transcription (Miller *et al.,* 2003a). *srd* is located immediate downstream of early promoter, which is supposed to be expressed at very early time after T4 infection (Mathews 1994; Miller *et al.,* 2003a). Although our attempt for detecting endogenous expression of Srd was not successful (data not shown), we could detect endogenous *srd* mRNA by semi-quantitative RT-PCR (Figure 13). This result indicates that the level of *srd* mRNA during infection is almost same as that derived from pBAD18-*srd*. As origin of pBAD33 is different from pBAD18, its copy number is less than pBAD18, level of *srd* mRNA should be lower than either those generated from pBAD18-srd or endogenous level. The observations that Δ*srd* mutant forms smaller plaques (Fig. 15A) and exhibits a much lower burst size (Fig. 15B) in comparison to wild-type phage indicate that Srd is required for efficient growth of T4 phage. Host

mRNA degradation by Srd-stimulated RNase E activity should contribute to the transition of gene expression from host to T4 immediately after T4 infection (a possible model was shown in figure 16). However, this would not be a sole reason for the reduction of T4 growth in the absence of Srd. Namely, the stimulation of RNase E activity may contribute to other processes in T4 propagation. RNase E degrades T4 mRNAs (Mudd *et al.,* 1990a) to facilitate the transition of gene expression from early to middle or from middle to late. If Srd functions throughout T4 infection, then it may contribute to the transition of T4 gene expression via stimulation of RNase E activity. Ribonucleotides provided by mRNA degradation can be converted to deoxyribonucleotides by T4-encoded ribonucleotide reductase (Nrd) to stimulate DNA replication (Greenberg *et al.*, 1994). Viral DNA synthesized after 30min-infection with Δ*srd* phage mutant was less than that produced in wild type T4-infected cells (data not shown). Thus, Srd may be able to stimulate DNA replication via stimulation of RNase E activity. At present, I have no evidence that the stimulation of RNase E activity is a sole function of Srd. Exogenous expression of T4 Srd is toxic to *E. coli* cell growth, which is consistent with Mosig' s work (1998). Srd shares partial sequence similarity with sigma 70. Mosig *et al.* assumed that Srd might act as a decoy of σ^{70} thus affect host transcription like the action of T4 Srh, which shares sequence similarity with *rpoH* (σ^{32}) and was postulated that it might modulate competitions of σ^{32} and T4 gp55 for core RNA polymerase for T4 late transcription at high temperature.

Considering that the effect of *srd* on host mRNA degradation is partial (Fig. 3C), T4 phage may have multiple mechanisms to sustain rapid degradation of host mRNAs. On the other hand, infection-induced host RNA degradation is not limited to T4 phage, similar phenomenon also occurs in both prokaryotic and eukaryotic viruses. There may be factor(s) with similar function to T4 Srd in those phages and viruses.

Figure 1

A


Uninfected
T4-infected
0'
2'
5'
10'
2'
5'
10'
ompA ▶
t1/2
15'
2.4'
lpp ▶
t1/2
24'
2.5'

B

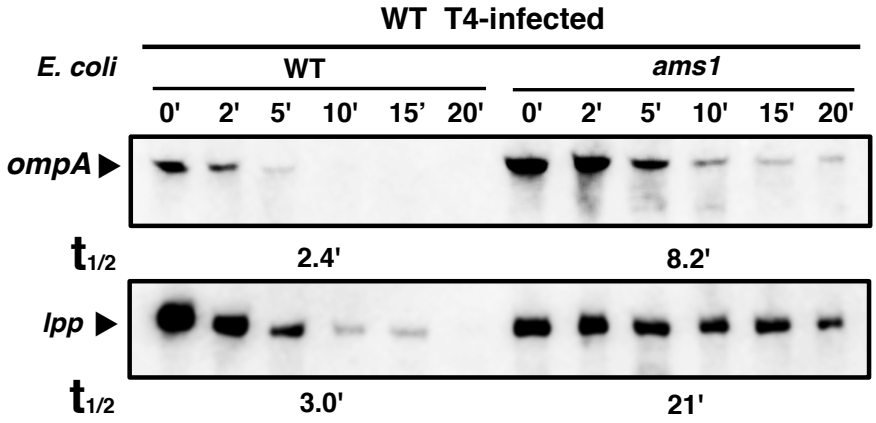
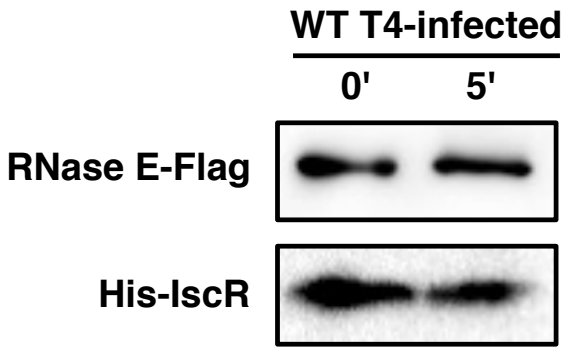

WT T4-infected
E. coli
WT
ams1
0'
2'
5'
10'
15'
20'
0'
2'
5'
10'
15'
20'
ompA ▶
t1/2
2.4'
8.2'
lpp ▶
t1/2
3.0'
21'

Figure 1. Destabilization of host mRNA degradation after T4 infection

(A) Change of host mRNA stability before and after T4 infection. MH1 (Wild-type) cells were grown in M9C (M9-glucose medium supplemented with 0.3% casamino acids, 1 μg ml^{-1} thiamine and 20 μg ml^{-1} tryptophan) medium until the OD_{600} reached 0.5 at 37˚C, and were divided into two aliquots. Transcription inhibitor, rifampicin, with a final concentration of 500 μg ml^{-1} was added to one aliquot (Uninfected cells), and the other was infected with T4 wild type at a m.o.i of 7. Total RNAs were isolated at the indicated times after infection and then analyzed by Northern blotting with probes for *lpp* or *ompA*. A Half-life ($t_{1/2}$) of each mRNA was shown below the figure.

(B) Involvement of *E. coli* RNase E. YT10 (Wild-type) cells or YT20 (*ams1*) cells were grown in M9C medium until the OD_{600} reached 0.3 at 30˚C, and shifted to 44˚C for another 30minutes. Both of them were then infected with T4 wild type at a m.o.i of 7. Total RNAs were isolated at the indicated times after infection and then analyzed by Northern blotting with probes for *lpp* or *ompA*. A Half-life ($t_{1/2}$) of each mRNA was shown below the figure.

C



(C) Amount of RNase E before and after T4 infection. TY1007 cells harboring pQ-orf2-95 were grown in LB medium until the OD600 reached 0.3 and treated with 0.02 mM IPTG for 30 min to induce IscR. Cells were harvested immediately before (0 min) or 5 min after T4 infection. Equal amount of cell extracts were electrophoresed through a 10% polyacrylamide gel containing SDS, followed by western blot analysis with antibodies against FLAG-tag and His-tag.

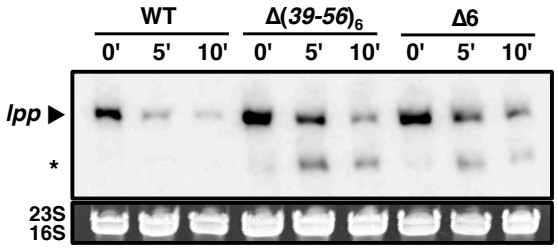
Figure 2

Figure 2. Repeat sequences generating $\Delta(39\text{-}56)_6$ phage mutant genome.

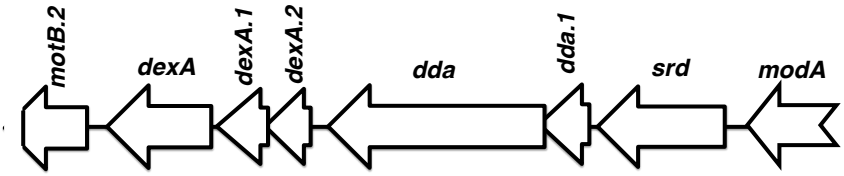
Part of sequences from 7724 to 12386 of T4 genome DNA (GenBank Accession No. NC_000866) from gene *motB.2* to gene *modA* are shown in figure. Sequences preceding the junction at deletion endpoints are boxed. The position of repeat sequences is labeled with numbers consistent with those under GenBank Accession No. NC_000866. Start sites of 10nt-repeat sequence are shown at nucleotide position 7737 and 12356.

Figure 3

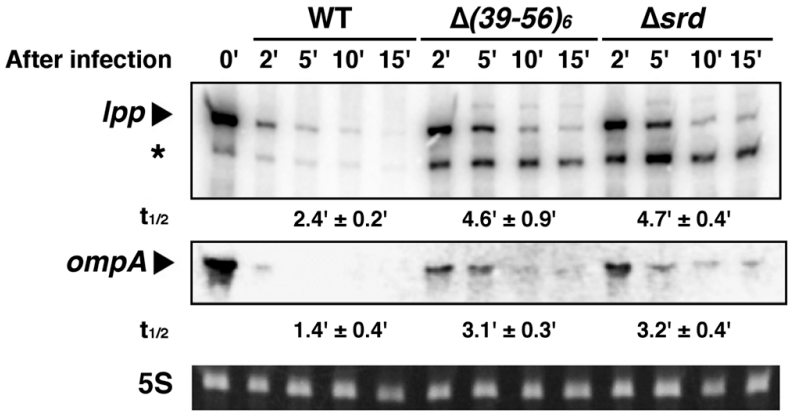
A



B



C



D

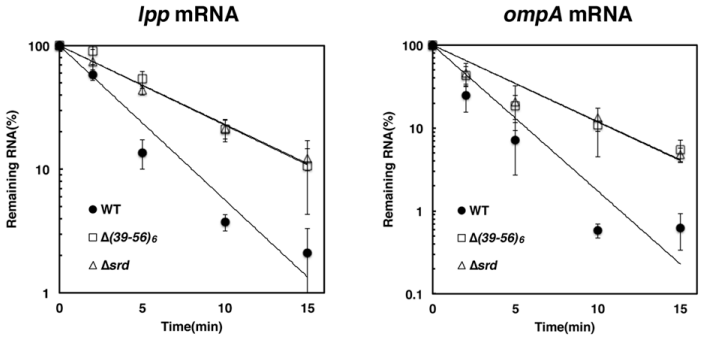


Figure 3. Effect of *srd* on T4-induced host mRNA degradation.

(A) Genetic organization of the chromosomal region deleted in Δ(*39-56*)$_6$ phage.

(B) MH1 cells were grown in M9C medium until the OD_{600} reached 0.5 at 37˚C and infected with T4 wild-type, Δ(*39-56*)$_6$ or Δ6 phage. Total RNAs were isolated at the indicated times after infection and electrophoresed through a 5% polyacrylamide gel containing 7M urea, followed by northern blot analysis with a probe for *lpp* or *ompA*. Ethidium bromide-stained 5S rRNA is shown as a loading control. Arrowheads and an asterisk indicate each full-length mRNA and a decay intermediate of *lpp* mRNA, respectively.

(C) MH1 cells were grown in M9C medium until the OD_{600} reached 0.5 at 37˚C and infected with T4 wild-type, Δ(*39-56*)$_6$ or Δ*srd* phage. Total RNAs were isolated at the indicated times after infection and electrophoresed through a 5% polyacrylamide gel containing 7M urea, followed by northern blot analysis with a probe for *lpp* or *ompA*. Ethidium bromide-stained 5S rRNA is shown as a loading control. Arrowheads and an asterisk indicate each full-length mRNA and a decay intermediate of *lpp* mRNA, respectively. The signal intensity was quantified using the Image J program and the time required for a 50% reduction was taken as a half-life ($t_{1/2}$) of mRNA shown below the figure.

(D) Quantification analysis of *lpp* and *ompA* mRNAs in the figure (C) was performed. Data points represent the mean ± SD of multiple measurements.

Figure 4

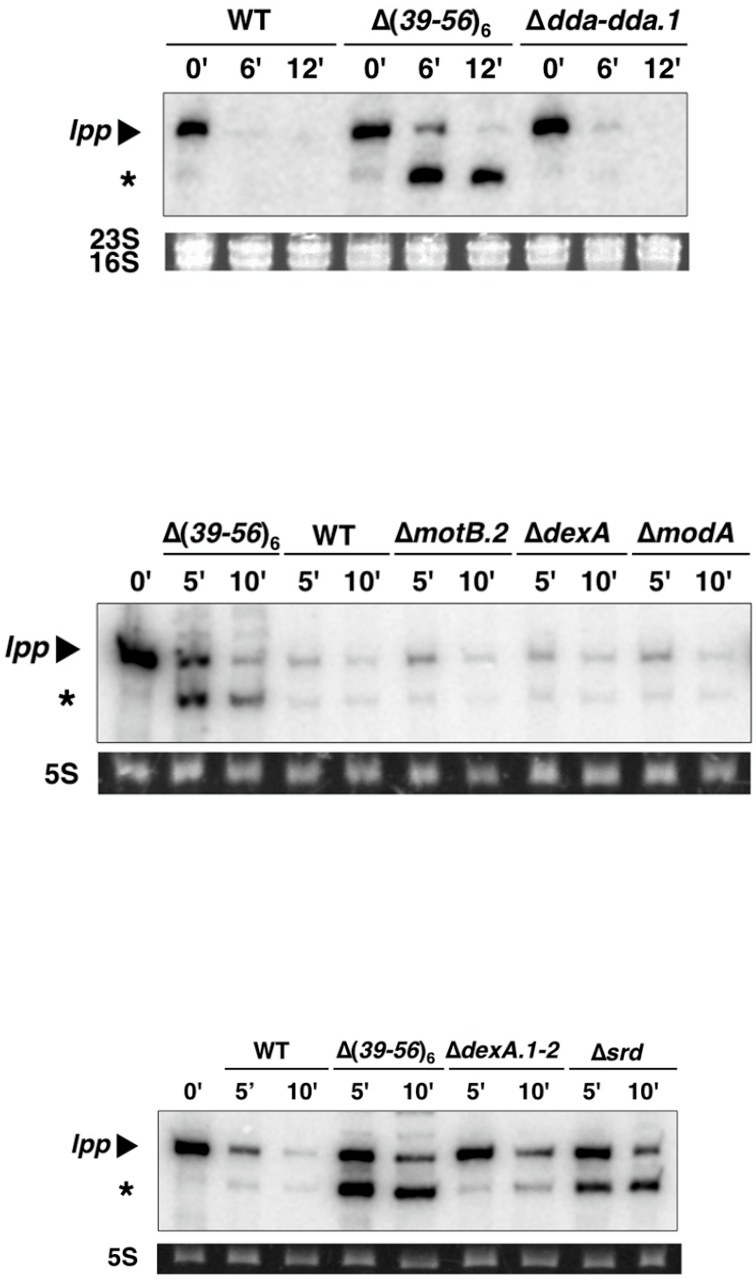
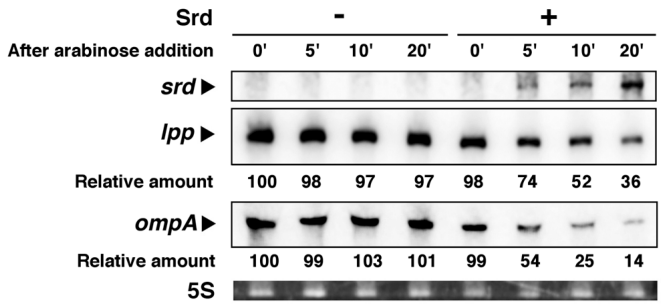

WT
Δ(39-56)6
Δdda-dda.1
0' 6' 12' 0' 6' 12' 0' 6' 12'
lpp
*
23S
16S
Δ(39-56)6
WT
ΔmotB.2
ΔdexA
ΔmodA
0' 5' 10' 5' 10' 5' 10' 5' 10' 5' 10'
lpp
*
5S
WT
Δ(39-56)6
ΔdexA.1-2
Δsrd
0' 5' 10' 5' 10' 5' 10' 5' 10'
lpp
*
5S

Figure 4. Effect of deleted genes on T4-induced host mRNA degradation.

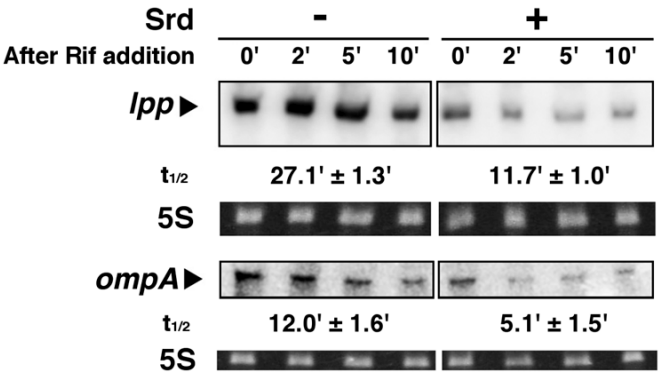
MH1 cells were grown in M9C medium until the OD_{600} reached 0.5 at 37˚C, and infected with T4 wild type, Δ(*39-56*)$_6$, Δ*dda-dda.1*, Δ*motB.2*, Δ*dexA*, Δ*modA*, Δ*dexA.1-dexA.2* or Δ*srd* mutant phage. Total RNAs were isolated at the indicated times after infection and then analyzed by northern blotting with a probe for *lpp*. An arrowhead and an asterisk indicate full-length and a decay intermediate of *lpp* mRNA. Ethidium bromide-stained 5S rRNA or 23S/16S rRNA as a loading control is shown at the bottom of each panel. The Δ*srd* mutant phage exhibited that *lpp* mRNA was stabilized and also the decay intermediate was highly accumulated, like a Δ(*39-56*)$_6$ phage.

Figure 5

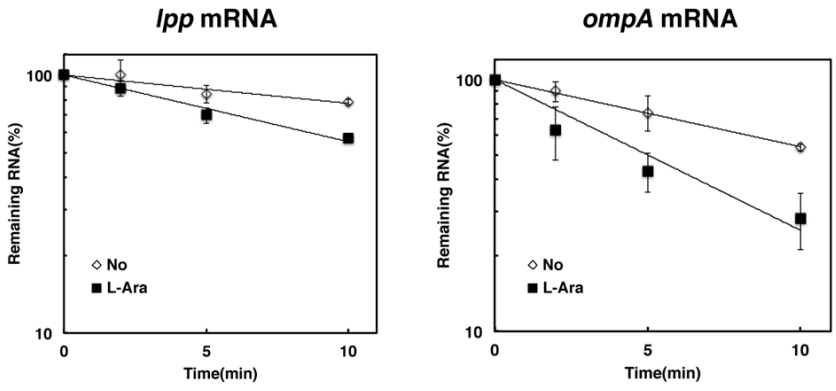
A


Srd
-
+
After arabinose addition
0' 5' 10' 20' 0' 5' 10' 20'
srd ▶
lpp ▶
Relative amount
100 98 97 97 98 74 52 36
ompA ▶
Relative amount
100 99 103 101 99 54 25 14
5S

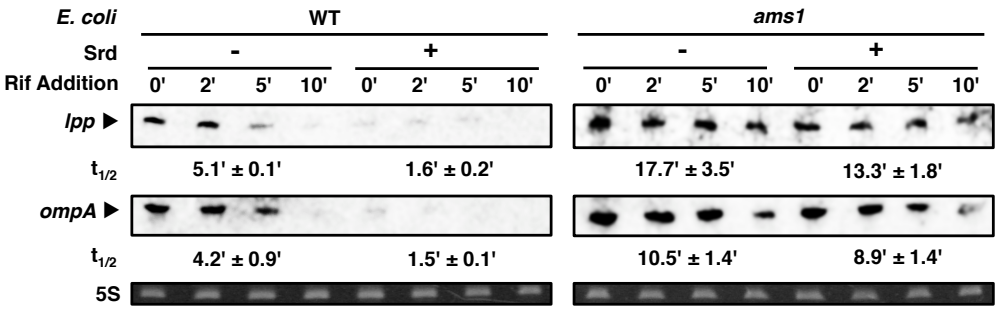
B


Srd
-
+
After Rif addition
0' 2' 5' 10' 0' 2' 5' 10'
lpp ▶
t1/2
27.1' ± 1.3'
11.7' ± 1.0'
5S
ompA ▶
t1/2
12.0' ± 1.6'
5.1' ± 1.5'
5S

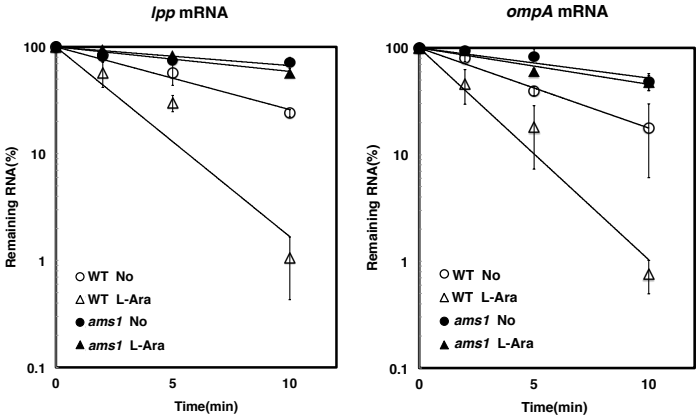
C


lpp mRNA
ompA mRNA
Remaining RNA(%)
100
10
0
5
10
Time(min)
◇ No
■ L-Ara

D


E. coli
WT
ams1
Srd
-
+
-
+
Rif Addition
0' 2' 5' 10' 0' 2' 5' 10' 0' 2' 5' 10' 0' 2' 5' 10'
lpp ▶
t1/2
5.1' ± 0.1'
1.6' ± 0.2'
17.7' ± 3.5'
13.3' ± 1.8'
ompA ▶
t1/2
4.2' ± 0.9'
1.5' ± 0.1'
10.5' ± 1.4'
8.9' ± 1.4'
5S

E


lpp mRNA
ompA mRNA
Remaining RNA(%)
100
10
1
0.1
0
5
10
Time(min)
WT No
WT L-Ara
ams1 No
ams1 L-Ara

F

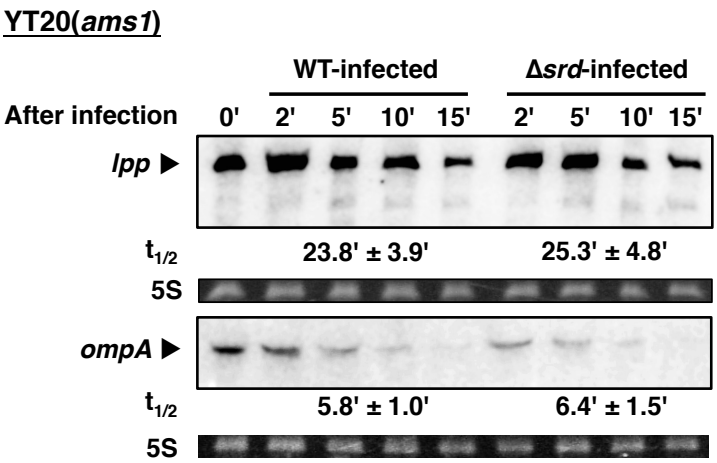

YT20(ams1)
WT-infected
Δsrd-infected
After infection
0' 2' 5' 10' 15' 2' 5' 10' 15'
lpp ▶
t1/2
23.8' ± 3.9'
25.3' ± 4.8'
5S
ompA ▶
t1/2
5.8' ± 1.0'
6.4' ± 1.5'
5S

Figure 5. Destabilization of *lpp* and *ompA* mRNAs by exogenous expression of Srd

(A) TY0807 cells harboring pBAD18 or pBAD18-*srd* were treated with 0.15% arabinose when the OD_{600} reached 0.5 at 30˚C. Total RNAs were extracted at the indicated times after induction and subjected to northern blotting with a probe for *srd*, *lpp* or *ompA* mRNA. The intensity of band at 0 time was set to 100% and relative intensity at each time was shown below the figure.

(B) TY0807 cells harboring pBAD18-*srd* were treated with (+) or without (-) 0.15% arabinose when the OD_{600} reached 0.5 at 30˚C and further incubated for 10 min. Total RNAs were extracted at various times after addition of rifampicin and subjected to northern blotting with probes for *lpp* or *ompA*. A Half-life ($t_{1/2}$) of each mRNA was shown below the figure.

(C) Quantification analysis of *lpp* and *ompA* mRNAs in the figure (B) was performed. Data points represent the mean ± SD of multiple measurements.

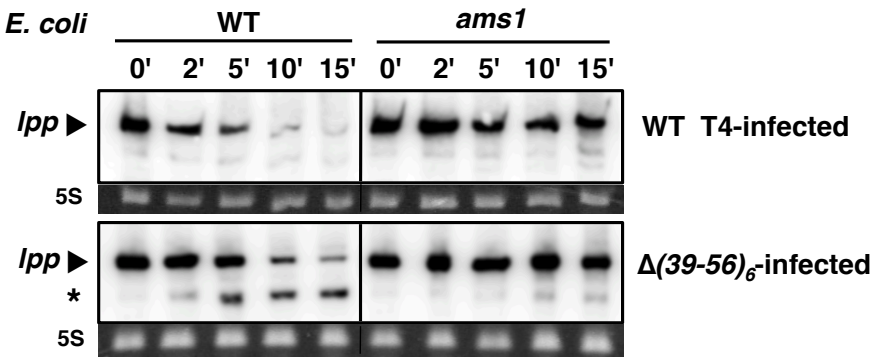
(D) TY0807 cells harboring pBAD18-*srd* or TY1009 (TY0807 *ams1*) cells harboring pBAD18-*srd* were grown in LB medium until the OD_{600} reached 0.3 at 30˚C and shifted to 44˚C for another 30 min, then divided into two aliquots followed by treating without or with 0.2% L-Arabinose for 10min. Total RNAs were extracted at various times after addition of rifampicin and subjected to northern blotting with probes for *lpp* or *ompA*. A Half-life ($t_{1/2}$) of each mRNA was shown below the figure.

(E) Quantification analysis of *lpp* and *ompA* mRNAs in the figure (D) was performed. Data points represent the mean ± SD of multiple measurements.

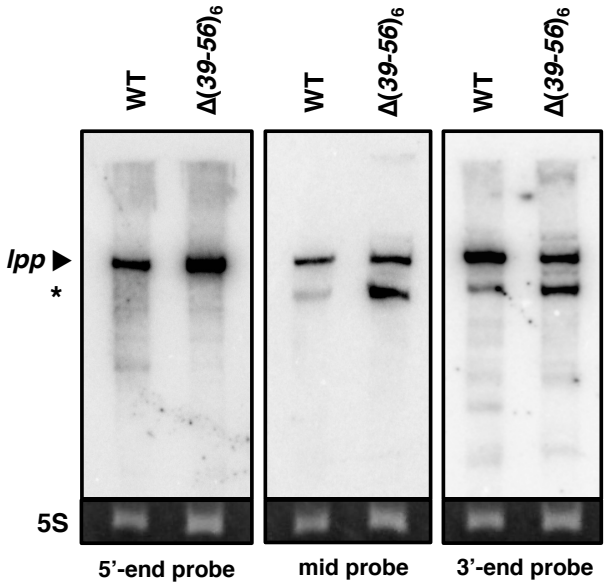
(F) YT20 (YT10 *ams1*), cells were grown in M9C medium until the OD600 reached 0.3 at 30˚C and shifted to 44˚C for another 30 min, then infected with wild-type or Δ*srd* mutant. Total RNAs extracted at the indicated times were analyzed by northern blotting with probes for *lpp* and *ompA*. An arrowhead indicates the full-length transcript of *lpp* or *ompA* mRNA. A Half-life ($t_{1/2}$) with SD of each mRNA was shown below the figure.

Figure 6

A


E. coli
WT
ams1
0' 2' 5' 10' 15' 0' 2' 5' 10' 15'
lpp ▶
5S
WT T4-infected
lpp ▶
*
5S
Δ(39-56)6-infected

B


WT
Δ(39-56)6
WT
Δ(39-56)6
WT
Δ(39-56)6
lpp ▶
*
5S
5'-end probe
mid probe
3'-end probe

C

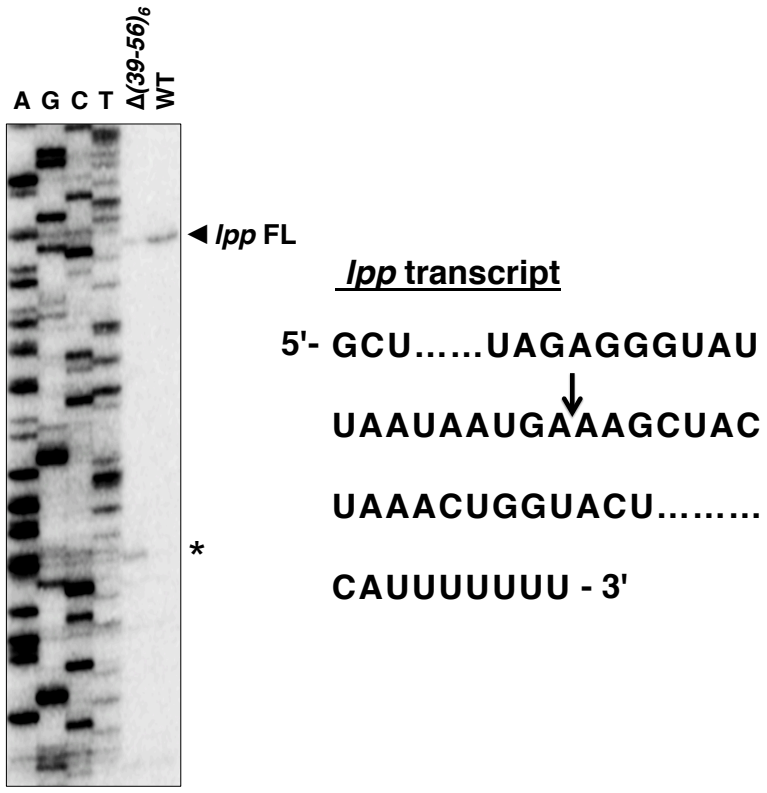

A G C T
Δ(39-56)6
WT
◀ lpp FL
*
lpp transcript
5'- GCU……UAGAGGGUAU
UAAUAAUGAAAGCUAC
UAAACUGGUACU……..
CAUUUUUUU - 3'

Figure 6. The decay intermediate of *lpp* mRNA generated by RNase E.

(A) YT10 (WT) or its derivative, YT20 (*ams1*), cells were grown in M9C medium until the OD_{600} reached 0.3 at 30˚C and shifted to 44˚C for another 30 min, then infected with wild-type or $\Delta(39\text{-}56)_6$ phage. Total RNAs were isolated at the indicated times after infection and then analyzed by northern blotting with a probe for *lpp*. An arrowhead and an asterisk indicate the full-length *lpp* mRNA and its decay intermediate.

(B)Total RNAs extracted from MH1 cells 2min and 5min after infected with wild-type or $\Delta(39\text{-}56)_6$ phage were electrophoresed and analyzed by northern blot using probes for 5′-end, middle part, and 3′-end of *lpp* transcript, respectively. (RNAs loaded were not quantified)

(C) 5 μg of total RNAs extracted from MH1 cells infected with wild-type or $\Delta(39\text{-}56)_6$ phage were used for primer extension analysis as described in *Experimental procedures*. FL or the asterisk denotes the full-length *lpp* mRNA or its decay intermediate. A set of sequence ladders for *lpp* obtained by dideoxy-sequencing method with the same primer was run in parallel. The RNA sequence around the start codon of *lpp* mRNA is shown in the right side and the arrow indicates the cleavage site generating the decay intermediate.

Figure 7

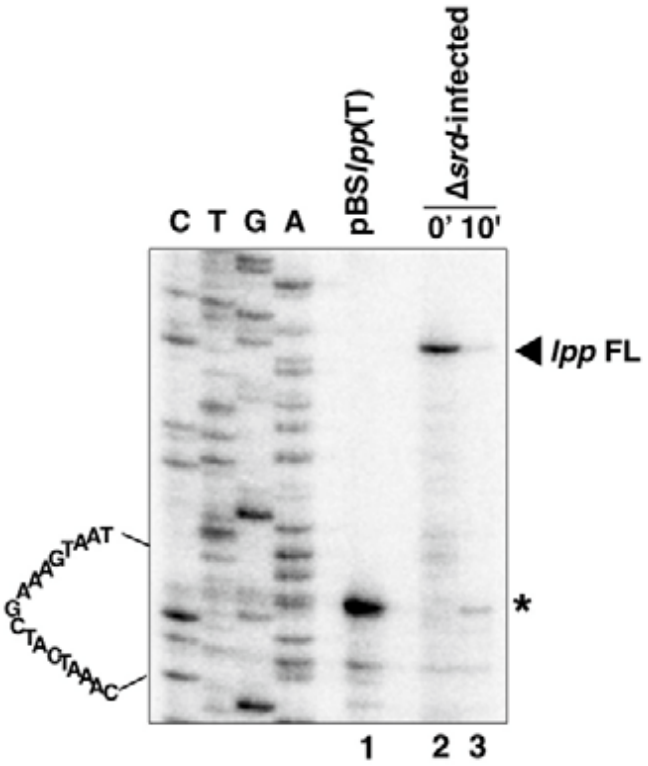
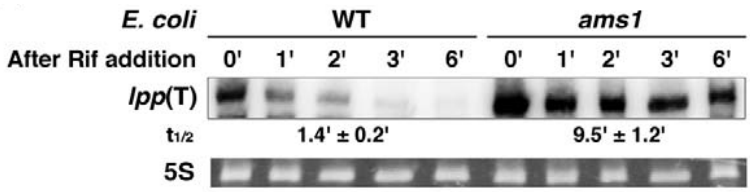


Figure 7. 5′-end of truncated *lpp* RNA driven from a pBS*lpp*(T) plasmid.

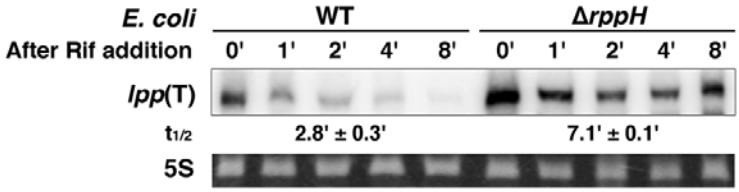
5 μg of total RNAs extracted from YT1001 cells harboring pBS*lpp*(T) treated with 1 mM IPTG for 10 min (lane 1), from MH1 cells at 0 min (lane 2) or at 10 min (lane 3) after Δ*srd* mutant infection were used for primer extension analysis. FL or an asterisk denotes the full-length *lpp* mRNA or its decay intermediate. 5′-end of truncated *lpp* RNA expressed from pBS*lpp*(T) was identical to that of the decay intermediate of *lpp* mRNA accumulated in Δ*srd* mutant-infected cells.

Figure 8

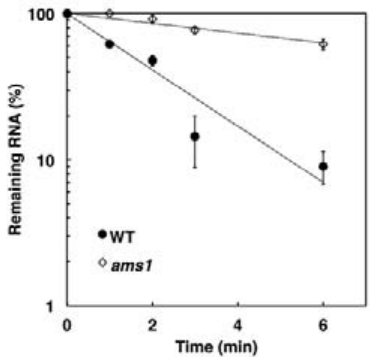
A



C



B



D

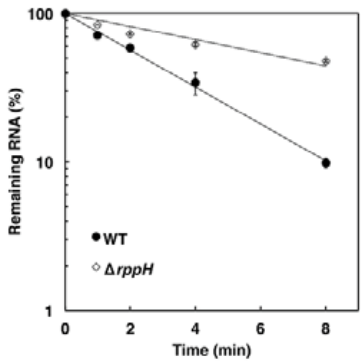


Figure 8. Degradation of truncated *lpp* RNA.

(A) TY1001 (Δ*lpp*::*kan*) or TY1002 (Δ*lpp*::*kan ams1*) cells harboring pBS*lpp*(T) were grown in LB medium until the OD_{600} reached 0.3 at 30˚C and shifted to 44˚C for another 30 min. After cells were treated with 1 mM IPTG for 10 min to induce truncated *lpp* RNA, total RNAs were extracted at various times after addition of rifampicin and subjected to northern blotting with a probe for *lpp*.

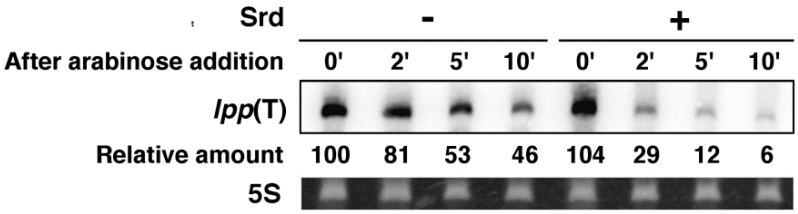
(B) Quantification analysis of *lpp*(T) RNA in the figure (A) was performed. Data points represent the mean ± SD of multiple measurements.

(C) TY1001 or TY1006 (Δ*lpp*::*kan* Δ*rppH*) harboring pBS*lpp*(T) were treated with 1 mM IPTG for 10 min when the OD_{600} reached 0.5 at 30˚C. Total RNAs were extracted at various times after addition of rifampicin and subjected to northern blotting with a probe for *lpp*.

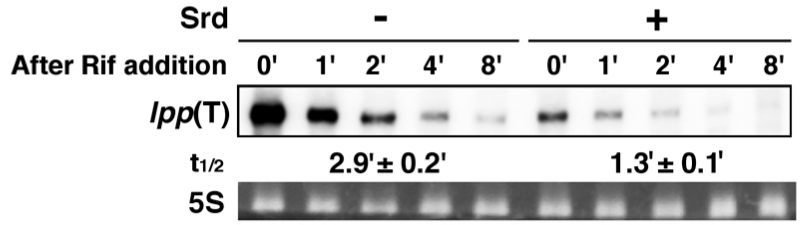
(D) Quantification analysis of *lpp*(T) RNA in the figure (C) was performed. Data points represent the mean ± SD of multiple measurements.

Figure 9

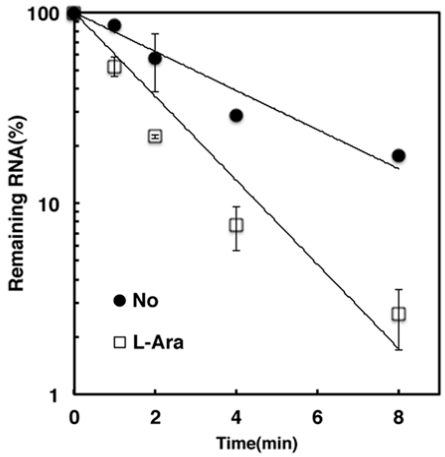
A


Srd
-
+
After arabinose addition
0' 2' 5' 10' 0' 2' 5' 10'
lpp(T)
Relative amount
100 81 53 46 104 29 12 6
5S

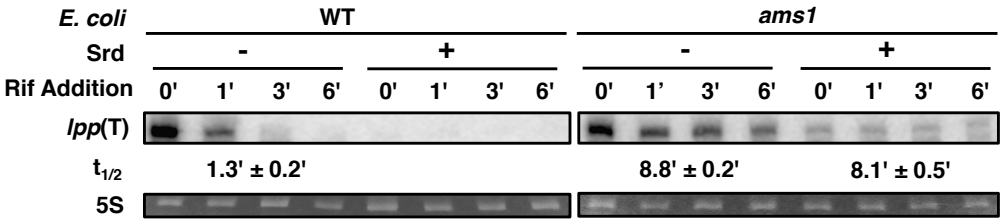
B


Srd
-
+
After Rif addition
0' 1' 2' 4' 8' 0' 1' 2' 4' 8'
lpp(T)
t1/2
2.9'± 0.2'
1.3'± 0.1'
5S

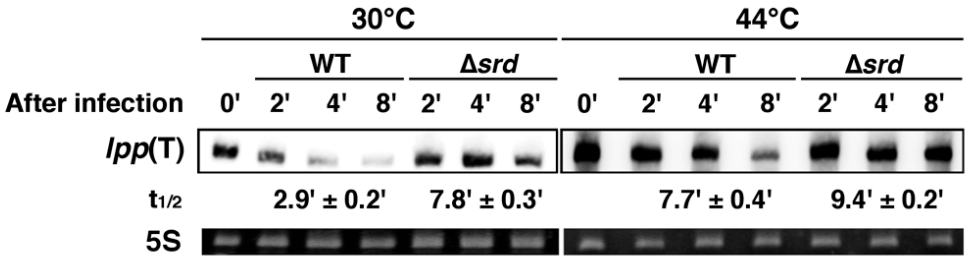
C


100
10
1
Remaining RNA(%)
No
L-Ara
0
2
4
6
8
Time(min)

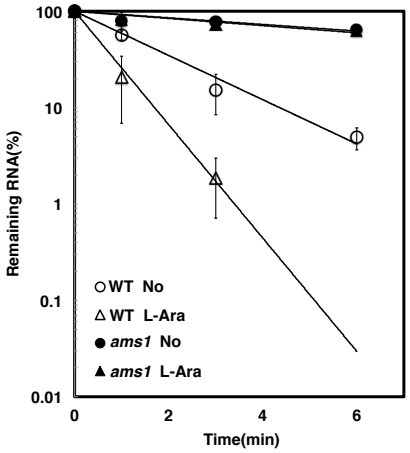
D


E. coli
WT
ams1
Srd
-
+
-
+
Rif Addition
0' 1' 3' 6' 0' 1' 3' 6' 0' 1' 3' 6' 0' 1' 3' 6'
lpp(T)
t1/2
1.3' ± 0.2'
8.8' ± 0.2'
8.1' ± 0.5'
5S

F


30°C
44°C
WT
Δsrd
WT
Δsrd
After infection
0' 2' 4' 8' 2' 4' 8' 0' 2' 4' 8' 2' 4' 8'
lpp(T)
t1/2
2.9' ± 0.2'
7.8' ± 0.3'
7.7' ± 0.4'
9.4' ± 0.2'
5S

E


100
10
1
0.1
0.01
Remaining RNA(%)
WT No
WT L-Ara
ams1 No
ams1 L-Ara
0
2
4
6
Time(min)

G

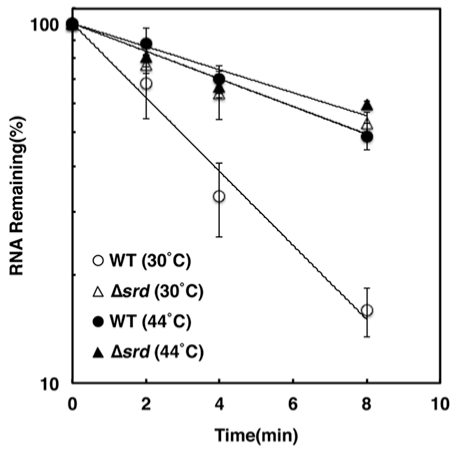

100
10
RNA Remaining(%)
WT (30˚C)
Δsrd (30˚C)
WT (44˚C)
Δsrd (44˚C)
0
2
4
6
8
10
Time(min)

Figure 9. Effect of Srd on degradation of truncated *lpp* RNA.

(A) TY1001 cells harboring pBS*lpp*(T) plus either pBAD33 or pBAD33-*srd* were grown at 30˚C until the OD_{600} reached 0.5 and added with 1 mM IPTG. At 10 min after IPTG addition, 0.2% arabinose was added to induce Srd. Total RNAs were extracted at various times after arabinose addition and subjected to northern blotting with a probe for *lpp*. The intensity of band at 0 time was set to 100% and relative intensity at each time was shown below the figure.

(B) TY1001 cells harboring pBS*lpp*(T) and pBAD33-*srd* were grown at 30˚C until the OD_{600} reached 0.5 and treated with 1 mM IPTG. At 10 min after IPTG addition, cells were treated with (+) or without (-) 0.05% arabinose for another 10 min. Total RNAs were extracted at various times after addition of rifampicin and subjected to northern blotting with a probe for *lpp*. A half-life ($t_{1/2}$) of *lpp*(T) RNA was shown below the figure.

(C) Quantification analysis of *lpp*(T) RNA in the figure (B) was performed. Data points represent the mean ± SD of multiple measurements.

(D) TY0807 cells harboring both pBS*lpp*(T) and pBAD33-*srd* or YT20 cells harboring both pBS*lpp*(T) and pBAD33-*srd* were grown in LB medium until the OD_{600} reached 0.3 at 30˚C and shifted to 44˚C for another 30 min. Then, cells were treated with 1 mM IPTG for 10 min, and further divided into two aliquots, and further treated without or with 0.05% L-Arabinose for 5min. Total RNAs were extracted at various times after addition of rifampicin and subjected to northern blotting with probes for *lpp*. A Half-life ($t_{1/2}$) of *lpp*(T) RNA was shown below the figure.

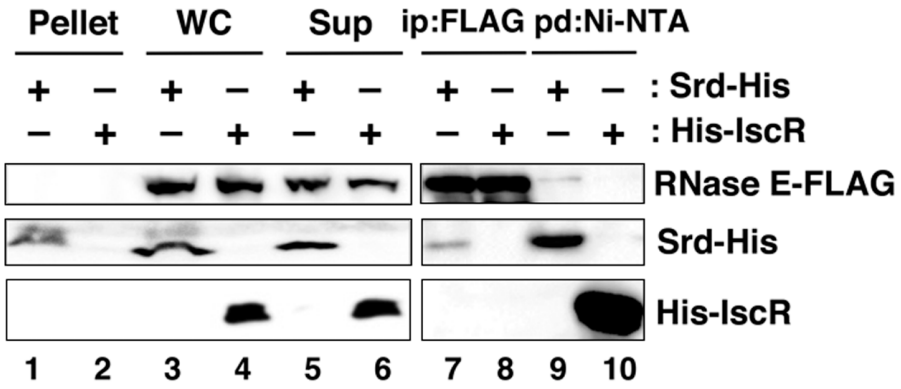
(E) Quantification analysis of *lpp*(T) RNA in the figure (B) was performed. Data points represent the mean ± SD of triplicate measurements.

(F) TY1002 cells harboring pBS*lpp*(T) were grown at 30˚C until the OD_{600} reached 0.3, divided into two aliquots, and incubated for 25 min at 30 or 44˚C, respectively. Then, cells were treated with 1 mM IPTG for 10 min, and further divided into two aliquots followed by infection with wild-type or Δ*srd* phage. 10 μg of each RNA sample was subjected to northern blotting with a probe for *lpp*.

(G) Quantification analysis of *lpp* and *ompA* mRNAs in the figure (B) was performed. Data points represent the mean ± SD of multiple measurements.

Figure 10

A


Pellet
WC
Sup
ip:FLAG
pd:Ni-NTA
+ − + − + − + − + − : Srd-His
− + − + − + − + − + : His-IscR
RNase E-FLAG
Srd-His
His-IscR
1 2 3 4 5 6 7 8 9 10

B

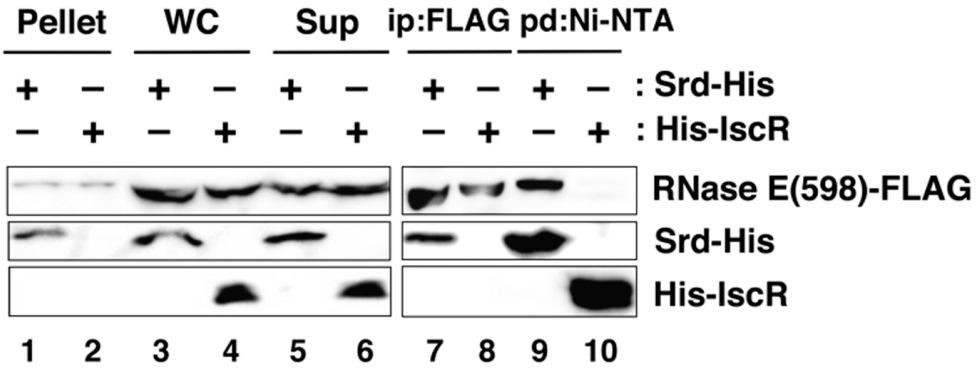
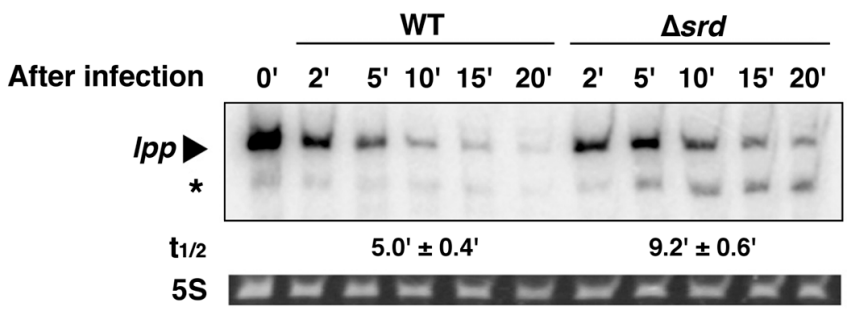

Pellet
WC
Sup
ip:FLAG
pd:Ni-NTA
+ − + − + − + − + − : Srd-His
− + − + − + − + − + : His-IscR
RNase E(598)-FLAG
Srd-His
His-IscR
1 2 3 4 5 6 7 8 9 10

Figure 10. Physical association between RNase E and Srd *in vivo.*

(A) TY1007 (rne-FLAG-cat) cells or (B) TY1008 (rne598-FLAG-cat) cells harboring either pQE80L-*srd*-His or pQ-orf2-95 were grown at 30°C until the OD600 reached 0.6, and IPTG was added to induce Srd-His or His-IscR. Preparation of cell extracts and fractions, immunoprecipitation with anti-FLAG M2 agarose beads and pull-down with Ni-NTA beads were carried out as described in *Materials and Methods*. 0.33% of whole cell extract (WC, lanes 3 and 4), the insoluble fraction (Pellet, lanes 1 and 2), and the soluble fraction (Sup, lanes 5 and 6), and 33.3% of the bound fractions (lanes 7-10) were analyzed by western blotting with antibodies against FLAG-tag (top panel) and His-tag (middle and bottom panels).

Figure 11

A



B

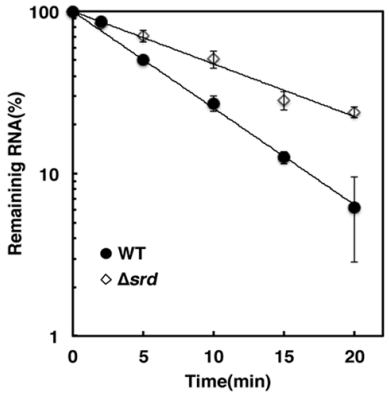


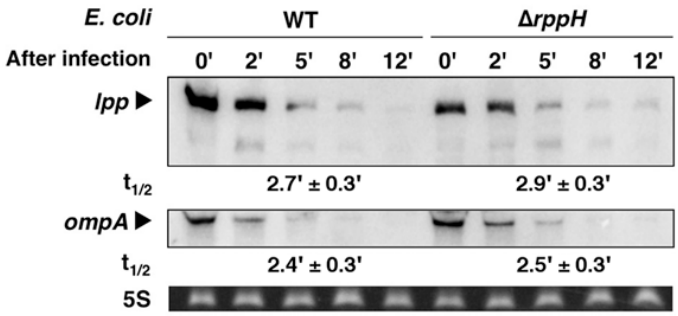
Figure 11. Effect of CTH of RNase E on T4-induced host mRNA degradation

(A) TY1008 cells were grown at 37˚C in M9C medium until the OD_{600} reached 0.5, divided into two aliquots, and infected with wild-type or Δ*srd* phage. Total RNAs were extracted at various times after infection and subjected to northern blotting with a probe for *lpp*.

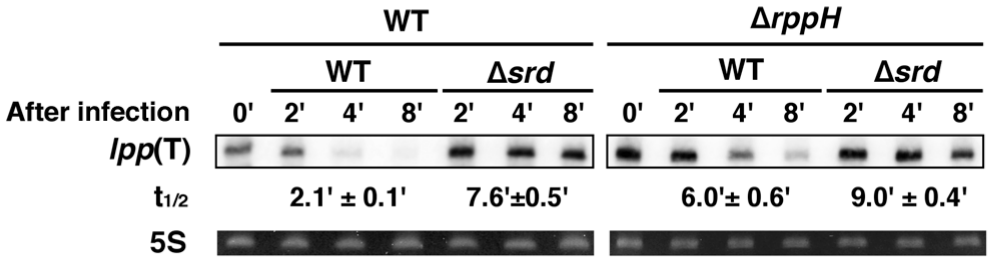
(B) Quantification analysis of *lpp* mRNA in the figure (A) was performed. Data points represent the mean ± SD of multiple measurements.

Figure 12

A



B



C

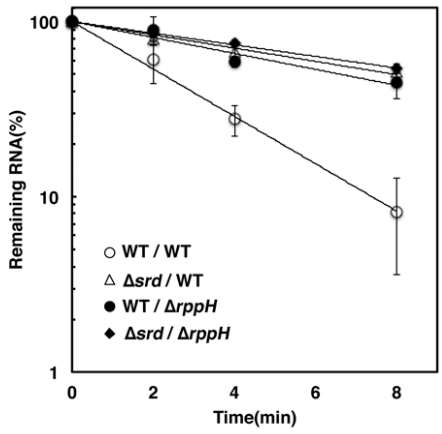


Figure 12. Effect of RppH on degradation of *lpp*, *ompA* mRNAs and truncated *lpp* RNA after T4 infection.

(A) Wild-type or Δ*rppH* cells were grown in M9C medium until the OD_{600} reached 0.5 at 37˚C, and infected with T4 wild type. Total RNAs were isolated at the indicated times after infection and then analyzed by Northern blotting with probes for *lpp* or *ompA*. A Half-life ($t_{1/2}$) of each mRNA was shown below the figure. RppH had no significant effect on the degradation of *lpp* and *ompA* mRNAs after T4 infection.

(B) TY1001 (Δ*lpp*::*kan*) or TY1006 (Δ*lpp*::*kan* Δ*rppH*) cells harboring pBS*lpp*(T) were grown at 30˚C until the OD600 reached 0.5. Then, the cells were treated with 1 mM IPTG for 10 min followed by infection with wild-type or Δ*srd* mutant. 10 μg of total RNAs extracted at the indicated times after infection were subjected to northern blotting with a probe for *lpp*.

(C) Quantification analysis of *lpp*(T) RNA in the figure (C) was performed. Data points represent the mean ± SD of multiple measurements.

Figure 13

A

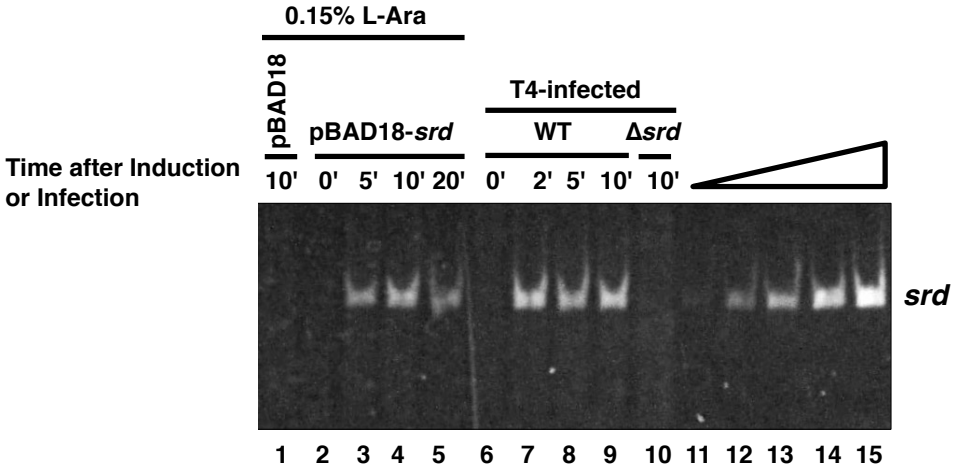


Figure 13. Semi-quantitative RT-PCR analysis to detect *srd* mRNA from T4 genome.

Total RNAs from TY0807 cells harboring pBAD18 at 10min or pBAD18-*srd* at 0, 5, 10 or 20 min after induction of 0.15% arabinose (lanes 1-5), or from MH1 cells after infection with wild-type phage (lanes 6-9) or Δ*srd* mutant (lane 10) were used for RT-PCR. A thermal cycle of 94 ˚C for 30 s, 54 ˚C for 15 s, and 72 ˚C for 20 s was repeated 15 times. The products were separated through a 6% polyacrylamide gel. Various amounts of pBAD18-*srd* were used as a template to demonstrate a semi‐quantitative profile of PCR conditions; lane 11: 0.5 ng; lane 12: 1 ng; lane 13: 2.5 ng; lane 14: 5 ng; lane 15: 10 ng.

Figure 14

A

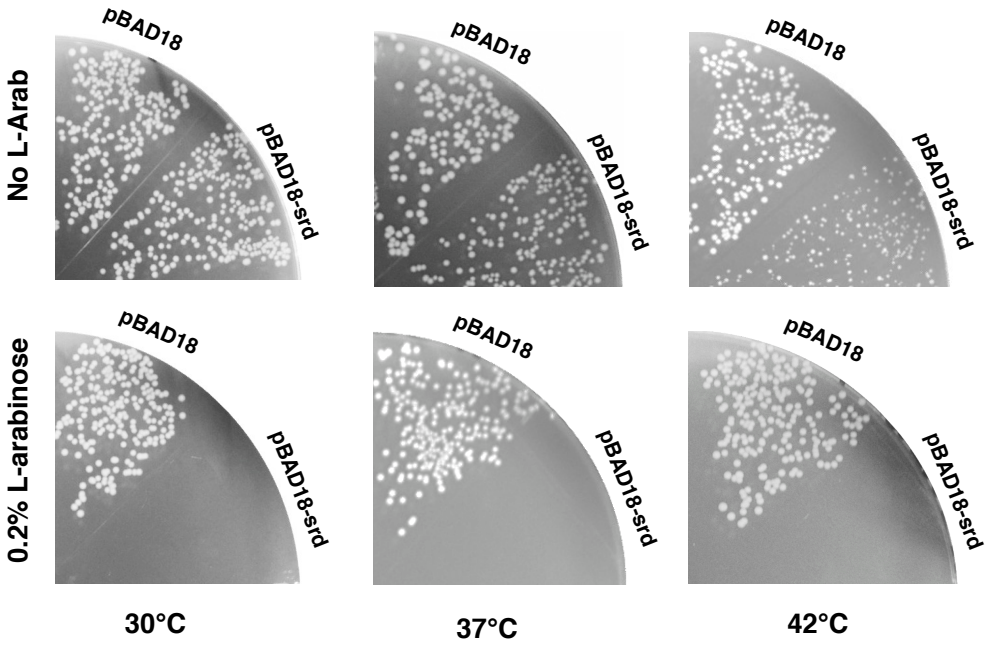
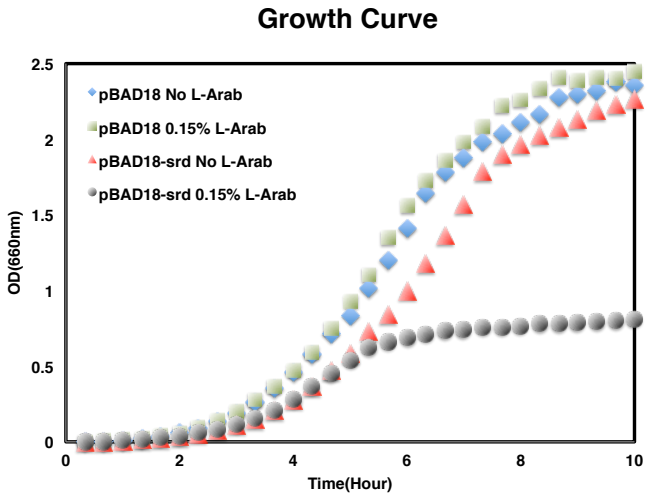


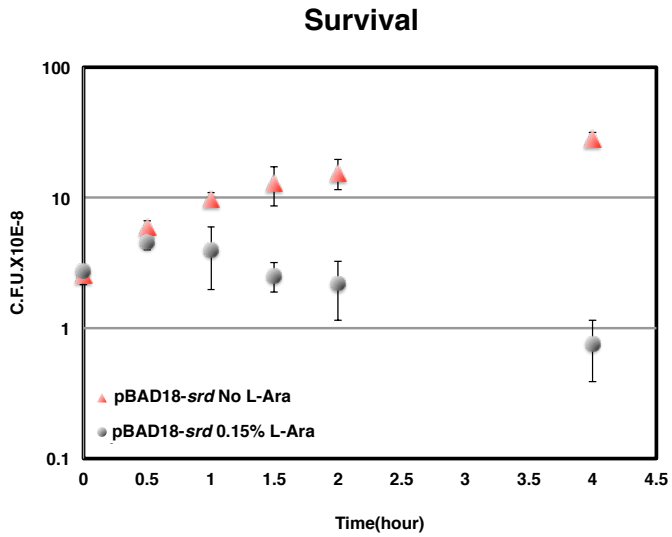
Figure 14. Toxicity of Srd on *E. coli* cell growth.

A) Diluted cultures of TY0807 cells harboring pBAD18 or pBAD18-*srd* were spread on LB plate containing 50 μg ml^{-1} ampicillin and 0% or 0.2% L-arabinose. Plates were incubated at 30, 37 and 42°C, respectively, overnight.

B



C



B) Growth of cells harboring pBAD18 or pBAD18-*srd* were monitored by optical density at 660nm every 20min.

C) Cells harboring pBAD18-*srd* was induced from time 0, cells were spread on LB plates at indicated times and incubated overnight. Colonies were calculated to investigate propagation ability of cells without or with T4 *srd* induction. Error bar was obtained from three independent experiments.

Figure 15

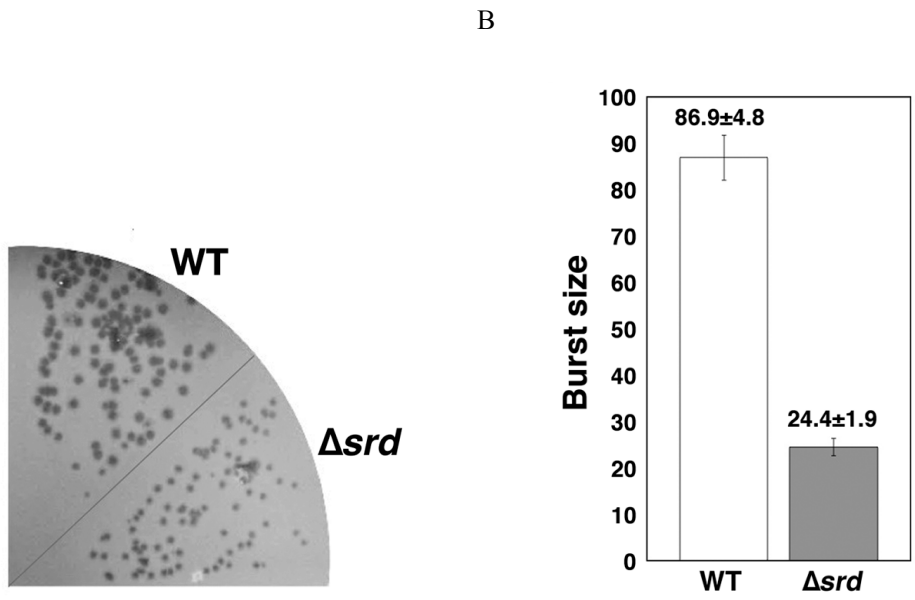


Figure 15. Growth of Δ*srd* phage

(A) Equal number of wild-type and Δ*srd* phage particles were plated onto a plate seeded with MH1 cells and incubated at 37 °C overnight.

(B) MH1 cells were grown in M9C medium until the OD_{600} reached 0.5, and infected with wild-type or Δ*srd* phage at an m.o.i. of 0.1 at 37˚C. At 8 min, the cells were diluted 10^4-fold with fresh M9C medium and further incubated for 70 min. After the cells were lysed with chloroform, the total number of progeny phage was determined by plating after an appropriate dilution with MH1 as an indicator. The burst size is the ratio of the number of progeny to the number of input phage and shown in bar graph. Each value indicates the mean and standard deviation obtained from at least three independent experiments.

Figure 16

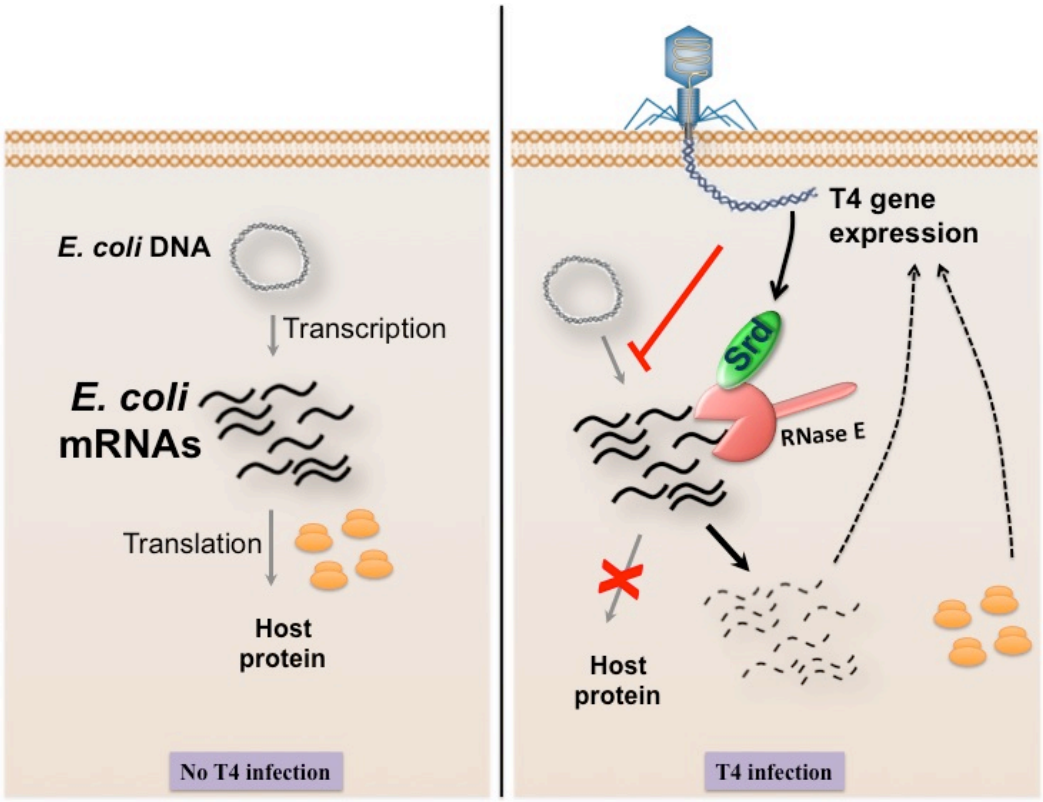


Figure 16. Possible model summarized from this work.

Immediately after T4 infection, *E. coli* transcription is stopped by T4 Alc protein. As a consequent effect, host macromolecular synthesis is shut off. Meanwhile, host mRNAs are subjected to drastic degradation. During this process, early-expressed Srd binds to N-terminal half of *E. coli* RNase E and stimulates its cleavage activity thus further accelerating decay of *E. coli* mRNAs. Subsequently generated ribonucleotides and free ribosomes become utilizable for T4 transcription and translation. This could be one of the important mechanisms that T4 induced to favor its very efficient propagation.

Appendix 1 —

Investigation of effect of Srd on decay of unstable host mRNAs

Abstract

E. coli stable *ompA* and *lpp* mRNAs are destabilized after Bacteriophage infection. Exogenous expression of Srd accelerates degradation of those mRNAs. Here, I also checked the effect of T4 Srd on decay of two host unstable mRNAs. We found that Srd also accelerates RNase E- dependent degradation of unstable *trxA* mRNA, though not all.

Introduction

E. coli ompA and *lpp* mRNAs are destabilized immediately after Bacteriophage T4 infection. Exogenous expression of Srd accelerates degradation of those mRNAs. Both these mRNAs are known as stable mRNAs of *E. coli*. Here, to address the effect of Srd on decay of host unstable mRNAs, I checked other two mRNAs - *trxA* and *rpsT* mRNAs, degradation of which is also RNase E-dependent.

Result

E. coli ompA and *lpp* mRNAs are destabilized immediately after Bacteriophage T4 infection. Exogenous expression of Srd accelerates degradation of those mRNAs. Besides *ompA* and *lpp*, host *ompC* mRNA also give the same results (see appendix 2 Fig 2 and 3). However, all these mRNAs are known as stable mRNAs of *E. coli*. Here, to address the effect of Srd on decay of host unstable mRNAs, I checked other two mRNAs - *trxA* and *rpsT* mRNAs, degradation of which is also RNase E-dependent.

Effect of T4 Srd expression on decay of host unstable mRNA - trxA

To address the effect of Srd on host unstable mRNAs degradation, I checked *trxA* mRNA. Degradation of host *trxA* mRNA is reported to be RNase E-dependent (Arraiano *et al.*, 1993). Half-life of *trxA* was around 1.5 min in uninfected *E. coli* cells (Fig. 1A). After wild-type T4 infection, its half-life was 0.8min, about half of that before infection. When Δ*srd* mutant infected, the half-lives of *lpp* and *ompA* mRNAs were increased to 1.2 min (Fig 1A). This result indicated that, like *ompA* and *lpp* mRNA, unstable *trxA* mRNA was destabilized after T4 infection, and T4 Srd was involved in this process.

TY0807 cells harboring pBAD18-*srd* were grown to mid-log phase, and total RNAs were then extracted from cells at 0, 5, 10, or 20 min after addition of arabinose and examined by northern blot analysis. As seen in Fig. 2A, the expression of *srd* mRNA results in amount reduction of *trxA* mRNA. To further explore whether the reduction of these mRNAs is caused by the increase of decay rate or not, I measured *trxA* mRNA stability (Fig. 2B). At 10 min after addition of arabinose, a transcription inhibitor, rifampicin, was added to the culture and total RNAs were extracted at various times. In the absence of arabinose, half-life of *trxA* transcripts was 3.6min. In contrast, its half-life was 2.2 min after addition of arabinose, indicating that its decay rate was increased approximately 1.6-fold after induction of Srd. Together with the result of Fig. 1A, it was strongly suggested that Srd also destabilized *unstable trxA* mRNA. Finally, I confirmed that Srd stimulated the RNase E-

dependent decay of *trxA* in RNase E-deficient cells (Fig. 3). At 44˚C, the half-life of *trxA* mRNA in the presence or absence of *srd* were 7.9 min or 6.9 min in RNase E temperature-sensitive cells. These results strongly suggest that Srd-accelerated *trxA* mRNA degradation is RNase E-dependent. Considering together with all above results, stimulation effect of Srd is not limited to stable mRNAs, it should also have effects on unstable mRNA.

Effect of T4 Srd expression on decay of host unstable mRNA - rpsT

To address the effect of Srd on host unstable mRNAs degradation, I also checked another mRNA, *rpsT*, which is alao known as target of *E. coli* RNase E (Mackie 2013b). Half-lives of *rpsT* P1 and P2 were 0.8 min and 1.2 min in uninfected *E. coli* cells (Fig. 1B). There were no significant change on half-life of *rpsT* before and after T4 infection, and absence of Srd also gave no effect on *rpsT* stability (Fig 1B). This result suggests that, dislike *ompA*, *lpp* and *trxA* mRNA, *rpsT* mRNA was not further destabilized after T4 infection.

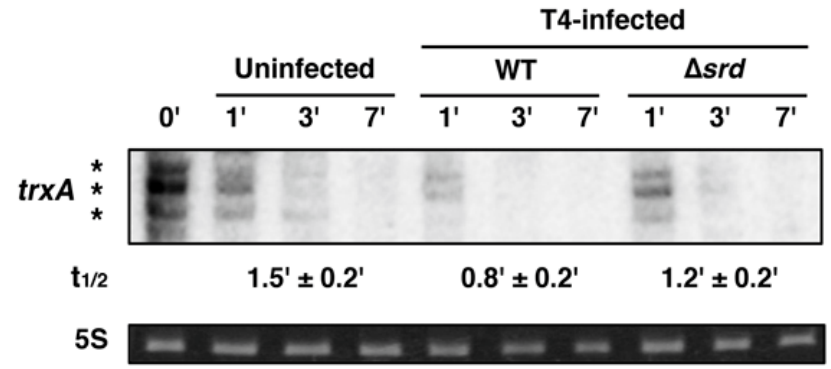
As seen in Fig. 4, half-life of *rpsT* mRNA didn't change much after induction of pBAD18-*srd*, suggesting that induction of Srd has no effect on *rpsT* decay rate. Together with Fig 1B, these results shows that T4 infection induce more rapid degradation of some unstable mRNA, such as *rpsT* mRNA and Srd doesn't accelerate *rpsT* mRNA.

Disscussion

E. coli ompA and *lpp* mRNAs are destabilized immediately after Bacteriophage T4 infection. Exogenous expression of Srd accelerates degradation of those mRNAs. Besides *ompA* and *lpp*, host *ompC* mRNA also give the same results (data not shown). However, all these mRNAs are known as stable mRNAs of *E. coli*. Here, to address the effect of Srd on decay of host unstable mRNAs, I checked other two mRNAs – *trxA* and *rpsT* mRNAs, degradation of which is also RNase E-dependent. Like *ompA* and *lpp* mRNAs, Srd also stimulates the degradation of unstable *trxA* mRNA. I also checked the effect of host RppH on *trxA* mRNA. My results showed degradation of *trxA* mRNA is RppH-independent (data not shown), suggesting *trxA* is another example of Srd stimulating 5′-end-independent activity of RNase E. On the other hand, Fig 1B and Fig 4 shows that there's no change on decay of unstable *rpsT* mRNA before and after T4 infection or absence and presence of Srd expression. However *rpsT* is one of the host ribosomal mRNAs, which are alwasys highly controlled responding to various changes of cell physiology (Parsons *et al.*, 1983). Like many ribosomal proteins, ribosomal protein S20 (Product of *rpsT*) is also an autogenous regulator of its own synthesis (Wirth, *et al.*, 1982; Donly *et al.*, 1988; Portier *et al.*, 1990) and therefore is presumed to be capable of binding either to 16S rRNA for ribosome assembly or its own messenger RNA to effect translational repression. Moreover, ribosomes are considerred to be important also for T4 gene expression after infection. Therefore, for decay of these kind of unstable mRNAs there may be additive effccts and it is difficult to simply summerize the function of T4 Srd on these kinds of mRNAs based on current understandings.

Figure 1

A


T4-infected
Uninfected
WT
Δsrd
0' 1' 3' 7' 1' 3' 7' 1' 3' 7'
trxA
*
*
*
t1/2
1.5' ± 0.2'
0.8' ± 0.2'
1.2' ± 0.2'
5S

B

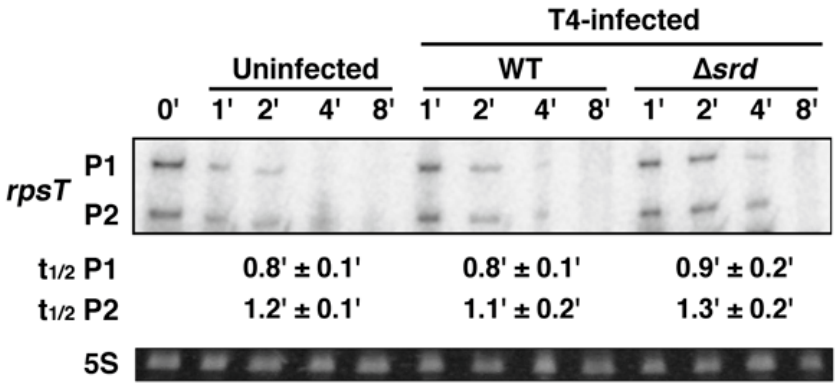
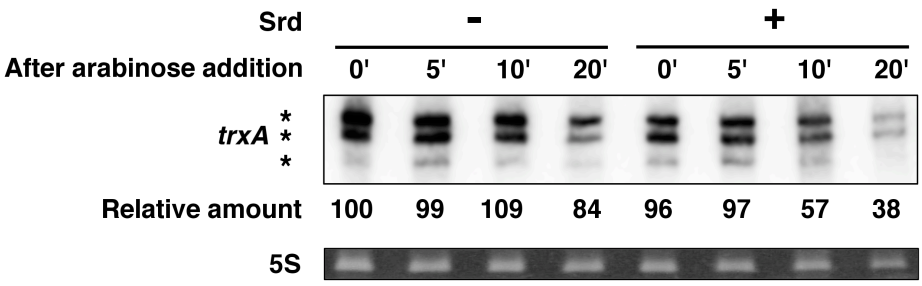

T4-infected
Uninfected
WT
Δsrd
0' 1' 2' 4' 8' 1' 2' 4' 8' 1' 2' 4' 8'
rpsT
P1
P2
t1/2 P1
0.8' ± 0.1'
0.8' ± 0.1'
0.9' ± 0.2'
t1/2 P2
1.2' ± 0.1'
1.1' ± 0.2'
1.3' ± 0.2'
5S

Figure 1. Effect of Srd on stabilities of unstable mRNAs.

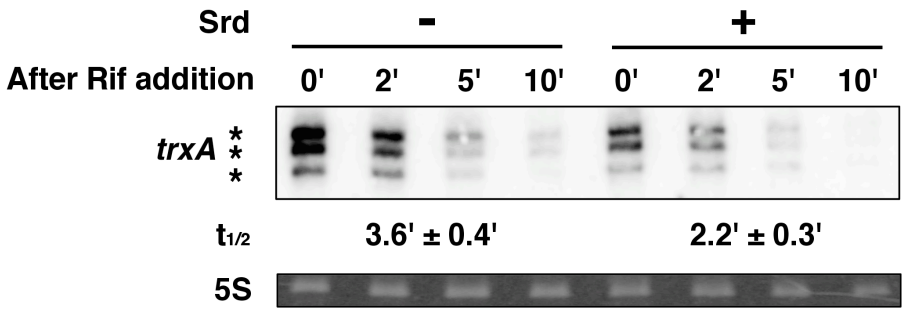
MH1 (wild-type) cells were grown in M9C medium until the OD600 reached 0.5 at 37℃, and infected with wild type T4 or Δ*srd* mutant or treated with rifampicin at a final concentration of 250 μg/ml. Total RNAs were extracted at the indicated times after infection or addition of rifampicin and then analyzed by northern blotting with an oligo-probe for *trxA* (A, 5′-^{32}P-CTGTCGTCAGTCAGGTGAATAATTTTATCGCTC) or *rpsT* (B, 5′-^{32}P-AGAGCGACGGCTTGCGTTGTGCTTACGAGCCTTTTCAGACTGAATGGCGC). Asterisks in the figure (A) indicate multiple *trxA* transcripts. Ethidium bromide-stained 5S rRNA as a loading control is shown at the bottom of each panel. Half-lives (t1/2) with the mean ± SD of duplicate measurements are shown below the figure. Host unstable *trxA* mRNA was destabilized after infection with wild-type T4 and this destabilization was recovered by the deletion of *srd*.

Figure 2

A


Srd
-
+
After arabinose addition
0'
5'
10'
20'
0'
5'
10'
20'
trxA
* * *
Relative amount
100
99
109
84
96
97
57
38
5S

B


Srd
-
+
After Rif addition
0'
2'
5'
10'
0'
2'
5'
10'
trxA
* * *
t1/2
3.6' ± 0.4'
2.2' ± 0.3'
5S

C

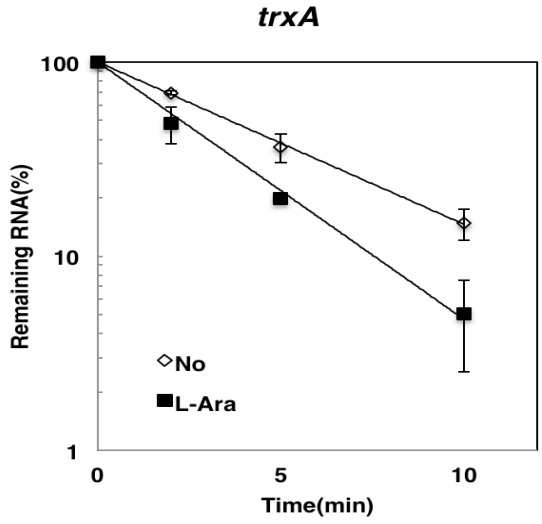

trxA
100
10
1
Remaining RNA(%)
No
L-Ara
0
5
10
Time(min)

Figure 2. Destabilization of *trxA* mRNA by exogenous expression of Srd.

Asterisks indicate multiple *trxA* transcripts. (A) TY0807 (wild-type) cells harboring pBAD18 (–) or pBAD18-*srd* (+) were treated with 0.15% arabinose when the OD600 reached 0.5 at 30˚C. Total RNAs extracted at the indicated times were subjected to northern blotting with probes for *trxA* mRNA. The intensity of band at time 0 was set to 100% and the relative intensity at each time was shown below the figure. (B) TY0807 cells harboring pBAD18-*srd* were treated with (+) or without (–) 0.15% arabinose for 10 min when the OD600 reached 0.5 at 30˚C. Total RNAs extracted at the indicated times after addition of the transcription inhibitor rifampicin were subjected to northern blotting with probes for *trxA*. Half-life (t1/2) with the mean ± SD of duplicate measurements are shown below figure. (C) Quantification analyses of *trxA* in the figure (B) were performed. Data points represent the mean ± SD of multiple measurements.

Figure 3

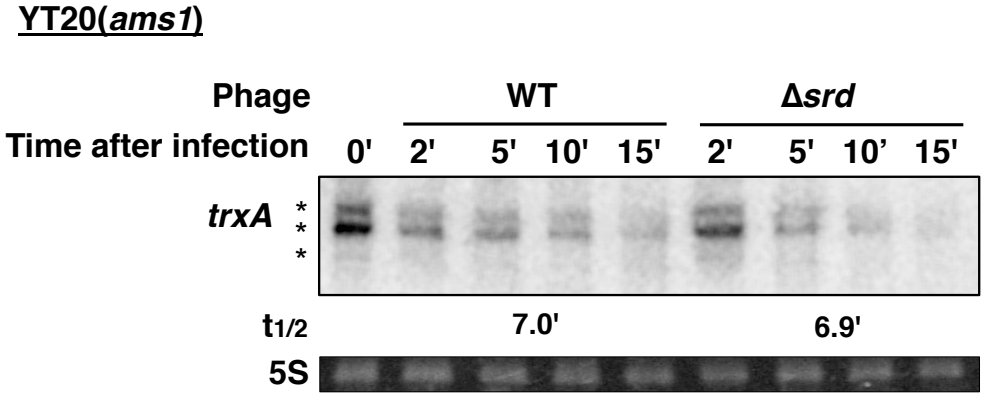


Figure 3. Stimulation of RNase E-dependent degradation of *trxA* by Srd after infection.

YT20 (ams1), cells were grown in M9C medium until the OD600 reached 0.3 at 30 ˚C and shifted to 44˚C for another 30 min, then infected with wild-type or Δ*srd* mutant. Total RNAs extracted at the indicated times were analyzed by northern blotting with a probe for *trxA*. Asterisks indicate multiple *trxA* transcripts. Half-life (t1/2) was shown below the figure.

Figure 4

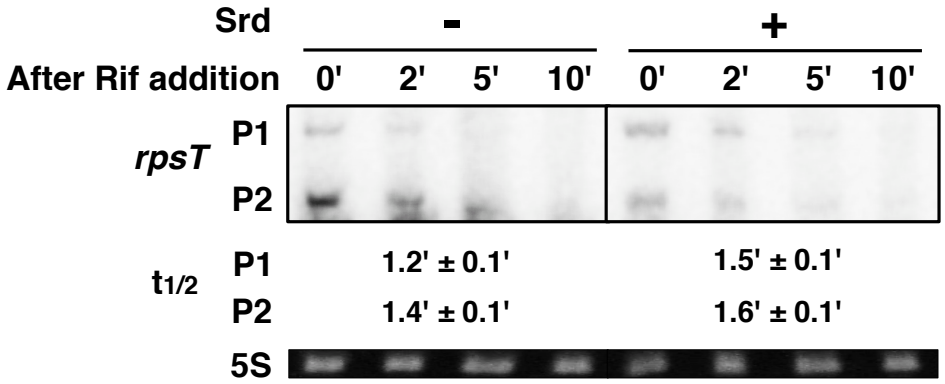


Figure 3. No effect of exogenous expression of Srd on stability of *rpsT* mRNA.

P1 and P2 repensent two transcripts generated from different promoters. TY0807 cells harboring pBAD18- *srd* were treated with (+) or without (–) 0.15% arabinose for 10 min when the OD600 reached 0.5 at 30˚C. Total RNAs extracted at the indicated times after addition of the transcription inhibitor rifampicin were subjected to northern blotting with probes for *rpsT* transcripts. Half-lives (t1/2) with the mean ± SD of duplicate measurements are shown below figure.

Appendix 2—

Attempt to find host factor that involved in T4-induced host mRNA degradation

Abstract

Bacteriophage T4 infection induces destabilization of host *ompA* and *lpp* mRNAs immediately after T4 infection. Previous work showed that gene expression immediate after infection should be involved (Ueno *et al.,* 2004). Host MicA and RybB sRNAs are reported to raise host membrane mRNA degradation by RNase E under envelope stress condition because of activated sigma E envelope stress resoponse (ESR). We checked if those sRNAs are involved in T4-induced host mRNA degradation. The results showed that destabilization of host mRNAs after T4 infection should occur without the participation of those small RNAs. Therefore, T4-induced rapid host mRNA degradation should not be induced through activating *E. coli* Sigma(E) ESR.

Introduction

Escherichia coli mRNAs are rapidly degraded immediately after bacteriophage T4 infection and the host RNase E contributes to this process. Previous work revealed that RNase E, which is an essential endoribonuclease in *E. coli* (Marcaida *et al.*, 2006), primarily functions in T4-induced host mRNA degradation (Ueno *et al.,* 2004). Previous work also showed that gene expression immediate after T4 infection either from host or T4 should contribute to destabilization of host *ompA* and *lpp* mRNAs (Ueno *et al.,* 2004).

E. coli are known to have several stress response systems, such as envelope stress systems (ESR) (Danese *et al.*, 1997; Johansen *et al.*, 2006; Gogol *et al.*, 2011) and phage shock response system *etc* (Darwin 2005). RpoE envelope stress reponse (ESR) system raises outer membrane protein mRNA degradation by recuiting RNase E cleavage. When *E. coli* cells are under envelop stress, RpoE protein (*E. coli* RNA polymerase sigma E factor) will be released from membrane, activating rapid expression of MicA and RybB small RNAs (Ades 2004; Gogol *et al.*, 2011). MicA and RybB subsequently binds to outer membrane protein mRNAs such as *ompA, ompC* and *lpp* mRNAs resulting in RNase E degradation of those mRNAs. Here, I invesitigated whether T4 infection induce *E. coli* ESR or not, and the results indicates that MicA and RybB sRNAs should not participate in T4-induced host mRNA degradation, suggesting that T4-induced rapid host mRNA degradation is not induced by activating *E. coli* RpoE ESR.

Result

Induced expression of MicA and RybB sRNAs under overexpression of E. coli RpoE

TY0807 cells harboring pBAD24-NFlag-*rpoE* plasmid was grown to mid-log phase and total RNAs were then extracted from cells at 0, 10, or 30 min after addition of arabinose and examined by northern blot analysis. As seen in Fig. 1, expression of MicA and RybB sRNAs are induced by overexpression of RpoE. Bands observed at time 0 represent their basic expression level in *E. coli* cells.

Detection of MicA and RybB sRNAs after T4 infection

MH1 cells were grown to mid-log phase and infected with wild-type T4 phage. Total RNAs were extracted at various time indicated in Fig. 2 and subjected to northern blot analysis. As seen in Fig.2, line 1 is control of MicA or RybB sRNAs; line 2-6 showed decrease of *ompA*, *ompC* and *lpp* mRNAs as well as MicA and RybB sRNAs. This result indicates that T4 infection didn't raise increased expression of host MicA and RybB.

MicA and RybB are not required in T4-induced host mRNA degradation

As deletion of *rpoE* is lethal to *E. coli* cell growth, to investigate whether T4 infection induced host envelope stress response, I measured stability of *ompA, ompC* and *lpp* mRNAs before and after T4 infection in Δ*micA* and Δ*rybB* cells. As seen in Fig 3, half-lives of *ompC*, *ompA* and *lpp* mRNAs are 19.2 min, 8.1 min and 21 min, respectively. After infection, whether in wild type cells or Δ*micA* or Δ*rybB* cells, harf-lives of those mRNAs are all around 3 min. There was no difference in the absence or presence of MicA or RybB sRNAs, considering together with Fig 2, MicA and RybB should not participate in T4-induced host mRNA degradation.

Discussion

Escherichia coli mRNAs are rapidly degraded immediately after bacteriophage T4 infection and the host RNase E contributes to this process (Ueno *et al.*, 2004). Moreover, previous work suggested that gene expression should be involved (Ueno *et al.*, 2004). *E. coli* RpoE envelope stress response system was reported to recirute RNasse E to cleave a series of outer membrane protein mRNAs via activating expression of small RNAs MicA and RybB *etc.* Since deletion of *rpoE* is lethal to E. coli cell growth, I checked if those sRNAs are involved in T4-induced host mRNA degradation. The results showed that destabilization of host mRNAs after T4 infection should occur without the participation of those small RNAs. Therefore, T4-induced rapid host mRNA degradation should not be induced through activating *E. coli* RpoE ESR.

To date, except RpoE ESR system, another two well-characterized response systems of *E. coli* cells against extracytoplasm stresses are Cpx (conjugative plasmid expression) and Psp (Phage shock protein) systems (Wulf *et al.*, 1999; Darwin 2005; Duguay *et al.*, 2004). However, based my knowledge, no report has been published that they are directly linked to RNase E activity. In Cpx response system, CpxA sensor kinase acts as an initiator protein when sensed stress signal (Hunke *et al.*, 2012). However, I measured stabilities of *ompA* and *lpp* mRNAs in T4-infected Δ*cpxA* cells and those mRNAs were rapidly degraded like those in T4-infected wild-type cells (data not shown). This result suggests that Cpx system should not be an important contributor to T4-induced rapid host mRNA destabilization. In Psp response system, PspA (most abundant), PspB, PspC or PspD protein has been reported to linked to PspF or PspG protein when responding to envelope stress (Brissette *et al.*, 1990; Darwin 2005). However, my result showed that *ompA* and *lpp* mRNAs were also drmasticly degraded in T4-infected Δ*pspA*, Δ*pspB* or Δ*pspF* cells like those in T4-infected wild-type cells (data not shown), suggesting that Psp system may also not participate in T4-induced rapid host mRNA destabilization. Considering above together, a high possibility is that T4 has a specific mechanism corresponding to rapid host mRNA degradation after infection other than inducing host envelope

response system. As previous work and my work both showed gene expression immediately after T4 infection should be involved, a very strong suggestive of T4 factor participation, which is later identified to be T4 Srd protein in my doctoral research though there might also be unkown involvement of other T4 factor or host factor.

Figure 1

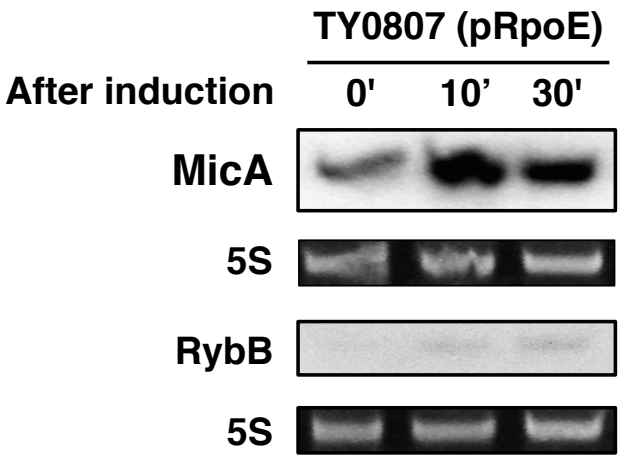


Figure 1. Expression level of MicA and RybB sRNAs under overexpression of *E. coli* RpoE.

TY0807 cells harboring pBAD24-NFlag-*rpoE* (A kind gift from Dr. Otsuka) were treated with 0.2% L-arabinose when the OD600 reached 0.5 at 37˚C. Total RNAs extracted at the indicated times and 5μg were subjected to northern blotting with a probe (5′-^{32}P- ACAAATGCGCGTCTTTC) for MicA sRNA and a probe (5′-^{32}P-GTTGATGGGCTCCACAA) for RybB sRNA. Ethidium bromide-stained 5S rRNA as a loading control is shown at the bottom of each panel.

Figure 2

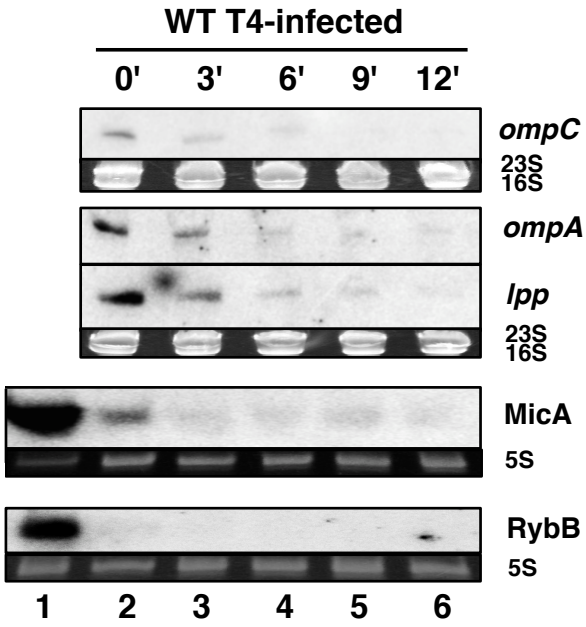


Figure 2. Rapid degradation of host mRNAs as well as small RNAs after T4 infection.

MH1 (wil-type) cells were grown in M9C medium until the OD600 reached 0.5 at 37℃, and infected with wild type T4. Total RNAs were extracted at the indicated times after infection (line2-6) and then 8μg were analyzed by northern blotting with oligo-probes for MicA and RybB small RNAs (line1: positive control for detection of MicA and RybB sRNAs) or 5μg were analyzed with probes for *ompC*, *ompA* and *lpp* mRNAs. Ethidium bromide-stained 23S and16S or 5S rRNA as a loading control is shown at the bottom of each panel. Radioactive probes for *ompA* and *lpp* were prepared as described (Ueno *et al.*, 2004). Probe *ompC* genes were amplified by PCR with primers as follows; 5′-^{32}P-CCAAGCTTTGTACGCTGAAAACAATG and 5′-CCGGTACCTAAAAAAGCAAATAAAGGCA. Template DNA was previously prepared by PCR using the above set of primers with *E. coli* MH1 DNA.

Figure 3

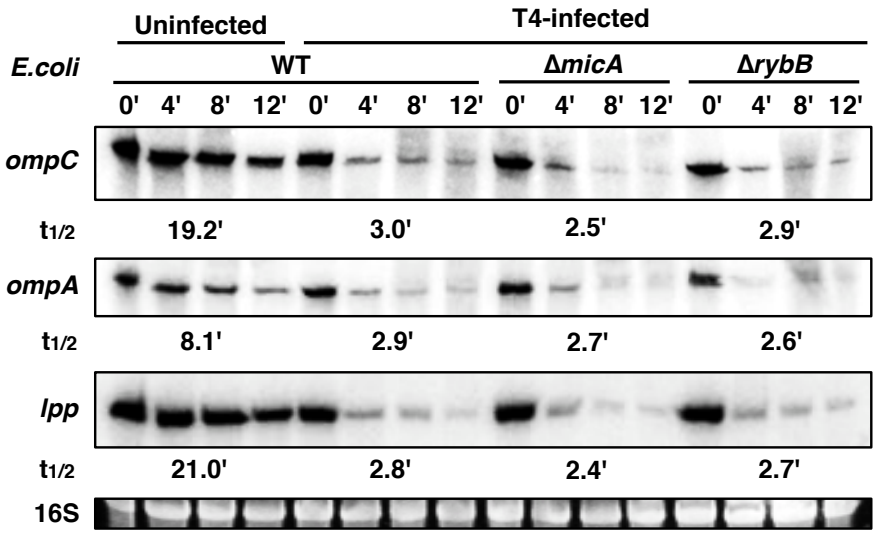


Figure 3. Effect of MicA or RybB on stabilities of host mRNAs.

TY0807 (wil-type) cells were grown in LB medium until the OD600 reached 0.5 at 37℃, and infected with wild type T4 mutant or treated with rifampicin. Δ*micA* or Δ*rybB* mutants were also grown in LB medium until the OD600 reached 0.5 at 37℃, and infected with wild type T4 mutant. Total RNAs were extracted at the indicated times after infection or addition of rifampicin and then analyzed by northern blotting and then 5μg were analyzed by northern blotting with probes for *ompC*, *ompA* and *lpp* mRNAs. Ethidium bromide-stained 16S rRNA as a loading control is shown at the bottom of each panel. The signal intensity was quantified with the NIH image J program. Relative intensities were plotted semilogarithmically and the time required for a 50% reduction was taken as the half-life of mRNA. Half-lives (t1/2) are shown below figure.

Appendix 3—

Preliminary Study on Function of Bacteriophage T4 sRNAs

Abstract

Bacteriophage T4 encodes two stable sRNAs-RNAC and RNAD, sizes of which are 139nt and 119nt, respectively. Function of them is still unknown. Deletion mutants of these two RNAs, Δ*C* and Δ*CD*, were separated from previous lab stock Δ*rnaC.ts*, and Δ*rnaCD.ts*, Δ*D* was renamed from lab stock Δ*rnaD*. Growth of Δ*C*, Δ*D* or Δ*CD* phage mutant shows no difference in comparison to that of wild type T4 in the usual laboratory strains of *E. coli*. Furthermore, I found that with exogenous expression of *rnaD* and *rnaC* together, *E. coli* K-12 TY0807 cells shows impaired growth at high temperature (42°C).

Introduction

Bacteriophage T4 has 10 genes encoding non-coding RNAs. Eight of them encode tRNAs and the remaining two encode small RNAs, RNAC and RNAD (Figure 1). The genes for these 10 RNAs have been mapped between genes e (lysozyme) and 57 on the T4 genome by genetic and structural methods (Wilson *et al.,* 1972; Abelson *et al.*, 1975). It is reported that *rnaC* and *rnaD* maybe transcribed with T4 tRNAs in a long transcription unit (Black *et al*., 1971). So far, there are only two reports describing sRNAs encoded by phage: the sRNA of phi29 is an essential component for packaging DNA into its head (Guo *et al.*, 1991) and the sRNA of P22 is a cis-encoded paring RNA to block expression of anti-repressor (Liao *et al.*, 1987; Wu *et al.*, 1987). However, T4 DNA packaging mechanism has been well described, in which *rnaC* and *rnaD* are not involved. There is no open reading frame in the vicinity of a region encoding *rnaC* or *rnaD*, suggesting that these RNAs should not be *cis*-encoded paring RNAs. Therefore, small RNAs of T4 may be quite different in biological function from these precedents. Nevertheless, similar molecules are encoded by bacteriophages T2 and T6, indicating that the molecules have been preserved during evolution. This retention may reflect a significant function for the RNAs (Paddock *et al.*, 1973; Plunkett III *et al.*, 1981).

Materials and methods

Phages and bacterial strains:

The wild-type phage T4 used was T4D. Δ*rnaC.ts* and Δ*rnaCD.ts* phage mutants are lab stocks, containing deletion of T4 gene *rnaC* only or both sRNAs, respectively, and a *ts* mutation in genome of Δ*rnaC.ts* and Δ*rnaCD.ts*. Δ*rnaD* phage mutant was also a previous lab stock with only gene *rnaD* deleted in phage genome. Δ*C* and Δ*CD* phages were separated by removing *ts* (temperature sensitive mutation) from Δ*rnaC.ts* and Δ*rnaCD.ts* phage mutants through phage recombination with wild type T4. For consistency of phage names, phage Δ*rnaD* was renamed as Δ*D*.

E. coli K-12 strains, MH1 (*sup*^{0} *araD139 hsdR* Δ*lacX74 rpsL*) and TY0807 (MH1 *araD*$^{+}$) (Koga *et al.*, 2011) were used as wild types.

Plasmids:

pBAD18-DC were a lab stock plasmid containing both genes *rnaD* and *rnaC* in the same expression direction to those in T4 genome DNA. Briefly, an 1163bp DNA fragment amplified using primers 5′-TAGAGGATCCCTGGAATGCTAAACCAG AAG, and 5′-GCCTAAGCTTCCGTCTTCATTACCCATTAC. Then the fragment was treated with *Eco*R I, and the resulting DNA was ligated to the corresponding site in pBAD18. Direction of genes cloned was checked with *Hinc* II, and the one with same direction as those in T4 genome was selected and named as pBAD18-DC (A kind gift From Dr. Otsuka).

RNA purification, Northern blot:

Isolation of total RNA and Northern blot analysis were carried out as described (Kai *et al.*, 1996). For Northern blots, radioactive probes for *rnaC* and *rnaD* were was 5′-^{32}P-TACGAAACGCCGGGATTCG and 5′-^{32}P-GGTGCGCCGTTTCTGCTG, respectively.

Result

Isolation of ΔC and ΔCD phage mutants

Our previous work has obtained deletion mutants of T4 sRNAs however with a temperature sensitive mutation in their genome, Δ*rnaC.ts* and Δ*rnaCD.ts,* and Δ*rnaD* lacking only *rnaD* gene as well. For Δ*rnaC.ts* and Δ*rnaCD.ts* (Fig 2), growth of them is temperature sensitive, compared to wild type T4, phage mutants show defective growth at 42°C. To remove unknown *ts* mutation from Δ*rnaC.ts* and Δ*rnaCD.ts* and obtain sRNA gene deletion mutants only, I carried out homologous recombination between phage mutants and wild type T4 at m.o.i ratio of 1: 9, where 1 is for phage mutant and 9 is for wildtype T4. MH1 cells were grown to mid-log phase in M9C medium at 30°C, phage mixture of phage mutant and wild type T4 was added and incubated for 1hour more with shaking. Chloroform was added to induce cell lysis. And then lysate was diluted and plated on MH1 cells. Plaques were selected at high temperature, genotype of those grow normally at 42°C was checked by PCR. And growth of candidates of deletion mutants of *rnaC* or both *rnaC* and *rnaD* was then checked by spot test. Finally, Δ*C*, single deletion of *rnaC*, and Δ*CD*, deletion of both *rnaC* and *rnaD* were isolated. Their genotype and growth were confirmed again (Figure 3A and B). Meanwile, phage mutant containing only a *ts* mutation in its genome was also obtained and named as TS. In addition, Δ*rnaD* phage mutant was renamed as Δ*D* for consistency.

Effect of deletion of T4 sRNAs on E. coli cell growth

As seen in Figure 3B and C, growth of Δ*CD*, Δ*C* or Δ*D* was the same as that of wild type at either normal or high temperatures. I also checked growth of newly got sRNA deletion mutants on most large-scale chromosomal deletion strains. As a result, deletion of T4 sRNAs has no effect on T4 growth at strains and growth condition I used (data not shown).

Effect of over expression of T4 sRNAs on E. coli growth

To investigate whether or not exogenous expression of T4 sRNAs affects *E. coli* cell growth, previous constructed pBAD18-DC expressing both RNAD and RNAC in their original expressing direction under the control of arabinose-inducible promoter was transformed into wild type TY0807 cells. Expression of *rnaC* and *rnaD* was confirmed by northern blotting (Fig 4), *rnaC* and *rnaD* were both detectable after about 15min induction of pBAD18-DC, sRNAs in T4-infected cells were detected as a positive control.

Then, first I observed the effect of T4 sRNAs on *E. coli* growth on LB solid medium. Same amount of cells harboring pBAD18 or pBAD18-DC were spotted on LB plates with or without 0.2% L-Arabinose and incubated at 30, 37 and 42°C overnight (Fig 5A). Cells harboring pBAD18-DC show impaired growth on plates containing 0.2% L-Arabinose at high temperature, revealing that expressing of exogenous T4 sRNAs is toxic to *E. coli* cells, though not that heavy. To confirm this, I measured growth curve of cells harboring pBAD18 or pBAD18-DC at 42°C. As seen in Fig 5B, growth of cells harboring pBAD18-DC with induction was slower, and cell density increased slowly as well within early 6 hours. These results suggest that over expression of T4 sRNAs is toxic to *E. coli* growth, though a little weak.

Discussion

In this primary study, I first isolated phage mutants: Δ*C* lacking T4 gene *rnaC* in its genome DNA, and Δ*CD* lacking both T4 genes *rnaC* and *rnaD* in its genome, by removing *ts* mutation from genome DNA of Δ*rnaC.ts* and Δ*rnaCD.ts* mutants respectively and also renamed previous lab stock Δ*rnaD* lacking T4 gene *rnaD* as Δ*D* for consistency. Second, growth of newly got phage mutants was investigated in usual laboratory stains of *E. coli* under usually used lab conditions, as a result, no difference compared to that of wild type T4 was observed. Third, I further investigated effect of over-expressed T4 sRNAs on *E. coli* cell growth, and *E. coli* cells shows impaired growth at high-temperature.

T4 *rnaC* and *rnaD* with sizes of 139nt and 119nt respectively, are two small stable RNAs encoding by T4 genome (Miller *et al.,* 2003a). Their homologs are also found in genome DNA of phage T2 and T6 (Paddock *et al.*, 1973; Plunkett III *et al.*, 1981). *rnaC* and *rnaD* are clustered in T4 tRNA encoding region, though they are both reported to have matured CCA 3′-terminis, in common with all tRNAs, but not possessing complete typical features of tRNAs (Paddock *et al.*, 1973; Plunkett III *et al.*, 1981). Therefore, functions of T4 sRNAs are still unknown. There might be three possibitilies: first, it is reported that although the entire region encoding these RNAs can be deleted from the T4 genome with little effect upon phage growth in the common laboratory strains of *E. coli* (Mcclain *et al.*, 1972; Wilson *et al.*, 1972), at least one tRNA, $tRNA^{Ile}$, is essential for growth of T4 in a natural (hospital) isolate of *E. coli* (Guthrie *et al.*, 1973). Thereby, T4 may encode these sRNAs to sustain its growth in certain stain(s) of *E. coli* other than usual laboratory ones. In addition, Paddock' work presumed a derived tRNA-like secondary structure of RNAC, which is, even though, not the most possible one. Therefore, RNAC might be a tRNA candidate under certain conditions, such as defective function of tRNAs or block tRNA binding through a structure-mimicking manner. Second, our previous work investigated the expression of these two RNAs. After T4 infection, RNAC and RNAD were detectable at about 3min after T4 infection, and went to a pick amount at about 10min, and quite stable in subsequent time (unpublished data). In addition,

Uzan and Miller's review (2010) mentioned that RNA structure influences translation initiation of T4 mRNAs, especially as they target protein binding in translational repression (Miller *et al.*, 1994 and 2003b) and some T4 mRNAs form intramolecular RNA structures that directly contribute to translation initiation efficiency of the respective mRNAs (Uzan *et al.,* 2010). Meanwhile, several papers have predicted secondary structure of T4 sRNAs (Paddock *et al.*, 1973; Plunkett III *et al.*, 1981), it is possible that sRNAs are expressed after several minutes of infection, and then participates in T4 gene expression regulation, probably just important for efficient T4 gene expression as deletion of them does not affect growth of T4. Finally, Some metabolic pathways during T4 growth were reported to be different at higher temperature (Ron *et al.*, 1971; Sandberg *et al.*, 2014), as over-expression of T4 sRNAs together impeded T4 growth at high temperature though not completely stop, stable T4 sRNAs might affect pathways described above.

This work didn't go further insight into action of T4 sRNAs, but since they are conserved between some kinds of bacteriophages, for instance, T2 and T6, reflecting a significant function for the sRNAs, thus more deeper investigations are expected to reveal their function.

Figure 1

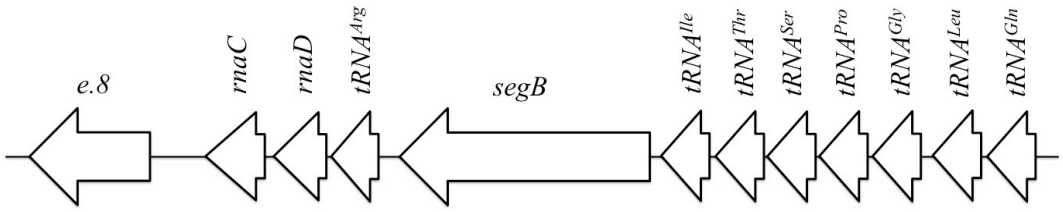


Figure 1. The map of the tRNA gene cluster on the T4 genome.

The tRNA gene cluster, for all the 8 T4 tRNAs and 2 stable RNAs, *rnaC* and *rnaD* (70908-71046 and 71053-71171 of GenBank Accession No. NC_000866), is mapped between genes e and 57 (Wilson *et al.*, 1972; Fukada *et al.*, 1980). The overall size of the tRNA gene cluster is about 1600 basepairs.

Figure 2

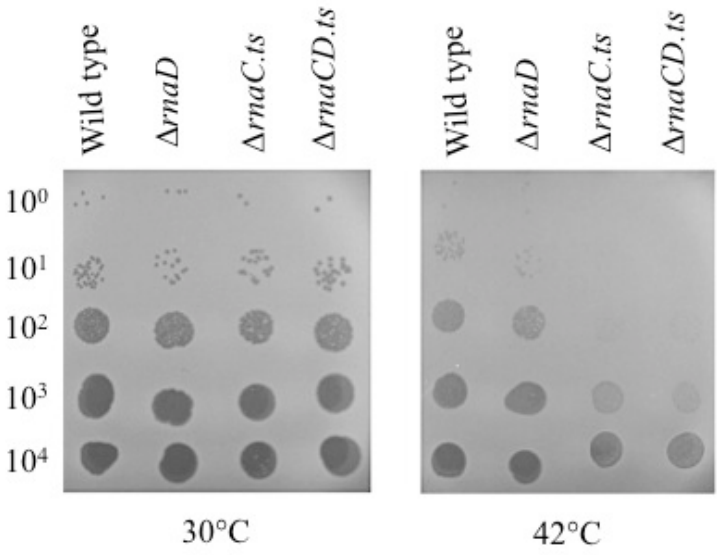
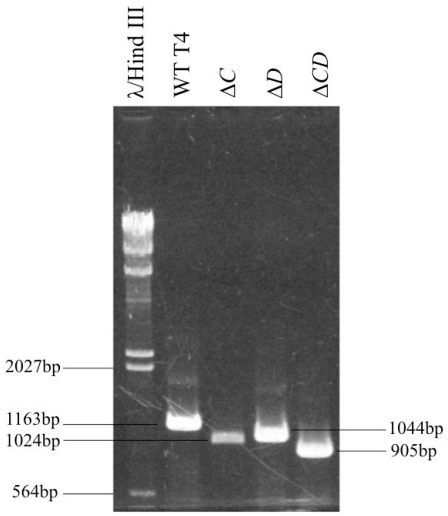


Figure 2. The growth ability of mutant phages at low or high-temperature.

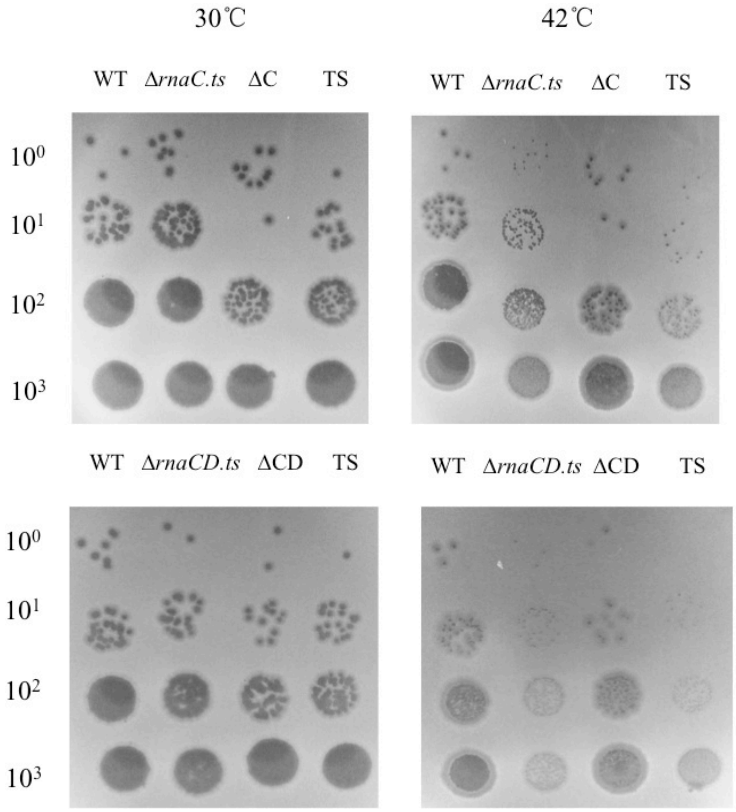
A solution containing mutant phage as indicated above the figure was serially diluted 10-fold, spotted onto an agar plate seeded with MH1 cells, and incubated overnight at 30 or 42°C. Phages were T4D, wild type; Δ*rnaC.ts*, gene *rnaC* deletion mutant with *ts* mutation; Δ*rnaD.ts*, gene *rnaD* deletion mutant with *ts* mutation; Δ*rnaCD.ts*, deletion mutant lacking both gene *rnaC* and *rnaD* and also containing *ts* mutation.

Figure 3

A


λ/Hind III
WT T4
ΔC
ΔD
ΔCD
2027bp
1163bp
1024bp
564bp
1044bp
905bp

B


30℃
42℃
WT ΔrnaC.ts ΔC TS
WT ΔrnaC.ts ΔC TS
10^0
10^1
10^2
10^3
WT ΔrnaCD.ts ΔCD TS
WT ΔrnaCD.ts ΔCD TS
10^0
10^1
10^2
10^3

C

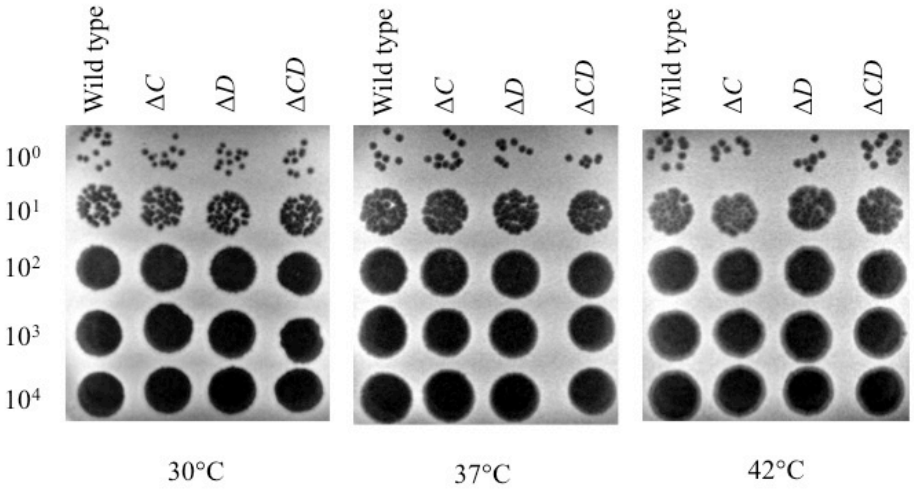


Figure 3. Genotype and growth confirmation of isolated phage mutants lacking gene *rnaC* or *rnaD* or both.

A) PCR fragments of genome DNA from wild type T4 and phage mutants amplified using two primers located within gene *e.8* and gene *segB*, respectively, were electrophoresed by a 1.2% agarose gel. Sizes of PCR fragments are shown in the figure.

B) A solution containing mutant phage as indicated above the figure was serially diluted 10-fold, spotted onto an agar plate seeded with MH1 cells, and incubated overnight at 30 or 42°C. Phages were WT, wild type; Δ*rnaC.ts*, gene *rnaC* deletion mutant with *ts* mutation in its genome as well; Δ*rnaCD.ts*, deletion mutant lacking both gene *rnaC* and *rnaD* and also containing *ts* mutation; TS, T4 phage only having a *ts* mutation in its genome.

C) A solution containing mutant phage as indicated above the figure was serially diluted 10-fold, spotted onto an agar plate seeded with MH1 cells, and incubated overnight at 30 or 42°C. Phages were wild type; Δ*C*, newly isolated phage mutant only lacking gene rnaC in its genome; Δ*CD*, newly isolated phage mutant lacking both *rnaC* and *rnaD* genes in its genome; Δ*D*, renamed from Δ*rnaD*, lacking *rnaD* gene in its genome.

Figure 4

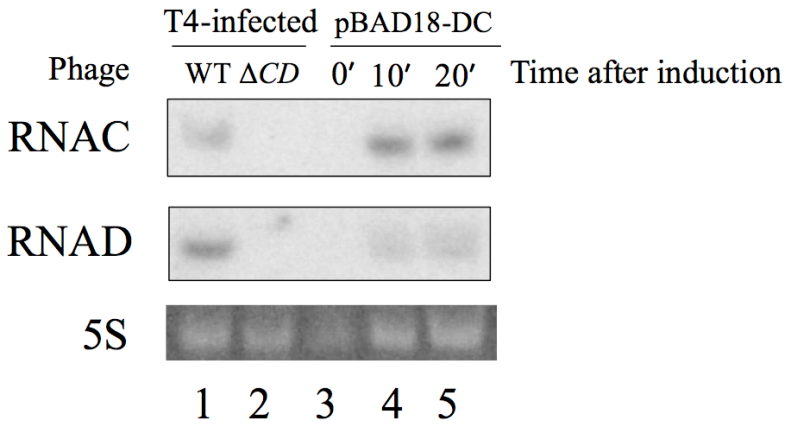
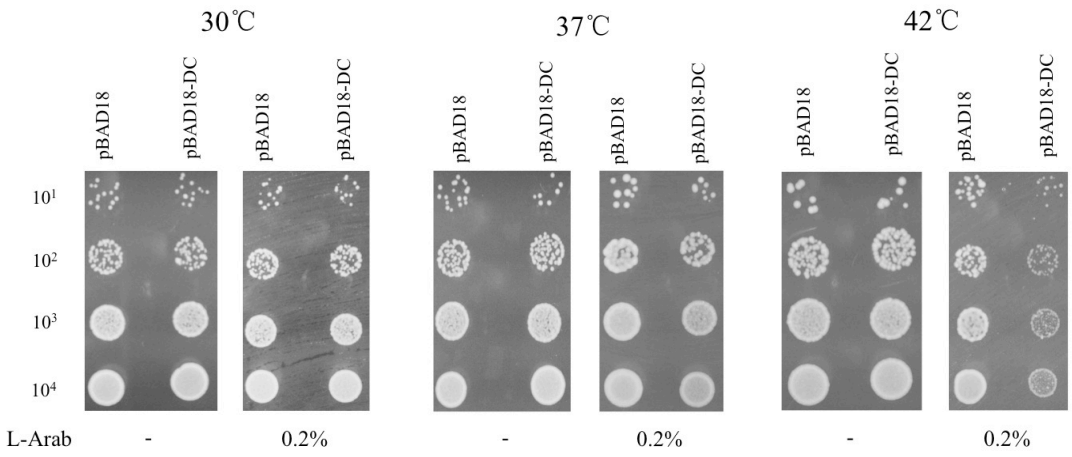


Figure 4. Expression of RNAC and RNAD from plasmid pBAD18-DC.

Line 1 and 2: MH1 cells were grown to mid-log phase at 37˚C and infected with wild-type T4 or ΔCD phage mutant, total RNAs were extracted after 10 minutes-infection and subjected to northern blotting with probes for RNAC or RNAD RNA. Line 3, 4 and 5: TY0807 cells harboring pBAD18-DC were treated with 0.2% arabinose when the OD_{600} reached 0.6 at 30˚C. Total RNAs were extracted at the indicated times after induction and subjected to northern blotting with probes for RNAC or RNAD RNA.

Figure 5

A


30℃
37℃
42℃
pBAD18
pBAD18-DC
10¹
10²
10³
10⁴
L-Arab
-
0.2%

B

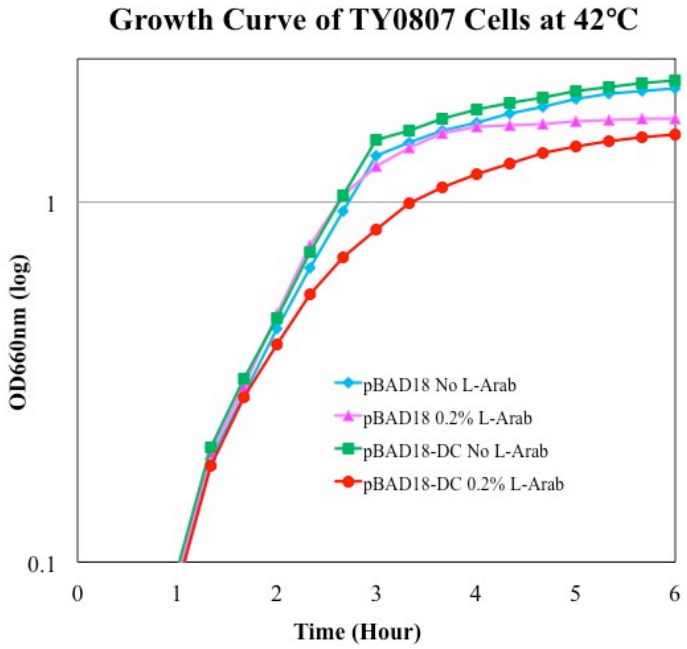

Growth Curve of TY0807 Cells at 42°C
OD660nm (log)
1
0.1
0
1
2
3
4
5
6
Time (Hour)
pBAD18 No L-Arab
pBAD18 0.2% L-Arab
pBAD18-DC No L-Arab
pBAD18-DC 0.2% L-Arab

Figure 5. Over expression of T4 sRNAs affects *E. coli* cell growth at high temperature.

A) A solution containing TY0807 harboring pBAD18 vector (left) or pBAD18-DC (right) was serially diluted in 10-fold steps, and 3μl containing the number of cells indicated in the left margin was spotted onto each plates and incubated at 30, 37 and 42°C, respectively.

B) TY0807 cells harboring pBAD18 or pBAD18-DC were grown in LB containing 50 μg ml^{-1} ampicillin with or without 0.2% L-arabinose. Cell growth was monitored by optical density at 660 nm every 20 min.

References

Abelson, J., Fukada, K., Johnson, P., Lamfrom, H., Nierlich, D.P., *et al.* (1975) Bacteriophage T4 tRNAs: structure, genetics, biosynthesis. *Brookhaven Symp Biol* **26**: 77-88.

Ades, S.E. (2004) Control of the alternative sigma factor σ^E in *Escherichia coli*. *Curr Opin Microbiol* **7**: 157-162.

Andrade, J.M., Pobre, V., Silva, I.J., Domingues, S. and Arraiano, C.M. (2009) The role of 3'-5' Exoribonucleases in RNA degradation. *Prog Mol Biol Transl Sci* **85**: 187-229.

Apirion, D. (1973) Degradation of RNA in *Escherichia coli*. A hepothesis. *Mol Gen Genet* **122**: 313-322.

Arraiano, C.M., Yancey, S.D. and Kushner. S.R. (1993) Identification of endonucleolytic cleavage sites involved in decay of *Esherichia coli trxA* mRNA.

Arraiano, C.M., Andrade, J.M., Domingues, S., Guinote, I.B., *et al.* (2010) The critical role of RNA processing and degradation in the control of gene expression. *FEMS Microbiol Rev* **34**: 883-923.

Babitzke, P. and Kushner, S.R. (1991) The Ams (altered mRNA stability) protein and ribonuclease E are encoded by the same structural gene of *Escherichia coli*. *Proc Natl Acad Sci* **88**: 1–5.

Babitzke, P., Granger, L., Olszewski, J. and Kushner, S. R. (1993) Analysis of mRNA decay and rRNA processing in *Escherichia coli* multiple mutants carrying a deletion in RNase III. *J Bacteriol* **175**: 229-239.

Bandyra, K.J. and Luisi, B.F. (2013) Licensing and due process in the turnover of bacterial RNA. *RNA Biol* **10**: 627-635.

Bernstein, J.A., Khodursky, A.B., Lin, P.H., Lin-Chao, S. and Cohen, S.N. (2002) Global analysis of mRNA decay and abundance in *Escherichia coli* at single-gene resolution using two-color fluorescent DNA microarrays. *Proc Natl Acad Sci* **99**: 9697-9702.

Bernstein, J.A., Lin, P.H., Cohen, S.N. and Lin-Chao, S. (2004) Global analysis of *Escherichia coli* RNA degradosome function using DNA microarrays. *Proc Natl Acad Sci* **10**: 2758-2763.

Black, L.W. and Gold, L.M. (1971) Pre-replicative development of the bacteriophage T4: RNA and protein synthesis *in vivo* and *in vitro*. *J Mol Biol* **60** : 365-388.

Bouvier, M., and Carpousis, A.J. (2011) A tale of two mRNA degradation pathways mediated by RNase E. *Mol Microbiol* **82:** 1305-1310.

Brissette, J.L., Russel, M., Weiner, L. and Model, P. (1990) Phage shock protein, a stress protein of *Escherichia coli*. *Proc Natl Acad Sci* **87**: 862-866.

Callaghan, A.J., Marcaida, M.J., Stead, J.A., McDowall, K.J., *et al.* (2005) Structure of *Escherichia coli* RNase E catalytic domain and implications for RNA turnover. *Nature* **437:** 1187-1191.

Celesnik, H., Deana, A. and Belasco, J.G. (2007) Initiation of RNA decay in *Escherichia coli* by 5' pyrophosphate removal. *Mol Cell* **27:** 79-90.

Carlson, K., Raleigh, E.A. and Hattman, S. (1994) Restriction and Modification. In *Molecular Biology of Bacteriophage T4*. Karam, J.D., Drake, J.W., Kreuzer, K.N., Mosig, G., Hall, D.H., Eiserling, F.A., Black, L.W., Spicer, E.K., Kutter, E., Carlson, K., Miller, E.S. (eds). American Society for Microbiology, Washington, D.C., pp. 369-381.

Carpousis, A. J., Van Houwe, G., Ehretsmann, C. and Krisch, H. M. (1994) Copurification of *E. coli* RNase E and PNPase: evidence for a specific association between two enzymes important in RNA processing and degradation. *Cell* **76**: 889–900.

Carpousis, A.J. (2007) The RNA degradosome of *Escherichia coli*: An mRNA-degrading machine assembled on RNase E. *Annu Rev Microbiol* **61:** 71-87.

Carpousis, A.J., Luisi, B.F. and McDowall, K.J. (2009) Endonucleolytic initiation of mRNA decay in *Escherichia coli*. *Prog Mol Biol Transl Sci* **85**: 91-135.

Cherepanov, P.P. and Wackernagel, W. (1995) Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* **158:** 9-14.

Clarke, J.E., Kime, L., Romero, A.D. and McDowall, K.J. (2014) Direct entry by RNase E is a major pathway for the degradation and processing of RNA in *Escherichia coli*. *Nucleic Acids Res* **42:** 11733-11751.

Covarrubias S., Richner, J.M., Clyde K., Lee, Y.J. and Glaunsinger, B.A. (2009) Host shutoff is a conserved phenotype of gammaherpevirus infection and is orchestrated exclusively from the cytoplasm. *J Virol* **83**: 9554-9566.

Danese P.N. and Silhavy, T.J. (1997) The sigma (E) and the Cpx signal transduction systems control the synthesis of periplasmic protein-folding enzymes in *Escherichia coli*. *Genes Dev* **11**: 1183-1193.

Darwin, A.J. (2005) The phage-shock-protein response. *Mol Microbiol* **57**: 621-628.

Deana, A., Celesnik, H. and Belasco, J.G. (2008) The bacterial enzyme RppH triggers messenger RNA degradation by 5' pyrophosphate removal. *Nature* **451:** 355-358.

Doherty, A.J., Serpell, L.C. and Ponting, C.P. (1996) The helix-hairpin-helix DNA-binding motif: a structural basis for non-sequence-specific recognition of DNA. *Nucleic Acids Res* **24**: 2488-2497.

Donly, B.C. and Mackie, G.A. (1988) Affinities of ribosomal protein S20 and C-terminal deletion mutants for 16S rRNA and S20 mRNA. *Nucleic Acids Res* **16**: 997-1010.

Duguay, A.R. and Silhavy, T.J. (2004) Quality control in the bacterial periplasm. *Biochim Biophys Acta* **1694**: 121-134.

Emory, S.A., Bouvet, P. and Belasco, J.G. (1992) A 5'-terminal stem-loop structure can stabilize mRNA in *Escherichia coli*. *Genes Dev* **6:** 135-148.

Folichon, M., Arluison, V., Pellegrini, O., Huntzinger, E., Régnier, P. and Hajnsdorf, E. (2003) The poly(A) binding protein Hfq protects RNA from RNase E and exoribonucleolytic degradation. *Nucleic Acids Res* **31**: 7302–7310.

Fukada, K. and Abelson, J. (1980) DNA sequence of a T4 transfer RNA gene cluster. *J Mol Biol* **139**: 377-391.

Gaglia, M.M., Covarrubias, S., Wong, W. and Glaunsinger, B.A. (2012) A common strategy for host RNA degradation by divergent viruses. J *Virol* **86**: 9527-9530.

Gao, J., Lee, K., Zhao, M., Qiu, J., Zhan, X., Saxena, A., *et al*. (2006) Differential

modulation of *E. coli* mRNA abundance by inhibitory proteins that alter the composition of the degradosome. *Mol Microbiol* **61:** 394-406.

Garrey, S.M., Blech, M., Riffell, J.L., Hankins, J.S., Stickney, L.M. *et al.* (2009) Substrate binding and active site residues in RNases E and G. Role of 5'-sensor. *J Biol Chem* **284**:31843-31850.

Georgellis, D., Barlow, T., Arvidson, S. and von Gabain, A. (1993). Retarded RNA turnover in *Escherichia coli*: a means of maintaining gene expression during anaerobiosis. *Mol Microbiol* **9**: 375-381.

Ghora, B.K. and Apirion, D. (1978) Structural analysis and *in vitro* processing to p5 rRNA of a 9S RNA molecule isolated from an *rne* mutant of *E. coli*. *Cell* **15**: 1055-1066.

Glaunsinger, B. and Ganem, D. (2004) Lytic KSHV infection inhibits host gene expression by accelerating global mRNA turnover. *Mol Cell* **13**: 713-723.

Gogol, E.B., Rhodius, V.A., Papenfort, K., Vogel, J. and Gross, C.A. (2011) Small RNAs endow a transcriptional activator with essential repressor functions for single-tier control of a global stress regulon. *Proc Natl Acad Sci* **108**: 12875-12880.

Górna, M.W., Carpousis, A.J. and Luisi, B.F. (2012) From conformational chaos to robust regulation: The structure and function of the multi-enzyme RNA degradosome. *Q Rev Biophys* **45**: 105-145.

Göpel, Y., Papenfort, K., Reichenbach, B., Vogel, J. and Görke, B. (2013) Targeted decay of a regulatory small RNA by an adaptor protein for RNase E and counteraction by an anti-adaptor RNA. *Genes Dev* **27:** 552-564.

Göpel, Y., Khan, M.A., and Görke, B. (2014) Ménage à trois: post-transcriptional control of the key enzyme for cell envelope synthesis by a base-pairing small RNA, an RNase adaptor protein, and a small RNA mimic. *RNA Biol* **11**: 433-442.

Greenberg, G.R., He, P., Hilfinger, J. and Tseng, M.J. (1994) Deoxyribonucleoside triphosphate synthesis and phage T4 DNA replication. In *Molecular Biology of Bacteriophage T4*. Karam, J.D., Drake, J.W., Kreuzer, K.N., Mosig, G., Hall, D.H., Eiserling, F.A., Black, L.W., Spicer, E.K., Kutter, E., Carlson, K., Miller, E.S. (eds). American Society for Microbiology, Washington, D.C., pp. 14-27.

Guisbert, E., Rhodius, V.A., Ahuja, N., Witkin, E. and Gross, C.A. (2007) Hfq modulates the sigmaE-mediated envelope stress response and the sigma32-mediated cytoplasmic stress response in *Escherichia coli*. *J Bacteriol* **189**:1963-1973.

Guo. P.X., Rajagopal B.S., Anderson D., *et al.* (1991) sRNA of phage phi29 of *Bacillus subtilis* mediates DNA packaging of phi 29 proheads assembled in *Escherichia coli*. *Virology* **185**: 395-400.

Guthrie, C. and McClain, W.H. (1973) Conditionally lethal mutants of bacteriophage T4 defective in production of a transfer RNA. *J Mol Biol* **81**:137-155.

Guzman, L.M., Belin, D., Carson, M.J. and Beckwith, J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J Bacteriol* **177:** 4121-4130.

Homyk, T. Jr., and Weil, J. (1974) Deletion analysis of two nonessential regions of the T4 genome. *Virology* **61:** 505-523.

Huang, C., Lokugamage, K.G., Rozovics, J.M., Narayanan, K., *et al.* (2011) SARS Coronavirus nsp1 Protein Induces Template-Dependent Endonucleolytic Cleavage of mRNAs: Viral mRNAs Are Resistant to nsp1-Induced RNA Cleavage. *PloS Pathog* **7**: e1002433.

Hunke, S., Keller, R. and Müller, V.S. (2012) Signal integration by the Cpx-envelope stress system. Signal integration by the Cpx-envelope stress system. *FEMS Microbiol Lett* **326**: 12-22.

Ikeda, Y., Yagi, M., Morita, T. and Aiba, H. (2011) Hfq binding at RhlB-recognition region of RNase E is crucial for the rapid degradation of target mRNAs mediated by sRNAs in *Escherichia coli*. *Mol Microbiol* **79**: 419-432.

Jain, C., Deana, A. and Belasco, J.G. (2002) Consequences of RNase E scarcity in *Escherichia coli*. *Mol Microbiol* **43:** 1053-1064.

Jerome, L.J., van Biesen, T., and Frost, L.S. (1999) Degradation of FinP antisense RNA from F-like plasmids: the RNA-binding protein, FinO, protects FinP from ribonuclease E. *J Mol Biol* **285**: 1457–1473.

Johansen, J., Rasmussen, A.A., Overgarrd, M. and Valentin-Hansen, P. (2006) Conserved small non-coding RNAs that belong to the σ^E regulon: role in down-

regulation of outer membrane proteins. *J Mol Biol* **364**: 1-8.

Kaberdin, V.R., Miczak, A., Jakobsen, J.S., Lin-Chao, S., *et al.* (1998) The endoribonucleolytic N-terminal half of *Escherichia coli* RNase E is evolutionarily conserved in *Synechocystis sp.* and other bacteria but not the C-terminal half, which is sufficient for degradosome assembly. *Proc Natl Acad Sci* **95**: 11637-11642.

Kaberdin, V. R. (2003) Probing the substrate specificity of *Escherichia coli* RNase E using a novel oligonucleotidebased assay. *Nucleic Acids Res* **31**: 4710–4716.

Kaempfer, R.O., and Magasanik, B. (1967a) Effect of infection with T-even phage on the inducible synthesis of β-galactosidase in *Escherichia coli*. *J Mol Biol* **27**: 453-468.

Kaempfer, R.O. and Magasanik, B. (1967b) Mechanism of β-galactosidase induction in *Escherichia coli*. *J Mol Biol* **27**:475-494.

Kamitani, W., Narayanan, K., Huang, C., Lokugamage, K., *et al.* (2006) Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. *Proc Natl Acad Sci* **103**:12885-12890.

Kai, T., Selick, H.E. and Yonesaki, T. (1996) Destabilization of bacteriophage T4 mRNAs by a mutation of gene *61.5*. *Genetics* **144:** 7–14.

Kennell, D. (1970) Inhibition of host protein synthesis during infection of *Escherichia coli* by bacteriophage T4. II. Induction of host messenger ribonucleic acid and its exclusion from polysomes. *J Viol* **6**: 208-217.

Khemici, V., Poljak, L., Toesca, I. and Carpousis, A.J. (2005) Evidence *in vivo* that the DEAD-box RNA helicase RhlB facilitates the degradation of ribosome-free mRNA by RNase E. *Proc Natl Acad Sci* **102**: 6913-6918.

Kime, L., Jourdan, S.S., Stead, J.A., Hidalgo-Sastre, A. and McDowall, K.J. (2009) Rapid cleavage of RNA by RNase E in the absence of 5'- monophosphate stimulation. *Mol Microbiol* **76**: 590–604.

Koga, M., Otsuka, Y., Lemire, S. and Yonesaki, T. (2011) *Escherichia coli rnlA* and *rnlB* compose a novel toxin-antitoxin system. *Genetics* **187:** 123-130.

Kwong, A.D., Kruper, J.A. and Frenkel, N. (1988) Herpes simplex virus virion host

shutoff function. *J Virol* **62**: 912-921.

Kushner, S.R. (2002) mRNA decay in *Escherichia coli* comes of age. *J Bacteriol* **184**: 4658-4665.

Kutter, E., Guttman, B. and Carlson, K. (1994a) The transition from host to phage metabolism after T4 infection, In *Molecular Biology of Bacteriophage T4*. Karam, J.D., Drake, J.W., Kreuzer, K.N., Mosig, G., Hall, D.H., Eiserling, F.A., Black, L.W., Spicer, E.K., Kutter, E., Carlson, K., Miller, E.S. (eds). American Society for Microbiology, Washington, D.C., pp. 343–346.

Kutter, E., White, T., Kashlev, M., Uzan, M., McKinney, J. and Guttman, B. (1994b) Effects on host genome structure and expression. In *Molecular Biology of Bacteriophage T4*. Karam, J.D., Drake, J.W., Kreuzer, K.N., Mosig, G., Hall, D.H., Eiserling, F.A., Black, L.W., Spicer, E.K., Kutter, E., Carlson, K., Miller, E.S. (eds). American Society for Microbiology, Washington, D.C., pp. 357-368.

Lee, K. and Cohen, S.N. (2003a) A *Streptomyces coelicolor* functional orthologue of *Escherichia coli* RNase E shows shuffling of catalytic and PNPase binding domains. *Mol Microbiol* **48**: 349–360.

Lee, K., Zhan, X., Gao, J., Qiu, J., Feng, Y., Meganathan, R., *et al*. (2003b) RraA: a protein inhibitor of RNase E activity that globally modulates RNA abundance in *E. coli. Cell* **114**: 623-634.

Lee, K., Bernstein, J.A. and Cohen, S.N. (2002) RNase G complementation of *rne* null mutation identifies functional interrelationships with RNase E in *Escherichia coli*. *Mol Microbiol* **43**: 1445-1456.

Li, Z., Pandit, S. and Deutscher, M.P. (1999) RNase G (CafA protein) and RNase E are both required for the 5' maturation of 16S ribosomal RNA. *EMBO J* **18**:2878-2885.

Li, Z. and Deutscher, M.P. (2002) RNase E plays an essential role in the maturation of *Escherichia coli* tRNA precursors. *RNA* **8**: 97-109.

Liao, S.M., Wu, T.H., Chiang, C.H., Susskind M.M. and McClure W.R. (1987) Control of gene expression in bacteriophage P22 by a small antisense RNA. I. Characterization in vitro of the P_{sar} promoter and the *sar* RNA transcript. *Genes Dev* **1**: 197-203

Lin-Chao, S., Wei, C. L. and Lin, Y.T. (1999) RNase E is required for the maturation of *ssrA* RNA and normal *ssrA* RNA peptide-tagging activity. *Proc Natl Acad Sci* **96**: 12406-12411.

Lundberg, U. and Altman, S. (1995) Processing of the precursor to the catalytic RNA subunit of RNase P from *Escherichia coli*. *RNA* **1**: 327-334.

Mackie, G.A. (1992) Secondary structure of the mRNA for ribosomal protein S20. Implications for cleavage by ribonuclease E. *J Biol Chem* **267**: 1054-1061.

Mackie, G.A. and Genereaux, J.L. (1993) The role of RNA structure in determining RNase E-dependent cleavage sites in the mRNA for ribosomal protein S20 *in vitro*. *J Mol Biol* **234**: 998–1012.

Mackie, G.A. (1998) Ribonuclease E is a 5′-end-dependent endonuclease. *Nature* **395**: 720–723.

Mackie, G.A. (2013a) RNase E: at the interface of bacterial RNA processing and decay. *Nat Rev Microbiol* **11:** 45-57.

Mackie, G.A. (2013b) Determinants in the *rpsT* mRNAs recognized by the 5'-sensor domain of RNase E. *Mol Microbiol* **89**: 388-402.

Marcaida, M.J., DePristo, M.A., Chandran, V., Carpousis, A.J. and Luisi, B.F. (2006) The RNA degradosome: life in the fast lane of adaptive molecular evolution. *Trends Biochem Sci* **31:** 359-365.

Marchand, I., Nicholson, A.W. and Dreyfus, M. (2001) Bacteriophage T7 protein kinase phosphorylates RNase E and stabilizes mRNAs synthesized by T7 RNA polymerase. *Mol Microbiol* **42:** 767-776.

Matos, R.G., Barbas, A., Gómez-Puertas, P. and Arraiano, C.M. (2011) Swapping the domains of exoribonucleases RNase II and RNase R: conferring upon RNase II the ability to degrade dsRNA. *Proteins* **79**: 1853-67.

Mathews, C.K. (1994) An overview of the T4 developmental program. In *Molecular Biology of Bacteriophage T4*. Karam, J.D., Drake, J.W., Kreuzer, K.N., Mosig, G., Hall, D.H., Eiserling, F.A., Black, L.W., Spicer, E.K., Kutter, E., Carlson, K., Miller, E.S. (eds). American Society for Microbiology, Washington, D.C., pp.1-8.

McClain, W. H., Guthrie, C. and Barrell, B.G. (1972) Eight Transfer RNA Induced by

Infection of *Escherichia coli* with Bacteriophage T4. *Proc Nat Acad Sci* **69**: 3703-3707.

McDowall, K.J., Kaberdin, V.R., Wu, S.W., Cohen, S.N. and Lin-Chao, S. (1995) Site-specific RNase E cleavage of oligonucleotides and inhibition by stem–loops. *Nature* **374**: 287–290.

Miczak, A., Kaberdin, V.R., Wei, C.L., and Lin-Chao, S. (1996) Proteins associated with RNase E in a multicomponent ribonucleolytic complex. *Proc Natl Acad Sci* **93**: 3865–3869.

Miller, E.S., Karam, J.D. and Spicer, E. (1994) Control of translation initiation: mRNA structure and protein repressors. In *Molecular Biology of Bacteriophage T4*. Edited by: Karam, J.D., Drake, J.D., Kreuzer, K.N., Mosig, G., Hall, D.W., Eiserling, F.A., Black, L.W., Spicer, E.K., Kutter, E, Carlson, K., Miller, E.S., Washington, D.C.: American Society for Microbiology, pp: 193-205.

Miller, E.S., Kutter, E., Mosig, G., Arisaka, F., Kunisawa, T. and Rüger W. (2003a) Bacteriophage T4 genome. *Microbiol Mol Biol Rev* **67:** 86-156.

Miller, E.S., Heidelberg, J.F., Eisen, J.A., Nelson W.C., *et al.* (2003b) Complete genome sequence of the broad-host-range vibriophage KVP40: comparative genomics of a T4-ralated bacteriophage. *J Bacteriol* **185**: 5220-5233.

Misra, T.K. and Apirion, D. (1979) RNase E, an RNA processing enzyme from *Escherichia coli. J Biol Chem* **254**: 11154-11159.

Mohanty, B.K. and Kushner, S.R. (1999) Analysis of the function of *Escherichia coli* polymerase I in RNA metabolism. *Mol Microbiol* **34**:1094-1108.

Mosig, G., Colowick, N.E. and Pietz, B.C. (1998) Several new bacteriophage T4 genes, mapped by sequencing deletion endpoints between genes *56* (dCTPase) and *dda* (a DNA-dependent ATPase-helicase) modulate transcription. *Gene* **223:** 143-155.

Mudd, E.A., Prentki, P., Belin, D. and Krisch, H.M. (1988) Processing of unstable bacteriophage T4 gene 32 mRNAs into a stable species requires *Escherichia coli* ribonuclease E. *EMBO J* **7**: 3601-3607.

Mudd, E.A., Carpousis, A.J. and Krisch, H.M. (1990a) *Escherichia coli* RNase E has a role in the decay of bacteriophage T4 mRNA. *Genes Dev* **4:** 873-881.

Mudd, E.A., Krisch, H.M. and Higgins, C.F. (1990b) RNase E, an endoribonuclease, has a general role in the chemical decay of *Escherichia coli* mRNA: evidence that *rne* and *ams* are the same genetic locus. *Mol Microbiol* **4:** 2127-2135.

Nilsson, G., Belasco, J.G., Cohen, S.N. and von Gabain, A.V. (1984) Growth-rate dependent regulation of mRNA stability in *Escherichia coli. Nature* **312**: 75-77.

Niyogi, S.K. and Datta, A.K. (1975) A novel oligoribonuclease of *Escherichia coli.* I. Isolation and properties. *J Biol Chem* **250**: 7307-7312.

Nomura, M., Okamoto, K. and Asano, K. (1962) RNA metabolism in *Escherichia coli* infected with bacteriophage T4. Inhibition of host ribosomal and soluble RNA synthesis by phage and effect of chloromycein. *J Mol Biol* **4**: 376-387.

Ono, M. and Kuwano, M. (1979) A conditional lethal mutation in an *Escherichia coli* strain with a longer chemical lifetime of messenger RNA. *J Mol Biol* **129:** 343-357.

Otsuka, Y., Miki, K., Koga, M., Katayama, N., Morimoto, W., Takahashi, Y. and Yonesaki, T. (2010) IscR regulates RNase LS activity by repressing *rnlA* transcription. *Genetics* **185:** 823-830.

Otsuka, Y. and Yonesaki, T. (2005) A novel endoribonuclease, RNase LS, in *Escherichia coli*. *Genetics* **169:** 13–20.

Ow, M.C., Liu, Q. and Kushner, S.R. (2000) Analysis of mRNA decay and rRNA processing in *Escherichia coli* in the absence of RNase E-based degradosome assembly. *Mol Microbiol* **38**: 854-866.

Ow, M.C. and Kushner, S.R. (2002) Initiation of tRNA maturation by RNase E is essential for cell viability in *E. coli. Genes Dev* **16**: 1102-1115.

Ow, M.C., Perwez, T. and Kushner, S.R. (2003) RNase G of *Escherichia coli* exhibits only limited functional overlap with its essential homologue, RNase E. *Mol Microbiol* **49:** 607-622.

Paddock, G. and Abelson, J. (1973) Sequence of T4, T2 and T6 Bacteriophage Species I RNA and Specific Cleavage by an *E. coli* Endonuclease. *Nat New Biol* **246**: 2-6.

Parsons, G.D. and Mackie, G.A. (1983) Expression of the gene for ribosomal protein

S20: effects of gene dosage. *J Bacteriol* **154**: 152-160.

Plunkett III, G., Mazzara, G.P., and McClain, W. (1981) Characteriazation of Bacteriophage T4 Band D RNA, a Low-Molecular-Weight RNA of Unknown Function. *Arch Biochem Biophys* **210**: 298-306.

Portier, C., Dondon, L., Grunberg-Manago, M. (1990) Transtational autocontrol of the *Escherichia coli* ribosomal protein S15. *J Mol Biol* **211**: 407-414.

Py, B., Higgins, C.F., Krisch, H.M. and Carpousis, A.J. (1996) A DEAD-box RNA helicase in the *Escherichia coli* RNA degradosome. *Nature* **381**: 169–172.

Ray, B.K. and Apirion, D. (1981) Transfer RNA precursors are accumulated in *Escherichia coli* in the absence of RNase E. *Eur J Biochem* **114**: 517-524.

Read G.S. and Frenkel, N. (1983) Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of alpha (immediate early) viral polypeptides. *J Virol* **46**: 498-512.

Richards, J., Luciano, D.J. and Belasco, J.G. (2012) Influence of translation on RppH-dependent mRNA degradation in *Escherichia coli*. *Mol Microbiol* **86:** 1063-1072.

Ron, E.Z. and Davis, B.D. (1971) Growth rate of *Escherichia coli* at elevated temperatures: limitation by methionine. *J Bacteriol* **107**: 391-396.

Rowe, M., Glaunsinger B, van Leeuwen D, Zuo J, Sweetman D, *et al*. (2007) Host shutoff during productive Epstein-Barr virus infection is mediated by BGLF5 and may contribute to immune evasion. *Proc Natl Acad Sci* **104**: 3366-3371.

Sandberg, T.E., Pedersen, M., LaCroix, R.A., Ebrahim, A. *et al.* (2014) Evolution of *Escherichia coli* to 42°C and Subsequent Genetic Engineering Reveals Adaptive Mechanisms and Novel Mutations. *Mol Biol Evol* **31**: 2647-2662.

Sarkar, N. (1997) Polyadenylation of mRNA in prokaryotes. *Annu Rev Biochem* **66**: 173-97.

Selick, H.E., Kreuzer, K.N. and Alberts, B.M. (1988) The Bacteriophage T4 Insertion/Substitution Vector System. *J Biol Chem* **263:** 11336-11347.

Selinger, D.W., Saxena, R.M., Cheung, K.J., Church, G.M. and Rosenow, C. (2003) Global RNA half-life analysis in *Escherichia coli* reveals positional patterns of transcript degradation. *Genome Res* **13**: 216-23.

Singh, D., Chang, S.J., Lin, P.H., Averina, O.V., Kaberdin, V.R. and Lin-Chao, S. (2009) Regulation of Ribonuclease E activity by L4 ribosomal protein of *Escherichia coli*. *Proc Natl Acad Sci* **106:** 864-869.

Snustad, D.P., Bursch, C.J., Parson, K.A. and Hefeneider, S.H. (1976) Mutants of Bateriophage T4 Deficient in the Ability to Induce Nuclear Disruption: Shutoff of Host DNA and Protein Synthesis, Gene Dosage Experiments, Identification of a Restrictive Host, and Possible Biological Significance. J *Virol* **18**: 268-288.

Stead, M.B., Marshburn, S., Mohanty, B.K., Mitra, J. *et al.* (2011) Analysis of *Escherichia coli* RNase E and RNase III activity *in vivo* using tiling microarrays. *Nucleic Acids Res* **39**:3188-203.

Stent, G.S. (1963) Molecular Biology of Bacterial Viruses. W. H. Freeman & Co., San Fransisco. pp. 345.

Strelow, L.I. and Leib, D.A. (1995). Role of the virion host shutoff (*vhs*) of herpes simplex virus type 1 in latency and pathogenesis. *J Virol* **69**: 6779–6786.

Suzutani, T, Nagamine, M., Shibaki, T., Ogasawara, M., *et al.* (2000) The role of the UL41 gene of herpes simplex virus type 1 in evasion of non-specific host defence mechanisms during primary infection. *J Gen Virol* **81**: 1763-1771.

Svenson, S.B. and Karlström, O.H. (1976) Bacteriophage T4-induced shut-off of host-specific translation. *Viology* **17**: 326-334.

Taddeo, B., Zhang, W. and Roizman, B. (2013) The herpes simplex virus host shutoff RNase degrades cellular and viral mRNAs made before infection but not viral mRNA made after infection. *J Virol* **87**: 4516-4522.

Tanaka, T., Kamitani, W., DeDiego, M.L., Enjuanes, L. and Matsuura, Y. (2012) Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA. *J Virol* **86**: 11128-11137.

Tomcsányi, T. and Apirion, D. (1985) Processing enzyme ribonuclease E specifically cleaves RNA I. An inhibitor of primer formation in plasmid DNA synthesis. *J Mol Biol* **185**: 713-720.

Tsai, Y.C., Du, D., Domínguez-Malfavón, L., *et al*. (2012) Recognition of the 70S ribosome and polysome by the RNA degradosome in *Escherichia coli*. *Nucleic*

Acids Res **40:** 10417-10431.

Ueno, H. and Yonesaki, T. (2004) Phage-induced change in the stability of mRNAs. *Virology* **329:** 134-141.

Uzan, M. and Miller, E.S. (2010) Post-transcriptional control by bacteriophage T4: mRNA decay and inhibition of translation inhibition. *Virol J* **7**: 360. doi: 10.1186/1743-422X-7-360.

Valentin-Hansen, P., Eriksen, M. and Udensen, C. (2004) The bacterial Sm-like protein Hfq: a key player in RNA transactions. *Mol Microbiol* **51**: 1525-1533.

Vogel, J. and Luisi, B. F. (2011) Hfq and its constellation of RNA. *Nature Rev Microbiol* **9**: 578–589.

Wachi, M., Umitsuki, G. and Nagai, K. (1997) Functional relationship between *Escherichia coli* RNase E and the CafA protein. *Mol Gen Genet* **253:** 515-519.

Wachi, M., Umitsuki, G., Shimizu, M., Takada, A. and Nagai, K. (1999) *Escherichia coli cafA* gene encodes a novel RNase, designated as RNase G, involved in processing of the 5' end of 16S rRNA. *Biochem Biophys Res Commun* **259**: 483-488.

Wilson, J.H., Kim, J.S. and Abelson, J.N. (1972) Bacteriaphage T4 transfer RNA.III. Clustering of the genes for the T4 transfer RNA's. *J Mol Biol* **71**: 547-556.

Wilson, G.G., Young, K.Y., Edlin, G.J. and Konigsberg, W. (1979) High-frequency generalized transduction by bacteriophage T4. *Nature* **280:** 80-82.

Wirth, R., Littlechild, J. and Böck, A. (1982) Ribosomal protein S20 purified under mild conditions almost completely inhibits its own translation. *Mol Gen Genet* **188**: 164-166.

Wu, T.H., Liao, S.M., McClure W.R. and Susskind M.M. (1987) Control of gene expression in bacteriophage P22 by a small antisense RNA. II. Characterization of mutants defective in repression. *Genes Dev* **1**: 204-12.

Wulf, P.D., Kwon, O., and Lin, E.C.C. (1999) The CpxRA signal transduction system of *Escherichia coli*: growth-related autoactivation and control of unanticipated targe operons. *J Bacteriol* **181**: 6772-6778.

Yeom, J.H., Go, H., Shin, E., Kim, H.L., Han, S.H., Moore, C.J., *et al*. (2008a)

Inhibitory effects of RraA and RraB on RNase E-related enzymes imply conserved functions in the regulated enzymatic cleavage of RNA. *FEMS Microbiol Lett* **285:** 10-15.

Yeom, J.H., Shin, E., Go, H., Sim, S.H., Seong, M.J. and Lee, K. (2008b) Functional implications of the conserved action of regulators of ribonuclease activity. *J Microbiol Biotechnol* **18:** 1353-1356.

Publications

Qi, D., Alawneh, A.M., Yonesaki, T. and Otsuka, Y. (2015) Rapid degradation of host mRNAs by stimulation of RNase E activity by Srd of bacteriophage T4. *Genetics* doi: 10.1534/genetics.115.180364 (Early online)

Alawneh, A.M., Qi, D., Yonesaki, T. and Otsuka, Y. (2015) An ADP-ribosyltransferase Alt of bacteriophage T4 negatively regulates the *Escherichia coli* MazF toxin of a toxin–antitoxin module. *Mol Microbiol* DOI: 10.1111/mmi.13225 (Article first published online)

Naka, K., Qi, D., Yonesaki, T. and Otsuka, Y. (2015) RnlB antitoxin of the *Esherichia coli* RnlA-RnlB toxin-antitoxin module requires RNase HI for inhibition of RnlA toxin activity. (Recently submitted)

Acknowledgements

I would like to express my best deepest gratitude to my supervisor, Dr. Tetsuro Yonesaki for his kind guidance and supporting my research during the course of this work. Everything I learned from him will certainly be helpful to my future development as a scientist.

I also wish to express my sincere gratitude to Dr. Yuichi Otsuka for continuous guidance, encouragement and valuable advices throughout all my five years' study.

I also wish to thank three supporting examiner of my doctoral thesis, Dr. Hiroshi Kanazawa, Dr. Hisao Masukata, and Dr. Tatsuro Kakimoto for advices to improve my doctoral thesis presentation.

I also thank all present and past members of Yonesaki's laboratory for their kind helps. My thanks especially go to Dr. Mitsunori Koga, Ms. Yasuko Tada, Mr. Kenta Naka, Ms. Ayaka Washizaki, and Mr. Abdulheem Alawneh for their valuable discussions and kind help during my doctoral course.

I thank Dr. Yuichi Otsuka (Dokkyo Medical University) for the plasmid pQ-orf2-95, pBAD18-DC, pRpoE and Δ*rnaC.ts*, Δ*rnaCD.ts* and Δ*rnaD* phage mutants as well as *E. coli* TY1009, Δ*micA*, Δ*rybB* mutants. I thank Dr. H. Takahashi (University of Tokyo) for T4 mutant $\Delta(39\text{-}56)_6$ and also Dr. H. Aiba (Nagoya University) for *E. coli* RNase E mutants TM338 and TM529. I thank Dr. John. W. Drake (the National Institute of Environmental Health Sciences) for a critical reading and the invaluable help with manuscript of my publication paper.

I thank all the staff of the Radioisotope Research Center at Toyonaka, Osaka University, where all my experiments using radioisotope were carried out, for facilitation of my research. And also I thank Ms. Takashima for supporting my research upon many detailed procedures.

Finally, I really wish to thank my dear parents, my dear husband and my parents-in-law for supporting my work with deep love.