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Phage-induced change in the stability of mRNAs

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Abstract

The stability of mRNA in *Escherichia coli* cells changed after phage T4 infection. Stable *E. coli* mRNAs such as *lpp* and *ompA* were drastically destabilized immediately after infection. In contrast, T4 phage *soc* mRNA that had been unstable before infection became stabilized after infection. The host RNases E and G both contributed to the destabilization of these mRNAs. Accordingly, these RNases may alter their target RNAs before and after infection. An RNA chaperon, Hfq, and polyadenylation at 3′ends of mRNA are known key factors for destabilization of *ompA* and *lpp* mRNAs in uninfected cells. However, they had no effect on the destabilization of *E. coli* mRNAs after infection. On the other hand, T4 infection in the presence of rifampicin or infection of a deletion mutant, Δ*tk*2, did not destabilize the host mRNAs. These results strongly suggest that a phage-encoded factor is responsible for the destabilization of host mRNAs. Destabilization of host mRNAs was also observed after infection by phages T2 and T7.

Keywords: mRNA degradation; Target selection; T2 phage; T4 phage; T7 phage; RNase E; RNase G; T4 factor; *Escherichia coli*

Introduction

Infection by bacteriophage T4 blocks the synthesis of host macromolecules such as DNA, RNA, and protein. Instead, T4 induces other mechanisms, converting a host cell into an efficient factory for the production of viral progeny (Mathews, 1994). Among these mechanisms, a shift of gene expression from host to T4 is one of the most important. The sequential modification of transcriptional activity after T4 infection is well characterized (Mathews, 1994; Miller et al., 2003; Mosig and Hall, 1994). Three different classes of promoters, early, middle, and late, initiate T4 transcription. Immediately after T4 infection, early genes are transcribed from early promoters by the *Escherichia coli* RNA polymerase. Early-gene products alter the host RNA polymerase to transcribe middle genes from middle promoters. By the expression of middle genes, the host RNA polymerase is further modified and ultimately transcribes late genes. Usually, the sequential modifications of the host RNA polymerase complete within 20 min. On the other hand, cessation of host gene expression ("shutoff") after T4 infection is very rapid (within 2 min). This "shutoff" is not explained solely by changes in transcriptional activity, and another mechanism, such as the rapid elimination of existing mRNAs, is required.

Degradation of T4 mRNAs has been studied and three endoribonucleases are found to initiate mRNA degradation. T4 RegB cleaves in the middle of 5′-GGAG of some early gene mRNAs, including *regB* mRNA (Sanson et al., 2000) and may contribute to a shift of gene expression from early to middle. *E. coli* RNase E has a substantial effect on the turnover of T4 mRNA, because a defect of RNase E considerably stabilizes many mRNAs (Mudd et al., 1990). This enzyme has additional functions to maturate gene *32* mRNA (Mudd et al., 1988) and block premature expression of late gene *soc* (Otsuka et al., 2003). *E. coli* RNase LS causes the rapid degradation of middle and late-gene mRNAs, when T4 *dmd* is mutated (Kai et al., 1996; Otsuka and Yonesaki, 2004). Thereby, RNase LS is considered to antagonize the T4 growth and *dmd* is required for

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overcoming the anti-T4 effect. In spite of the progress in our knowledge about T4 mRNA degradation, the fate of *E. coli* mRNAs after T4 infection has not been well characterized. In this article, we addressed the stabilities of host and phage mRNAs before and after T4 infection. As expected, the *E. coli lpp* and *ompA* mRNAs that were stable before infection were quickly destabilized after infection. Evidence is presented that a T4 gene product is involved in the destabilization of host mRNAs. In contrast, T4 *soc* mRNA was unstable before infection but became more stable after infection. Two different RNases contributed to the destabilization of *E. coli* mRNAs after infection and T4 mRNAs before infection. These results suggest that the selection of RNase targets is highly regulated.

Results

Destabilization of host mRNAs after T4 infection

E. coli lpp mRNA and *ompA* mRNA are known to be stable (see Fig. 1). These mRNAs are useful for testing our hypothesis that preexisting host mRNAs are rapidly degraded after T4 infection. To determine the decay rate of host mRNA in uninfected cells, rifampicin was added to block transcription and total RNA was then extracted at various times for Northern blot analysis. The decay rate after T4 infection could be measured without rifampicin because host-gene transcription is immediately repressed by the T4 Alc protein. This protein is synthesized within a few minutes after infection and inhibits the transcription of cytosine-containing host DNA but not of HMC-containing T4 DNA (Kashlev et al., 1993). The *lpp* mRNA in uninfected cells degraded with a half-life of 31 min (Fig. 1A). In contrast, its half-life was as short as 2.3 min immediately after infection. A similar result was obtained for *ompA* mRNA (Fig. 1B); the half-life was 30 min in uninfected cells and only 2.5 min after T4 infection. Thus, *lpp* and *ompA* mRNAs were quickly destabilized after T4 infection and were degraded more than 10-fold faster than before infection.

Identification of ribonucleases for host mRNA degradation after infection

The degradation of most mRNAs in *E. coli* is triggered by endonucleolytic cleavage. It is reasonable to assume that

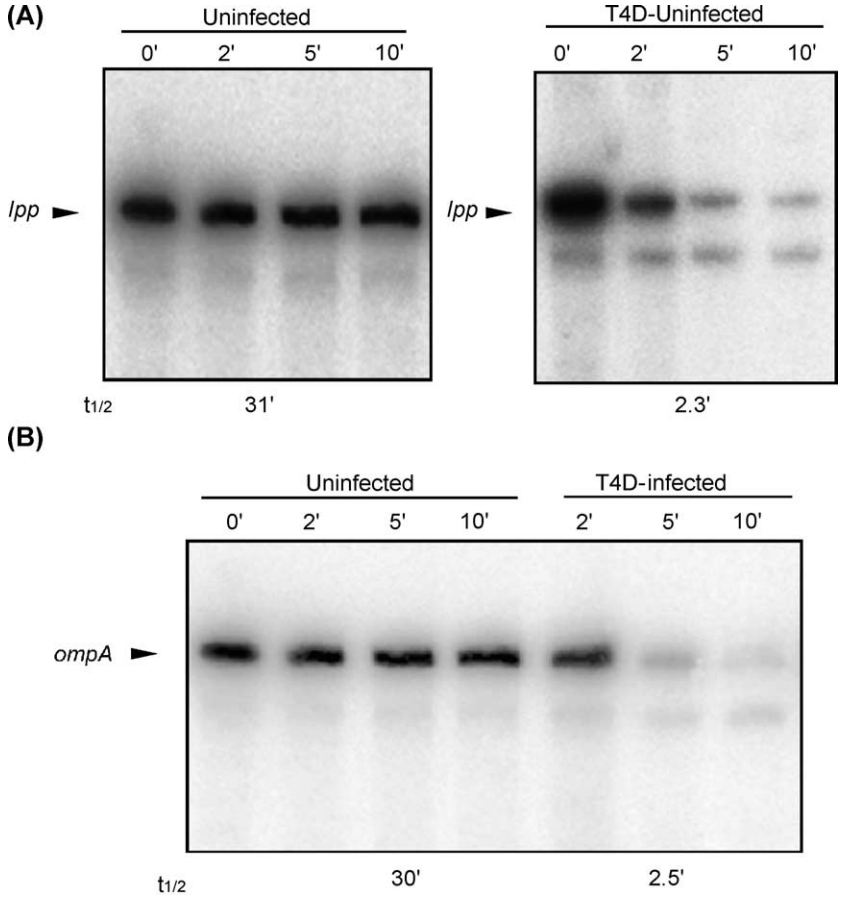

Fig. 1. Stability of *E. coli lpp* and *ompA* mRNAs before and after T4 infection. A culture of logarithmically growing GW10 cells was divided into two cultures. Rifampicin was added at time 0 to one culture (uninfected cells) or wild-type T4 phage was added at time 0 to another culture (T4D-infected cells). Total RNAs were isolated at the indicated times and 7 μg from each were analyzed by Northern blotting with a probe for (A) *lpp* or (B) *ompA*. An arrowhead indicates each full-length transcript. Half-lives ($t_{1/2}$) of full-length transcripts are shown below the figure.

a T4-encoded endoribonuclease was responsible for the rapid degradation of host mRNAs after T4 infection. RegB is the only known T4 endoribonuclease and is expressed immediately after infection (Sanson et al., 2000; Uzan et al., 1988). To examine the effect of RegB, we constructed a mutant, Δ*regB-denV*, lacking the structural gene for RegB. The Δ*regB-denV* mutant degraded *ompA* and *lpp* mRNAs as rapidly as wild-type phage (data not shown). In T4-infected cells, *dmd* is required for the control of mRNA stability (Kai et al., 1996; Ueno and Yonesaki, 2001). We found no significant effect of the *dmd* mutation on the destabilization of host mRNAs after infection (data not shown). We next investigated *E. coli* endoribonucleases. RNase E is essential for cell growth, and its inactivation stabilizes most mRNAs and impairs the processing of a variety of RNAs (Kushner, 2002). Another ribonuclease, RNase G, degrades a limited set of mRNAs (Lee et al., 2002). We found that both RNases E and G exhibited a profound effect on the decay of host mRNAs after infection. As shown in Fig. 2, *lpp* mRNA degraded with a half-life of 2.5 min in wild-type GW10 cells, whereas its half-life was 8.0 min in GW20 cells expressing a temperature-sensitive RNase E. The half-life was 8.2 min in GW11 cells that were deficient in RNase G. Furthermore, the half-life was 26 min in GW21 cells in which both RNase E and RNase G were defective, suggesting that the effects of RNase E and RNase G are additive. We obtained a similar result for *ompA* mRNA; its half-life was 2.3 min in GW10 cells, 15 min in GW20 cells, 10 min in GW11 cells, and 20 min in GW21 cells (data not shown). These results reveal that both RNases E and G play a substantial role in the degradation of *lpp* and *ompA* mRNAs in T4-infected cells.

Stability of T4 soc mRNA before and after infection

The observation that host mRNAs were quickly destabilized after T4 infection prompted us to examine whether the stability of T4 mRNA is changed by T4 infection. In T4-infected cells, transcription of the *soc* gene is initiated from its own late promoter by a modified RNA polymerase (Miller et al., 2003; Mosig and Hall, 1994). pTK44 cloned the whole *soc* gene including its promoter and terminator of transcription, into a promoterless site of pBSPLO+ (Selick et al., 1988). Because the *soc* promoter region overlaps an *E. coli* promoter sequence, *soc* can be transcribed even in uninfected cells. The transcription in uninfected cells starts from two different points: one is the same as in T4-infected cells and another is two nucleotides upstream of the former point (Otsuka et al., 2003), yielding nearly equal amounts of two transcripts with different 5′ end structures (data not shown). We analyzed a mixture of these transcripts as *soc* mRNA, because the difference of two nucleotides cannot be distinguished by Northern blotting. The half-life of the plasmid-encoded *soc* mRNA in uninfected cells was 3 min (Fig. 3). During normal infection, *soc* is expressed at late stages during which *soc* mRNA remains stable. Therefore, we expected that the stability of *soc* mRNA should be changed from unstable in uninfected cells to stable at late stages of T4 infection. However, because a plasmid is destroyed within 10 min after T4 infection, it would be impossible to follow the stability of plasmid-encoded *soc* mRNA until late stages of infection. To overcome this limitation, we utilized a T4 mutant deficient in both *denA* and *denB*. *denA* encodes endonuclease II, which introduces a nick in dsDNA (Sadowski and Hurwitz, 1969a), in a sequence-dependent manner (Carlson et al., 1999). *denB* encodes endonuclease IV, which breaks ssDNA (Sadowski and Hurwitz, 1969b). These two enzymes are specific to cytosine-containing DNA and promote the breakdown of plasmid DNA as well as host DNA (Souther et al., 1972). Therefore, mutations in these genes leave a plasmid intact and allow it to serve as a transcription template. In addition, to avoid a burst of expression from the T4 genome, the mutant phage was also deleted for *soc*. The half-life of *soc* mRNA derived from the pTK44 plasmid was measured at 2, 9, and 18 min after T4 infection. In this experiment, the amount of *soc* mRNA at 9 min was less than that at 2 min because of degradation. Its amount was, however, increased at 18 min, since transcription from the late promoter had begun. The half-life was 5.8 min at the early stage, 6.2 min at the middle stage, and 14 min at the late stage. Thus, the half-life of *soc* mRNA at early and middle stages was about twofold longer than in uninfected cells. Moreover, its half-life at late stages became about fivefold longer than in uninfected cells.

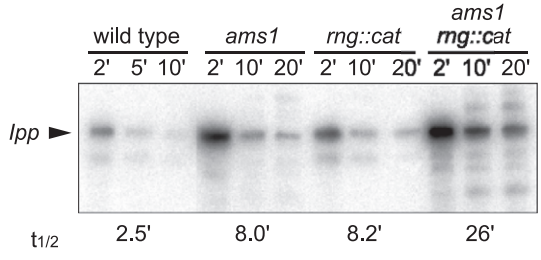

Fig. 2. Effects of RNases E and G on the stability of *lpp* mRNA in T4-infected cells. GW10, GW20, GW11, or GW21 cells were infected with wild-type T4 at time 0. Total RNAs were isolated at indicated times and 4 μg from each were analyzed by Northern blotting with a probe for *lpp* gene. Alleles of RNase gene in each strain are indicated above the figure. An arrowhead indicates the full-length *lpp* transcript. The half-lives of *lpp* full-length transcripts in each strain are indicated below the figure.

Identification of ribonucleases for soc mRNA degradation in uninfected cells

We also found that RNases E and G had marked effects on the decay of *soc* mRNA in uninfected cells (Fig. 4). The *soc* mRNA degraded with a half-life of 3 min in wild-type cells, while the half-life was 7.2 min when RNase E was inactivated. RNase G had a more profound effect than RNase E; the half-life of *soc* mRNA in the absence of RNase G was 18 min, which was sixfold longer than in the wild-type strain. Finally, *soc* mRNA was very stable when

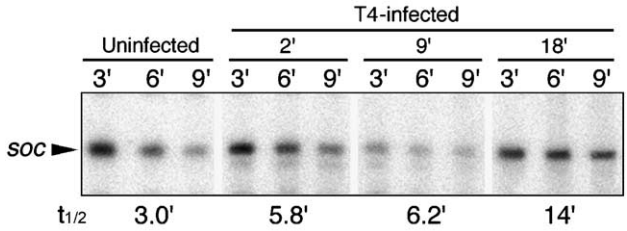

Fig. 3. Stability of T4 *soc* mRNA before and after T4 infection. A culture of GW10 cells carrying pTK44 was divided into four cultures. Cells in three cultures were infected with *nd28* (*denA*$^-$) ΔHTP8 (*denB*$^-$) Δ*soc* phage. Rifampicin was added at time 0 to uninfected cells or at 2, 9, or 18 min to infected cells. At various times after addition of rifampicin, total RNA was isolated at indicated times and 3.5 μg from each was analyzed by Northern blotting with a probe for *soc* gene. Half-lives of *soc* full-length transcripts are shown below the figure. An arrowhead indicates the full-length *soc* transcript.

both RNase E and RNase G were defective. The half-life of 26 min was longer than in each single mutant. Therefore, we conclude that both RNases E and G play a central role in *soc* mRNA degradation in uninfected cells, in addition to their role in the degradation of *lpp* and *ompA* mRNA after T4 infection.

Phage-induced host mRNA degradation requires the degradosome

RNase E forms a huge complex called the degradosome (Vanzo et al., 1998). The degradosome is composed of RNase E, polynucleotide phosphorylase, RhlB RNA helicase, and enolase. The amino-terminal half of RNase E (amino acid residues 1–498) contains an active site for RNA cleavage, while the carboxyl-terminal half (amino acid residues 499–1061) serves as a scaffold for the assembly of other components (Kido et al., 1996; Vanzo et al., 1998). We attempted to determine whether degradosome formation is essential for the RNase E activity to promote the T4-induced host mRNA destabilization. *E. coli* strain Bz189 has a nonsense mutation at amino acid position 593 in *rne*, the structural gene of RNase E, and thereby the degradosome cannot be formed in this strain. The half-life of *lpp* mRNA in parental strain SH3208 cells and in Bz189 cells after infection was 1.6 and 8.0 min, respectively (Fig. 5). The latter value is close to that of the strain defective in RNase E activity as described in Fig. 3. This result suggests that RNase E requires the degradosome to function in T4-induced *lpp* mRNA degradation.

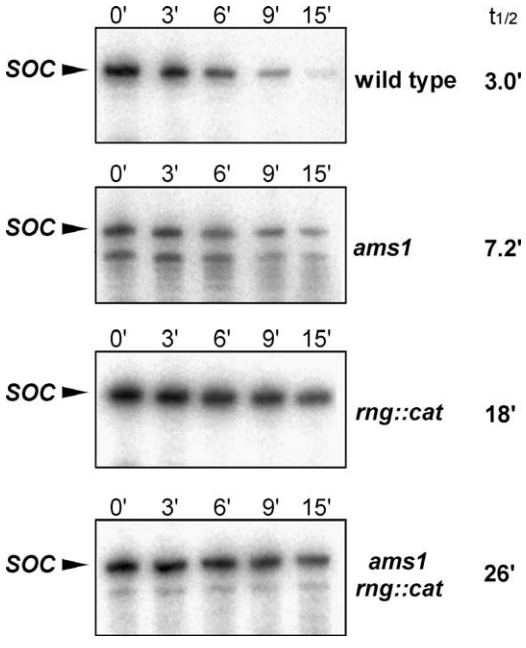

Fig. 4. Effects of RNases E and G on the stability of *soc* mRNA in uninfected cells. Rifampicin was added to GW10, GW20, GW11, or GW21cells harboring pTK44 at time 0. Total RNAs were isolated at the indicated times and 3.5 μg from each were analyzed by Northern blotting with a *soc* probe. Bacterial strains and the half-lives of full-length *soc* transcripts in each strain are shown in the right margin. An arrowhead indicates the full-length *soc* transcript.

The effects of Hfq and PAPI on the stability of host mRNAs after infection

Although *ompA* mRNA is usually stable in exponentially growing cells, it becomes destabilized under certain conditions: when cell culture conditions are shifted from rich in nutrition to poor or from aerobic to anaerobic, the decay rate of *ompA* mRNA increases several fold. Such destabilization requires an RNA chaperon, Hfq (Vytvytska et al., 1998). This protein binds to *ompA* mRNA and interferes with ribosome binding and with translation (Vytvytska et al., 2000). On the other hand, polyadenylation at the 3′-end of *lpp* mRNA promotes its destabilization (Mohanty and Kushner, 1999; O'Hara et al., 1995). Poly(A) polymerase I (PAPI) encoded by *pcnB* accounts for over 90% of polyadenylation in *E. coli* (O'Hara et al., 1995). We examined whether these factors affect the stabilities of *ompA* and *lpp* mRNAs after T4 infection. The half-life of *ompA* mRNA in T4-infected cells was 3.0 min for wild-type MH1 cells and 3.9 min for MH101 cells defective in Hfq. Thus, the stability of *ompA* was not significantly affected by Hfq. The half-life of *lpp* mRNA in T4-infected cells was

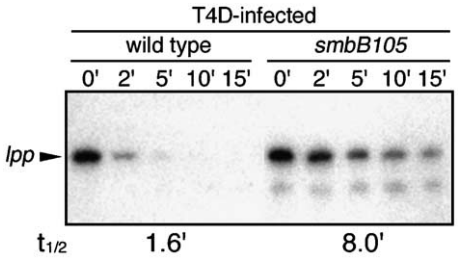

Fig. 5. Effect of the degradosome on the stability of *lpp* mRNA after T4 infection. Cells of SH3208 or its derivative, Bz189, were infected at time 0 with wild-type T4. Total RNAs were isolated at the indicated times and 5 μg from each were analyzed by Northern blotting with a probe for *lpp*. The half-lives of *lpp* full-length transcripts in each strain are shown below the figure. An arrowhead indicates the full-length *lpp* transcript.

almost same regardless of PAPI: 2.5 min for wild-type MRi7 cells and 2.6 min for MRi80 cells with a defect in PAPI. Therefore, these factors, known to be involved in mRNA destabilization in response to host physiological changes, had no effect on the destabilization of mRNAs after T4 infection.

Involvement of a T4 factor in the destabilization of host mRNAs after infection

To investigate whether a T4 gene product is required for the rapid degradation of *lpp* mRNA, we added rifampicin before T4 infection to inhibit the expression of T4 genes, and measured the decay rate of *lpp* mRNA after infection (Fig. 6A). The result showed that the half-life of *lpp* mRNA was 29 min, more than 10-fold longer than the half-life of 2.5 min in the absence of rifampicin and as long as in uninfected cells (Fig. 1). We also observed that prior addition of rifampicin substantially blocked the destabilization of *ompA* mRNA after T4 infection (data not shown). These results strongly suggested that a T4 gene product was involved in the destabilization of *lpp* and *ompA* mRNAs. To seek the T4 gene, we examined five T4 mutants available from our laboratory stock, dK Δ*24.1* (Kai et al., 1998), ΔeG506, ΔNB5060, Δ(*39–56*)11, and Δ*tk*2 (Kutter et al., 1994). We chose these mutants because they have a deletion of multiple genes and unknown orfs in various genomic regions. We found that Δ*tk*2 phage was unable to destabilize *lpp* mRNA, although the other mutants did so as rapidly as wild-type phage (Fig. 6B; data not shown). This result suggests that the 11.3-kb region missing in Δ*tk*2 encodes a key factor for the degradation of *lpp* mRNA in T4-infected cells.

Induction of host mRNA degradation by other phages

The destabilization of host mRNAs may promote a quick shift of gene expression from host to T4 genomes, which undoubtedly gives an advantage for efficient production of phage progeny. Such a strategy might be adopted by other phages. To explore this possibility, we examined phages T2 and T7 for their ability to induce the destabilization of host mRNAs. T2 is closely related to T4, but various genetic variations such as different host ranges, deletions of genomic DNA (Desplats and Krisch, 2003; Kim and Davidson, 1974), and gene displacement (Selick et al., 1993) are discernible between them. For this reason, we included T2 to examine if the destabilization mechanism is conserved. T7 is quite different from T4; no significant homology is found between them in any gene. After infection by T2 or T7, *lpp* and *ompA* mRNAs were degraded rapidly in wild-type cells (Fig. 7). When both RNases E and G were inactivated, these mRNAs were as stable as those in uninfected cells. Thus, like T4, T2 and T7 also have a mechanism for degrading host mRNAs, in which RNases E and G substantially contribute to the degradation.

Discussion

In this study, we showed that the *E. coli lpp* and *ompA* mRNAs, which are very stable in normally growing cells, were drastically destabilized immediately after infection by T4 phage. Considering the fact that host gene expression is rapidly shut off, our observation suggests that most, if not all, host mRNAs are degraded quickly after T4 infection. In contrast, T4 *soc* mRNA that was unstable in uninfected cells was rather stabilized after infection. Thus, the change in an mRNA decay rate after T4 infection was different between host and T4 mRNAs. RNases E and G were major determinants of the destabilization of host and phage mRNAs. These results strongly suggest that the targets of these RNases are changed by T4 infection. Because T4 Δ*tk*2 was not able to induce the destabilization of *lpp* and *ompA* mRNAs, a T4 factor may be involved in the target change of RNases E and G. How can a T4 factor control the host ribonucleases? At present, three possibilities can be raised. The first is that an interaction of the T4 factor with mRNAs leads to their degradation. The second is that host ribonucleases are modified by the T4 factor to alter their selectivity for substrates. According to Marchand et al.

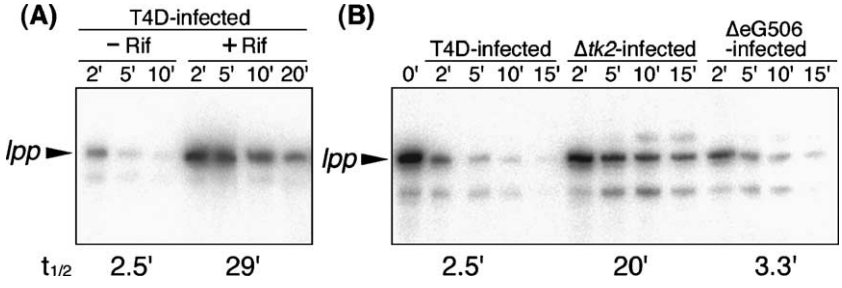

Fig. 6. Effect of a T4 gene product on the stability of *lpp* mRNA after infection. (A) Two cultures of GW10 cells were infected with wild-type T4 at time 0, with (+) or without (−) addition of rifampicin 3 min before infection. Total RNAs were isolated at various times and 4 μg from each were analyzed by Northern blotting with a probe for *lpp*. (B) GW10 cells were grown and infected at time 0 with T4 wild type, Δ*tk*2 mutant or ΔeG506 mutant. Total RNAs were isolated at indicated times and each 2 μg were analyzed as in (A). Half-lives of *lpp* full-length transcripts are shown below the figures. An arrowhead indicates the full-length *lpp* transcript.

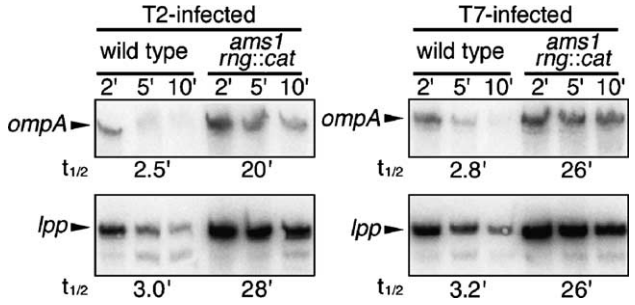

Fig. 7. Destabilization of *E. coli* mRNAs by infection with phages T2 and T7. GW10 or GW21 cells were infected at time 0 with T2 or T7. Total RNAs were prepared at indicated times and 5 μg from each were analyzed by Northern blotting with a mixture of probes for *lpp* and *ompA*. Half-lives of *lpp* and *ompA* full-length transcripts are shown below the figures.

(2001), T7 PK protein encoded by gene *0.7* phosphorylates RNase E in the C-terminal half. The phosphorylation reduces the decay rate of T7 mRNAs but does not block RNA cleaving activity for the maturation of 9S rRNA. Similarly, T4 also might modify RNases E and G to untarget *soc* mRNA and, instead, target host mRNAs. The third possibility is that the T4 factor changes the preference of translation. T4 infection modifies the host translation machinery so as to impair host mRNA translation (Dube and Rudland, 1970). RNase E favors mRNA for cleavage, which is not actively translated (Baker and Mackie, 2003). Consequently, RNase E may change its target. RNase G could behave similarly to RNase E.

T4 Δ*tk*2 was unable to destabilize host mRNAs. This phage lacks a 11.3-kb genomic region (TK2 region) containing 21 orfs from *nrdC.2* to *tk*. At least one of the orfs may encode a protein that plays an essential role in the regulation of RNases E and G. Except for *rI* and *tk*, however, there is no gene of known function in the TK2 region (Miller et al., 2003). Because four early promoters lie in this region, transcription of the unknown orfs could be initiated from them. Hence, the products of these orfs are possibly expressed immediately after infection and promote the destabilization of host mRNAs, which starts a few minutes after T4 infection. Because both T2 and T4 phages have the mechanism of host mRNA degradation, this mechanism would be conserved among T4-related phages. Although TK2 region is highly variable, one orf in the region, *nrdC.11*, is conserved in six T4-related phage genomes posted at http://www.phage.bioc.tulane.edu/. This orf could be responsible for the destabilization of the host mRNAs. Interestingly, the N-terminal 120 residues of NrdC.11 contain a conserved domain of predicted nucleotidyltransferase.

The half-life of *soc* mRNA increased twofold immediately after infection and fivefold at late stages compared uninfected cells. Because *soc* is normally expressed at late stages of infection, it should be noted that *soc* mRNA is most stabilized at the proper stages. There are four classes of mRNAs in T4-infected cells, *E. coli* mRNAs, early T4 mRNAs, middle T4 mRNAs, and late T4 mRNAs including *soc* mRNA. Our previous work revealed that the stabilities of middle and late mRNAs are differently controlled (Ueno and Yonesaki, 2001). Together with the observations in this study, we may surmise that the stabilities of all four classes of mRNAs are discriminated and differently controlled.

A quick shift of gene expression from host to phage genomes would be especially important for virulent phages to sustain a maximal production of progeny in a minimal time. Previously, we found that when T4-infected cells in which mRNA degradation had been impaired by a defect of RNase E, the transcription of T4 genes was apparently impaired (Otsuka et al., 2003). This effect could be explained by reduction of the ribonucleotide pool upon T4 infection. The generation of ribonucleotides by degrading unnecessary mRNA would assist the efficient transcription of phage genes. Thus, the destabilization of host mRNAs immediately after infection may promote the quick shift of gene expression. A similar strategy is also adopted by animal viruses such as herpes simplex virus. After infection, the virion host shutoff protein destabilizes all mRNAs to facilitate the shift from host to viral gene expression (Everly et al., 2002). In the present study, we showed that RNases E and G contributed considerably to the destabilization of host mRNAs by T2, T4, and T7. Because we found no similarity between T4 genes in TK2 region and any of T7 genes, the responsible genes of T4 and T7 may not be related. In this connection, it would be worth noting that RraA, Hfq, and T7 PK, the known modulators of RNase E activity, have no homology to each other and to the proteins encoded by genes in T4 TK2 region. In fact, the modulators control the RNase E activity in quite different ways. As noted above, T7 PK phosphorylates RNase E. RraA binds to RNase E and inhibits RNase E endonucleolytic cleavages (Lee et al., 2003). Hfq stimulates the RNase E cleavage of *ompA* mRNA by interfering with ribosome binding and with translation (Vytvytska et al., 2000). Similarly, T4 and T7 might modulate RNases E and G by different mechanisms. Genes that encode homologs of *E. coli* RNases E or G are conserved throughout prokaryote phylogeny (Aravind and Koonin, 2001). Therefore, host mRNA destabilization by these RNases could be widely used by other virulent phages with host ranges other than *E. coli*, although the mechanisms might be diverged.

Materials and methods

Bacterial strains and phages; growth and infection conditions

Wild-type T4D and *nd28* (*denA*$^-$) Δ*rII-denB* (ΔHTP8) were laboratory stocks. dK Δ*24.1* was described previously (Kai et al., 1998). Δ(*39–56*)11 and Δ*tk*2 were kindly provided by Dr. H. Takahashi at the University of Tokyo.

ΔeG506 was kindly provided by Dr. L. W. Black at the University of Maryland Medical School. ΔNB5060 was isolated by crossing wild type and GT7 (Wilson et al., 1979). To construct Δ*regB-denV*, successive PCRs were performed. In the first PCR, two primers, 5′-cgtattctcgctcttcctgg [complementary to T4 nucleotides 65555–65574 (GenBank accession no. NC_000866)] and 5′-gcttacctcattgagcctgtagttgatagg (T4 nucleotides 62272–62286 connected with 65367–65381), were used for amplifying a fragment with T4 DNA as a template. The amplified fragment and 5′-actccgccagagctttcttg (T4 nucleotides 62051–62070) were used for the second PCR with T4 DNA as a template. The fragment thus obtained encompassed a sequence from 62051 to 65574 with a deletion of *regB*, *vs.3–vs.8*, and *denV* from 62287 to 65366 in the T4 sequence. This sequence context was transferred to the T4 genome with the insertion/substitution system (Selick et al., 1988). Δ*soc* was constructed as follows: The *soc* coding region was deleted from pTK44 (Otsuka et al., 2003) using PCR and a primer for the deletion, 5′-catgtaatttaaataataactcaaggac, and the deletion was transferred to the T4 genome with the insertion/substitution system. T2 phage was a laboratory stock. T7 phage was kindly provided by Dr. K. Shimizu at Osaka University. The *E. coli* K-12 strains are listed in Table 1. MH101 was constructed by GT7-mediated transduction (Wilson et al., 1979) of the *hfq*::*cat* mutation (Wachi et al., 1999) into MH1. Cells were grown at 30 °C to a density of 3×10^8 cells/ml in M9 minimal medium supplemented with 0.3% casamino acids, 1 μg/ml thiamine, and 20 μg/ml tryptophan. In all the experiments except for Fig. 1, cells were shifted to 41 °C for 20 min before use. This temperature is nonpermissive for the temperature-sensitive RNase E in GW20 cells. When cells were infected with phage, the multiplicity of infection was 7. When rifampicin was used to block transcription, it was added to a final concentration of 200 μg/ml.

Determination of the region lacking in Δtk2 phage

Based on the map position of the deletion in Δ*tk2* (Kutter et al., 1994), we prepared two primers, 5′-gtgttcattataataagc complementary to a region in *tk* and 5′-cttaagcctgctattgatac complementary to a region in *nrdC.1*. These two primers are 11.7 kb apart in the T4 sequence. When Δ*tk2* phage DNA was used as a template, PCR with the primers yielded only one prominent band, corresponding to 340 bp, upon polyacrylamide gel electrophoresis. Because this fragment was not produced with wild-type T4 DNA as a template, the production of the short fragment could be attributable to a deletion in Δ*tk2*. The 340-bp fragment was recovered from the gel and subjected to sequencing. The sequence context clearly showed that the nucleotide at 48709 in the T4 sequence was connected with that at 60052, indicating a deletion of 11.3 kb from 48710 to 60051 in Δ*tk2*.

Table 1
Bacterial strains

Strain	Genotype	Source
GW10	*zce-726*::*Tn10*	Wachi et al., 1997
GW11	same as GW10 except for *rng*::*cat*	Wachi et al., 1997
GW20	same as GW10 except for *ams1*	Wachi et al., 1997
GW21	same as GW10 except for *ams1 rng*::*cat*	Wachi et al., 1997
MH1	*araD139 galU galK hsdR* Δ*lacX74 rpsL*	Kai et al., 1996
MH101	same as MH1 except for *hfq*::*cat*	This study
MRi7	*rpsL150* (strR)	Lopilato et al., 1986
MRi80	same as MRi7 except for *pcnB80*	Lopilato et al., 1986
SH3208	wild type	Kido et al., 1996
Bz189	same as SH3207 except for *smbB105*	Kido et al., 1996

Northern blot analysis

RNAs were extracted as described previously (Kai et al., 1996) and Northern blotting was performed as described (Ueno and Yonesaki, 2001). A radioactive probe for each gene transcript was prepared by PCR using one primer 5′-end-labeled with T4 kinase and [γ-^{32}P]ATP (Institute of Isotopes of the Hungarian Academy of Sciences, Hungary, 259 TBq/mmol) and another unlabeled primer. Probes for *lpp*, *ompA*, and *soc* genes were amplified by PCR with the sets of primers as follows; for *lpp*, 5′-[^{32}P]ttacttgcggtatttagtag and 5′-gaaagctactaaactggtac; for *ompA*, 5′-[^{32}P]ttggatttagtgtctgcacg and 5′-atgaaaaagacagctatcgc; for *soc*, 5′-[^{32}P]cctgcagtactctcctctata and 5′-cctgcagtaacaagttcggctc. Template DNA was previously prepared by PCR using the above sets of primers with either *E. coli* MH1 DNA for *lpp* and *ompA* or T4 DNA for *soc* as a template. The labeled probes were heat-denatured immediately before use. After an autoradiograph had been taken with a Bio-Image Analyzer, the signal intensity was quantified with the NIH image program. Relative intensities were plotted semilogarithmically and the time required for a 50% reduction was taken as a half-life of mRNA. In all experiments, we performed Northern blot analysis at least two times and similar results were obtained for each measurement. A representative result was shown in each figure.

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