Abstract of Thesis

Name (Qi Dan)	
Title	T4 Srd accelerates *E. coli* mRNA degradation via stimulating the activity of host RNase E (T4 Srd は宿主RNase Eの活性を促進することにより大腸菌mRNAの分解を加速する)

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*. RNase E is a protein consisting of two domains: highly conserved N-terminal catalytic half (NTH) and C-terminal scaffold half (CTH). Three components are recruited and bind to the CTH to form a complex called RNA degradosome. 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 *lpp* and *ompA* mRNAs, otherwise stable in uninfected cells. 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.

First, comparison of the amount of RNase E before and after T4 infection using western blotting showed no change, suggesting involvement of other factor(s). T4 infection may cause a damage of *E. coli* membrane through T4 DNA injection, implying the envelope stress response (ESR) system that raises degradation of *lpp* and *ompA* mRNAs by recruiting RybB and MicA, respectively, small RNA-mediated RNase E cleavage. However, deletion of these small RNA showed no effect on T4-induced host mRNA degradation. This result leaded to explore another hypothesis that T4 factor(s) activate RNase E activity. After checking some T4 mutants with a deletion of multiple genes, I found that *srd* with unknown function was involved in T4-induced host mRNA degradation. The rapid degradation of host *ompA* and *lpp* mRNAs was partially

alleviated and a decay intermediate of *lpp* mRNA remarkably accumulated in cells infected with T4 Δ*srd* phage. Exogenous expression of Srd in uninfected cells impairs the cell growth and accelerated the decay of long-lived *lpp* and *ompA* mRNAs and a short-lived *trxA* mRNA. In addition, *lpp*(T) RNA, which had a sequence identical to the decay intermediate of *lpp* mRNA, was also considerably 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 or by 5'-end-dependent mechanism. The latter mechanism requires removal of a pyrophosphate from triphosphate at the 5'-end of the transcript by RNA pyrophosphohydrolase (RppH) prior to RNase E cleavage. *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 analyses demonstrated that Srd physically associated with the N-terminal half of RNase E containing the 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 phage growth.

To date, only three *E. coli* factors were identified as negative regulators of RNase E, and in most cases they function under specific stress conditions. On the other hand, Srd may be the first example of a general positive regulator of RNase E activity. In future, investigation in vitro to gain further insight into the regulation mechanism of Srd should be quite valuable to understand the control of RNase E function.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

mRNA 量の調節は遺伝子発現調節において最も重要な要素であり、転写調節と mRNA 分解の調節によって行われる。転写調節については詳細な知見が蓄積されてきたが、mRNA 分解調節は研究の歴史が浅く不明な点が多い。mRNA 分解の主役として機能する RNase はいくつかの生物で同定されているが、その中でも大腸菌の RNase E は最も活発に研究されている。申請者は T4 ファージ感染後に著しく高まる宿主 mRNA の分解が宿主の RNase E によることに注目して解析したところ、この現象が RNase E 量の変化を伴わない活性上昇であることを確認した。このことから RNase E の活性を促進する因子の存在が予想されたので、宿主因子と T4 ファージ因子のそれぞれの可能性について検討したところ、T4 ファージ因子としてそれまで機能未知であった Srd を同定した。この因子を非感染大腸菌で発現させたところ、RNase E に依存して宿主 mRNA の分解速度が高まったことから、Srd は単独で RNase E 活性促進機能を示すことがわかった。RNase E は mRNA 分子の内部領域を直接切断する機構と 5' 末端のーリン酸に依存して切断する機構を有することが知られているが、申請者は Srd により両方の機構が促進されることを見出した。また、RNase E は N 末側に活性ドメイン、C 末側に RhlB ヘリケースや Polynuceotide phosphorylase などと複合体を形成する足場ドメインを有するが、申請者は Srd が N 末側ドメインに結合することを明らかにするとともに、RNase E の活性促進効果は N 末側ドメインのみに対しても生じることを確認した。これらのことから、Srd は RNase E 活性を正に調節する因子であることが明らかとなった。RNase E 活性を正に調節する因子はこれまで知られていなかったことから、Srd が最初の例となった。さらに、申請者は Srd が T4 ファージの効率良い増殖に必須であることも示した。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。