Control of persistent infection of bacteria by two-component regulatory systems: EnvZ-OmpR-mediated reduction of pathogenicity in Escherichia coli

Dissertation Abstract
（学位論文要旨）

Control of persistent infection of bacteria by two-component regulatory systems: EnvZ-OmpR-mediated reduction of pathogenicity in *Escherichia coli*
（二成分制御系による細菌感染の持続性の調節：EnvZ-OmpR を介した大腸菌病原性の減少）

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Abstract

Bacteria adapt to environmental changes by altering gene expression patterns with the aid of signal transduction machinery called the two-component regulatory system (TCS), which consists of the sensor kinase and response regulator. I examined the role of the TCS in bacterial adaptation to host environments using genetically tractable organisms, *Escherichia coli* as a pathogen and *Drosophila melanogaster* as a host. I first determined the strength of the transcription promoters of TCS-encoding genes in adult flies by abdominally injecting *E. coli* that harbored plasmid for the expression of green fluorescent protein driven by the promoters of genes coding for 28 sensor kinases and 33 response regulators followed by the measurement of fluorescence intensities. I chose five TCS among those encoded by genes having relatively active promoters and analyzed them for the effect on bacterial pathogenicity to *Drosophila*. Mutant *E. coli* strains lacking EnvZ-OmpR, QseC-QseB, and NarQ-NarP showed higher pathogenicity than the parental strain while the lack of PhoQ-PhoP made *E. coli* less virulent, and EvgS-EvgA did not seem to influence bacterial virulence. I then further characterized EnvZ-OmpR: the forced expression of *envZ* and *ompR* in the mutant strain lowered its pathogenicity; the mRNA of EnvZ and OmpR were detectable in infected flies; and there was no difference in growth rate *in vitro* and in the level of colony-formable *E. coli* in flies between the parental and mutant bacteria. Furthermore, host immunity, either the humoral or cellular response, seemed unrelated to the actions of EnvZ-OmpR in the control of *E. coli* virulence. These results collectively indicated that EnvZ-OmpR mitigates the virulence of *E. coli* in *Drosophila* by a mechanism not accompanied by a change of bacterial burden in the host. I claim this behavior of *E. coli* to be a bacterial strategy to achieve persistent infection.

Introduction

As bacteria reside in various places such as in the air, soil, water and living organisms, they need to adapt themselves to changes in environmental conditions that are often hostile to their survival. Bacteria recognize new environments and change their structure, metabolism, and motility for adaptation. This is mostly achieved through the alteration of gene expression. Among machineries controlling gene expression in bacteria is the two-component regulatory system (TCS) that consists of two protein components, the sensor kinase and response regulator. Sensor kinases residing in the cell membrane recognize environmental changes and report the incidence to cytoplasmic response regulators. Upon receiving external stimuli, sensor kinases undergo autophosphorylation at histidine residues and subsequently transfer the phosphates to the aspartate residues of response regulators. Phosphorylated response regulators become able to bind to *cis*-acting DNA sequences and induce, or sometimes inhibit, the transcription of a variety of genes. As a consequence, kinds and concentrations of proteins in bacteria change for the adaptation to new environmental conditions.

It is most probable that bacteria enter the host seeking for nutrients, temperature, humidity, etc. suitable for their survival and proliferation. In contrast, bacterial infection is unfavorable to host organisms, with an exception of commensal bacteria residing in the digestive tract. The host organism is therefore equipped with immunity that attacks and eliminates invading bacteria to prevent the development of infectious diseases. Bacteria, on the other hand, possess a variety of ways to evade immune responses of the host, but it remains to be clarified how bacteria gain such a strategy. I anticipated the involvement of the TCS and pursued this study to identify and characterize the TCS responsible for the survival and persistent infection of bacteria. It is widely appreciated that the fundamental mechanism of immunity evoked against invading microbial pathogens is common among

species from the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* to mice and humans. The use of *Drosophila* provides the advantage that genetically tractable experiments are feasible using whole animals infected with microorganisms. In this study, I adopted *Escherichia coli* as a model bacterium and *Drosophila* as a model host because a genetic approach is applicable to both organisms in tackling the above-described issues.

Results

There appear to exist 30 sensor kinases and 34 response regulators in terms of the analysis of *E. coli* genome. I first determined which TCS are more expressed than others in *E. coli* after infecting host organisms. For this purpose, I measured the promoter strength of genes coding for the components of the *E. coli* TCS, 28 sensor kinases and 33 response regulators. Male adult flies received an abdominal injection with *E. coli* harboring plasmids that expressed green fluorescent protein (GFP) driven by the transcription promoters of *E. coli* genes. One hour after the injection, the flies were examined under a fluorescence microscope for the level of fluorescence intensities derived from GFP. The results suggested that genes coding for the components of *E. coli* TCS were differentially expressed in adult flies, and the activity of the promoter was not always consistent between genes encoding the sensor kinase and response regulator that constitute functional TCS. I chose five TCS, i.e., EnvZ-OmpR, QseC-QseB, NarQ-NarP, EvgS-EvgA, and PhoQ-PhoP for further analyses because these appeared to be expressed at relatively high levels in *E. coli* injected into flies, and the downstream genes they activate have been known.

I then examined the pathogenicity of *E. coli* with mutations on genes coding for the five TCS. I found that the loss of EnvZ-OmpR, QseC-QseB, and NarQ-NarP made *E. coli* more virulent than the parental strain (Figure 1). In contrast, *E. coli* with mutation on PhoQ-PhoP-encoding genes killed less flies than did the parental strain, and EvgS-EvgA did not seem to influence the virulence of *E. coli*. These results suggested that EnvZ-OmpR, QseC-QseB, and NarQ-NarP act to reduce the pathogenicity while PhoQ-PhoP contributes to the maintenance of virulence. I continued to analyze EnvZ-OmpR as a representative of the TCS that mitigates the virulence of *E. coli* in *Drosophila*. To confirm a decrease in the virulence of *E. coli* after the loss of EnvZ-OmpR, I conducted a gene complementation experiment in which both *envZ* and *ompR* were forcedly expressed in the *envZ-ompR* mutant (Δ*envZ-ompR*). The successful expression of *envZ* and *ompR* in Δ*envZ-ompR* was shown by the determination of the mRNA of EnvZ and OmpR. The results in a bacterial pathogenicity assay showed that the forced expression of *envZ* and *ompR* reduced the virulence of Δ*envZ-ompR* to the level of the parental strain. From these results, I concluded that EnvZ-OmpR plays a role in reducing the virulence of *E. coli* to *Drosophila*.

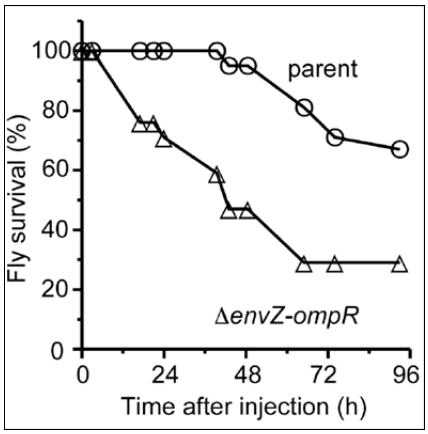

Figure 1. Increase of *E. coli* virulence to adult flies by loss of EnvZ-OmpR.

To clarify the actions of EnvZ-OmpR in the control of *E. coli* virulence, I first determined the growth rate of Δ*envZ-ompR* in Luria-Bertani liquid medium in comparison with the parental strain and found no significant differences between the two *E. coli* strains. I next examined if the absence of EnvZ-OmpR caused a difference in bacterial burden in *Drosophila*. Adult flies were injected with either Δ*envZ-ompR* or the parental strain, and the level of

colony-formable bacteria existing in flies was determined. The results showed that there was only a marginal difference between the two strains: the number of colony-formable *E. coli* remained almost the same for 3 days after injection (Figure 2), suggesting that increased virulence after the loss of EnvZ-OmpR was not due to an increase in the level of bacterial burden in flies. I finally examined a possible relationship between the actions of EnvZ-OmpR and host immunity. The involvement of a humoral response was first tested using *imd*[1] flies, a fly line defective in the Imd-mediated production of antimicrobial peptides. Δ*envZ-ompR* was more pathogenic to *imd*[1] than the parental strain, as observed in the experiment using the wild-type flies. Next, flies lacking hemocytes were generated by inducing apoptosis specifically in hemocyte and used as the host for *E. coli* infection. The results indicated that the loss of EnvZ-OmpR made *E. coli* more virulent even to flies that had no hemocytes. These results collectively suggested that host immunity is not involved in the EnvZ-OmpR-mediated control of *E. coli* virulence.

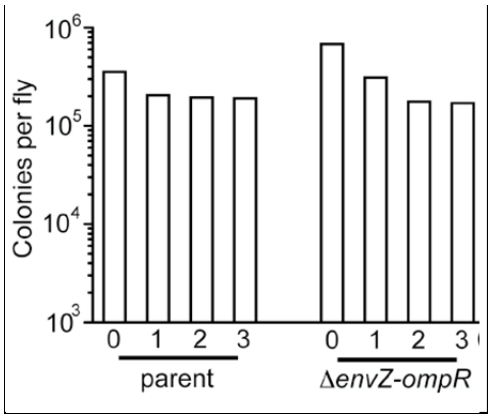

Figure 2. No change of bacterial burden in adult flies by loss of EnvZ-OmpR.
The numerals indicate days after infection.

Discussion

Among the TCS-encoding genes with relatively active promoters, I chose EnvZ-OmpR, QseC-QseB, NarQ-NarP, EvgS-EvgA, and PhoQ-PhoP, and analyzed them for the involvement in the virulence of *E. coli* to adult flies. An assay for fly deaths after the abdominal injection of *E. coli* showed the possibility that PhoQ-PhoP is necessary for *E. coli* virulence while EnvZ-OmpR, QseC-QseB, and NarQ-NarP act to decrease the pathogenic effect of *E. coli* in *Drosophila*. I took an interest in the latter TCS because the reduction in the level of virulence might help bacteria to adapt to and get along with host environments. I further characterized EnvZ-OmpR that had been more intensively studied than the others. The data showed that *E. coli* injected into adult flies expressed the mRNA of both EnvZ and OmpR, and that the forced expression of *envZ* and *ompR* returned the level of virulence of *E. coli* lacking EnvZ-OmpR down to the level seen for the parental bacteria. From these results, I concluded that EnvZ-OmpR acts to mitigate the pathogenic effect of *E. coli* in *Drosophila*.

There are preceding studies in which a similar approach was taken to examine the involvement of the TCS in the virulence of a variety of bacterial species to the host organisms. Most TCS analyzed so far were positively involved in the virulence of bacteria. However, the findings in my study were different: EnvZ-OmpR appeared to function to decrease the virulence of *E. coli*. I have interpreted this phenomenon as a host-pathogen interaction for both organisms to survive. It is hard at present to explain the mode of EnvZ-OmpR action because the loss of this TCS did not bring about a change in bacterial burden in flies as well as the susceptibility of bacteria to host immunity. EnvZ-OmpR recognizes a change in osmolarity and subsequently alters the level of transcription of over a dozen genes. Such downstream genes include those coding for proteins involved in the synthesis of curli and flagella. I speculate that EnvZ-OmpR reduces the virulence of *E. coli* by altering such extracellular structures. It is of importance to identify and characterize the genes located downstream of EnvZ-OmpR that are responsible for the reduction of *E. coli* virulence. In addition, the characterization of QseC-QseB and NarQ-NarP, which are apparently involved in the pathogenicity of *E. coli* in a way similar to EnvZ-OmpR, will be necessary for gaining

an overview of the TCS regulation of bacterial virulence.

Conclusion

EnvZ-OmpR, a two-component regulatory system, functions to mitigate the virulence of *E. coli* in *Drosophila*, most likely for the persistent infection of bacteria.

学位論文審査結果の要旨

審査委員による査読と公開での発表・討論の結果に基づき、標記学位論文は以下のように判定された。

本論文は、病原性発揮に関わる細菌遺伝子の同定と機能解析に関する研究を記述したものである。二成分制御系とよばれる情報伝達系は、細菌の環境適応において主たる役割を果たす。論文提出者は、ショウジョウバエをモデル宿主として二成分制御系の細菌病原性への関与を検証した。プッカレイ氏はまず、大腸菌二成分制御系をコードする 60 余種の遺伝子の転写プロモーター活性をショウジョウバエ内で調べた。そして、比較的強いプロモーターを持つ 5 種類の二成分制御系に着目して、それらの欠損菌の病原性を検討した。その結果、3 つの変異菌で病原性が増大し、1 つでは低下し、そして残り 1 つでは変化しなかった。続いて、欠損が強病原性をもたらした 3 種の二成分制御系のうち EnvZ-OmpR について解析を進めた。まず、EnvZ と OmpR の mRNA 発現はショウジョウバエに注入した大腸菌においても確認された。また、EnvZ-OmpR 欠損菌に両遺伝子を強制発現させると、病原性が野生型菌の程度まで低下した。さらに、EnvZ-OmpR 欠損は宿主内での細菌量に変化を及ぼさず、また欠損による高病原性は免疫を低下させた宿主においても観察された。以上より、EnvZ-OmpR は、宿主内での細菌量を変化させることなく、また宿主免疫に関わることなく、大腸菌の病原性を低下させる役割を担うと考察された。

細菌病原性を低下させる二成分制御系の初めての同定を記述する本論文は、免疫学や微生物学の発展に寄与すると評価され、博士（理学）の学位に値すると判定された。