博士論文（要約）

ユビキチン E3 リガーゼ CBL-C による腸管病原性大腸菌の

病原性抑制作用に関する研究

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Establishment of infection by enteric bacterial pathogens are contingent on the performance of the bacteria such as colonization, replication and dissemination in the host. While Pathogens like enteropathogenic and enterohaemorrhagic *Escherichia coli* (EPEC and EHEC, respectively) use a type Ⅲ secretion system to translocate virulence effector proteins into host cells during infection for enhancing colonization and manipulating antimicrobial host downstream signal, The host use their ability of post-translational protein modification such as ubiquitylation (Protein degradation system) for antimicrobial defence. Here, we report that host E3 ubiquitin ligase , CBL-C binds to the T3SS effector Tir (Translocated intimin receptor) from EPEC and it leads to Tir degradation. Protein interaction studies shows us that CBL-C is binding partner of Tir by phosphorylation dependent manner. Ring-finger type E3 ligase activity of CBL-C ubiquitinated Tir with L48-linked polyubiquitination and leads to Tir degradation by Ubiquitin-Proteasome System. An *in vitro* kinase assay indicated that Tir Tyr 454 was the major phosphorylation site by FYN, one of the Src family kinases, in Tir, and its phosphorylation was required for the interaction of Tir with CBL-C. The ubiquitination and degradation of Tir depends on Tir tyrosine 454 phosphorylation site which its Y454F mutant shows us unefficient ubiquitination and delayed degradation in the cell lines. The unique physiological role of CBL-C in Tir degradation demonstrated by using CBL-C silencing cell lines which showed that no significant different ubiquitination, degradation, pedestal structure and colonization level between WT Tir and Tir Y454F. EPEC-like mouse pathogen *Citrobacter rodentium* infection model shows that *C. rodentium* Δ*tir*/*tir* WT Y451F which has homology with EPEC Δ*tir*/*tir* TirY454F, increased colonization and death rate. These data demonstrate that Tyr-phosphorylation at EPEC Tir Tyr454/*C. rodentium* Tir Tyr 451 is critical for interaction with host CBL-C by which it down regulates bacterial attachment to host cells.

In this study, we investigated the fate of Tir in EPEC infected host cells, and provided *in vitro* and *in vivo* evidence that EPEC/*C. rodentium* delivers Tir proteins into host cells, as bacterial receptors. We also demonstrated that these Tir proteins were phosphorylated at Tyr 454 of EPEC/Tyr 451 of *C. rodentium* by host FYN, and targeted by host CBL-C and degraded by UPS. Importantly this host activity greatly contributed to diminished bacterial colonization of the colonic epithelium, where CBL-C played a critical role. Compared to the other CBL kinase family members CBL and CBL-B, CBL-C lacks an ubiquitin-associated (UBA) domain and is also distinct in its length and amino acid sequence. Although gene expression patterns have previously revealed that *CBLC* localized within intestinal epithelial cells while *CBLB* and *CBL* were ubiquitously expressed, functions of CBL-C *in vivo* have been poorly investigated. Our findings shed light on the novel biological function of CBL-C as a host defense mechanism against enteric pathogen like EPEC.