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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

食中毒原因菌である腸炎ビブリオの3型分泌装置(T3SS2)は、下痢原性に必須である。T3SSの最も重要な機能は、エフェクターと総称される細菌由来の機能性タンパク質を直接宿主細胞に注入することである。T3SSがエフェクターを効率よく標的細胞に注入するには、細菌が宿主細胞との接触を認識し、細胞との接触に応じて分泌タンパク質の量を制御する必要があると考えられる。しかしながら、T3SS2の分泌制御機構は全く不明であった。本研究ではまず、T3SS2の分泌制御を担う分子の同定に成功した。また、本菌が宿主細胞内の高カリウムイオン濃度を感知することで宿主細胞との接触を認識し、T3SS2のタンパク分泌スイッチの切換を行っていることを明らかにした。さらに、この機構はT3SS2がエフェクターを効率よく宿主細胞に注入し、病原性を発揮するために必要であった。これらの知見は、病原細菌による新たな宿主細胞認識機構を提唱するものであり、博士（医学）の学位授与に値する。

論　文　内　容　の　要　旨
Synopsis of Thesis

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論文題名 Title	*Vibrio parahaemolyticus* senses intracellular K^+ to translocate T3SS2 effectors effectively (腸炎ビブリオのT3SS2は宿主細胞内の高カリウムイオン濃度を感知することで、効率的にエフェクターを注入する)

論文内容の要旨

〔目　的(Purpose)〕

Many Gram-negative symbionts and pathogens utilize a type III secretion system (T3SS) for their benefit and/or pathogenesis. The T3SS is a sophisticated secretion system for direct delivery of effectors into the host cell cytosol. For the efficient translocation of effectors, T3SS substrate secretion is generally divided into three phases, which are regulated by specific components in a specific order. First, extracellular secretion of needle protein (the early substrate) leads to the formation of tube-like structures. When the needle of a bacterium reaches an appropriate length, molecular ruler proteins switch secretion to the second phase. Translocators are the middle substrates: they localize at the tip of the T3SS needle and form a pore in the host plasma membrane to create a pathway for the effectors. Bacteria promote to secrete the late substrates, effectors, after the organism contacts the host cells to achieve the most efficient translocation. The switching of T3SS secretion from the middle (translocators) to the late substrates (effectors) is controlled by a "gatekeeper" protein.

V. parahaemolyticus is a causative agent of food poisoning worldwide. Most clinical isolates from patients with diarrhea possess two sets of T3SS gene, one set on each chromosome. A number of reports indicate that T3SS2, which is encoded in an 80-kb pathogenicity island called Vp-PAI, is essential for enterotoxicity in animal infection models. T3SS2 is considered to be a major virulence factor for enteropathogenicity of this bacterium. However, the precise mechanism of action of T3SS2 remains unknown because there are many functionally uncharacterized genes encoded in Vp-PAI. In this study, we have characterized two hypothetical proteins in Vp-PAI as gatekeeper proteins of T3SS2. In addition, we have identified a host factor that switches T3SS2 secretion from the middle to late substrates.

〔方法ならびに成績(Methods/Results)〕

We show that the protein secretion switch of *Vibrio parahaemolyticus* T3SS2, which is a main contributor to the enteropathogenicity of a food-poisoning bacterium, is regulated by two gatekeeper proteins, VgpA and VgpB. In the absence of these gatekeepers, effector secretion was activated, but translocator secretion was abolished, causing the loss of virulence. We found that K^+ concentrations, which are high inside the host cell but low outside, are a key factor for VgpA- and VgpB-mediated secretion switching. Exposure of wild-type bacteria to K^+ ion provoked both gatekeeper and effector secretions but reduced the level of secretion of translocators. The secretion protein profile of wild-type bacteria cultured under K^+ conditions was similar to that of gatekeeper mutants. Furthermore, depletion of K^+ ions in host cells diminished the efficiency of T3SS2 effector translocation and the T3SS2-depend biological activities.

〔総　括(Conclusion)〕

VgpA and VgpB play a role as gatekeepers of T3SS2, which regulate the secretion in the free-living state by promotion secretion of translocators and suppression of effector secretion. Furthermore, intracellular K^+ concentration is a critical host factor for converting T3SS2 substrate secretion from translocators to effectors when infection of host cells. Also, this signal transduction facilitates the active translocation of T3SS2 effectors into host cells.