Distinct roles of the alternative sigma factor σH in Listeria monocytogenes

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論文の内容の要旨
Abstract of thesis

In this thesis, the author aimed to examine the role of the alternative sigma factor H (σ^H) in *L. monocytogenes*. σ^H is widely distributed in the *Firmicutes* group of Gram-positive bacteria and is known for being the main regulator of sporulation and genetic competence. However, *L. monocytogenes* is a non-spore forming species in which competence has not been detected. *L. monocytogenes* lacks most of the genes required to form spores but it has a series of homologous genes that form the DNA-uptake machinery (*comG, comE* and *comF* operons). Attempts to detect natural transformation in *L. monocytogenes* have failed so far, but the efforts have only focused on the ComK driven competence (from *B. subtilis* model). σ^H does not have a defined role yet, but some of the competence-related genes have been reported to participate in *L. monocytogenes* phagosomal escape. The author hypothesized that *L. monocytogenes* σ^H may act as the regulator of the DNA-uptake machinery and induce the development of competence for transformation.

The author used a combination of overexpressing and deletion strains of σ^H and the transcription factor ComK to investigate the role of σ^H in *L. monocytogenes*. She evaluated the contribution of σ^H to genetic competence (common role among non-sporulating *Firmicutes*) by gene expression and reporter assays of the DNA-uptake machinery genes and transformation experiments. Finally, she tested the effect of a *sigH* mutant during intracellular survival (an important facet of *L. monocytogenes* life style) by infection of macrophages and epithelial cells.

She performed gene expression analysis to show a unique regulation scheme in which σ^H and the transcription factor ComK were involved in the regulation of the DNA-uptake machinery genes. She found that σ^H and ComK induces the expression of the *comG* operon and that σ^H was essential for *comEA* expression but had no effect on *comEC* and the *comF* operon. She also showed that both regulators induced the expression at a subpopulation level.

She detected synthetic transformation for the first time in *L. monocytogenes.* She achieved transfer of an extracellular plasmid by the artificial overexpression of ComK, and unexpectedly detected transformants only in the absence of σ^H (deletion mutant). She found that σ^H was not required for transformation but rather its presence inhibited the import of extracellular DNA.

L. monocytogenes is an intracellular pathogen and the ComK-mediated activation of some of the DNA-uptake machinery (*comG* and *comEC*) genes was previously shown to contribute to optimal phagosomal escape. In this thesis, the author found that σ^H was essential for phagosomal escape in phagocytic and non-phagocytic cells and that the suppressive effect caused by the deletion of σ^H could not be compensated by ComK and was independent of the activity of virulence factors. Therefore, she concluded that σ^H should regulate phagosomal escape by a novel mechanism.

The σ^H factor in *L. monocytogenes* can induce the expression of the DNA-uptake machinery genes, but it is not required for synthetic transformation. Moreover, it seems to negatively regulate the process. The author's gene expression analysis showed that σ^H is essential for the DNA-receptor (ComEA) expression, and therefore, she suggests that in *L. monocytogenes,* ComEA possibly binds to the extracellular DNA but instead of bringing it to the channel it prevents its access, which would explain why the deletion of *sigH* is required for transformation. She also suggests that the observed negative effect of σ^H may also be caused by the induction of an nc-RNA that has been showed to bind to mRNA and hide the SD region of some of the DNA-uptake machinery genes.

L. monocytogenes σ^H is essential for intracellular survival. A recent report showed that a σ^H deletion mutant is affected during growth in minimal medium, which was attributed to a deficiency in the acquisition or utilization of nutrients. The author concluded that the observed impaired intracellular growth might be attributed to a broad physiological role and suggests that σ^H possibly regulates phagosomal escape by a novel mechanism since the deletion of *sigH* had no effect on the activity of virulence factors.

審査の結果の要旨
Abstract of assessment result

（批評 General Comments）

The author first showed that *L. monocytogene* is capable of DNA transformation, and then analyzed the role of the alternative sigma factor H (σ^H) in *L. monocytogenes* to find that σ^H regulates DNA uptake in a negative manner. She also demonstrated that σ^H is essential for survival within phagocytes and epithelial cells by allowing the bacteria to escape from phagosomes. These investigations by the author provide novel findings into the role of σ^H in diverse physiological aspects of *L. monocytogenes.* The author's investigations are technically sound and not only provide some novel insights for the role of σ^H but also raise many new interesting questions potentially important for further research in this field.

（最終試験の結果 Assessment）

The final examination committee conducted a meeting as a final examination on January 5, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論 Conclusion）

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.