論文 / 著書情報
Article / Book Information

題目(和文)	枯草菌ゲノムベクターシステムを用いたクラスⅠ嗅覚受容体遺伝子の発現制御領域の解析
Title(English)	Studies on the cis-element for mouse class I odorant receptor genes using the Bacillus subtilis genome vector system
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論文の要約

専攻：　　生物プロセス専攻
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【要約 (Summary)】

Technological developments in chromosome engineering are essential for the manipulation and functional analysis of genomic DNA fragments. The *Bacillus subtilis* genome (BGM) vector is a novel cloning system for large DNA fragments, in which the entire 4.2 Mb genome of *B. subtilis* functions as a vector. The BGM vector system has several attractive properties, such as a large cloning capacity of over 3 Mb, stable propagation of cloned DNA and various modification strategies using RecA-mediated homologous recombination. However, genetic modifications using the BGM vector system have not been fully established, and this system has not been applied to transgenesis. In this study, I developed important additions to the genetic modification methods of the BGM vector system. To explore the potential of the BGM vector, I focused on the mouse class I odorant receptor (class I OR) gene family, which consists of 158 genes and forms a single gene cluster. Although a *cis*-acting locus control region is expected to regulate transcription, this has not yet been determined experimentally.

Using two contiguous bacterial artificial chromosome clones containing several class I OR genes, I constructed two transgenes in the BGM vector by inserting a reporter gene cassette into one class I OR gene. Because they were oriented in opposite directions, I performed an inversion modification to align their orientation and then fused them to enlarge the genomic structure. DNA sequencing revealed that no mutations occurred during gene manipulations with the BGM vector. I demonstrated that the modified, reconstructed genomic DNA fragments could be used to generate transgenic mice. Transgenic mice carrying the enlarged transgene recapitulated the expression and axonal projection patterns of the target class I OR gene in the main olfactory system. Further transgenic analyses shortened and identified a putative

cis-acting element for the class I OR gene.

Through this study, I offered a complete genetic modification method for the BGM vector system, including insertion, deletion, inversion and fusion, to engineer genomic DNA fragments without any trace of modifications. In addition, I demonstrated that this system could be used for mouse transgenesis. Thus, the BGM vector system can be an alternative platform for engineering large DNA fragments in addition to conventional systems such as bacterial and yeast artificial chromosomes. Using this system, I provided the first experimental evidence of a *cis*-acting element for a class I OR gene.