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Development of a targeted gene integration procedure for the production of biopharmaceutical proteins

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論 文 名　：Development of a targeted gene integration procedure for the production of biopharmaceutical proteins
（バイオ医薬品タンパク質生産のための部位特異的遺伝子組込法の開発）

区　　分　：甲

論 文 内 容 の 要 旨

As a major classification of biologics, recombinant mammalian protein plays an important role in biopharmaceutical manufacturing. Production yield and quality of recombinant proteins are manufacturing critical parameters to meet the industrial requirements. Chinese hamster ovary (CHO) cells are the most commonly used mammalian hosts for producing recombinant proteins, and they share 70% market due to the ability of post-translational modifications such as proper folding and glycosylation. The conventional DHFR/MTX system for obtaining high-producer clones is labor-intensive and time-consuming, the high-producer clones obtained using this system sometime exhibit instability of target protein synthesis as culture time progresses. In our laboratory, an accumulative site-specific gene integration system (AGIS) using Cre-recombinase and mutated *loxP* sites was developed previously. In this thesis study, an improved version of AGIS was developed to accelerate the establishment of high-producer Chinese hamster ovary (CHO) cells. The usage of minicircle vectors lacking bacterial backbone sequences was also examined to show effectiveness in generation producer cells.

In Chapter 1, background, bottlenecks and current techniques in the field of biopharmaceutical manufacturing, particularly for recombinant protein production using the mammalian cells, are described as an introduction.

In Chapter 2, the techniques and mechanisms related to generation of cell lines producing recombinant protein are reviewed, including adoption of *hprt* locus as a transgene integration site, and irreversible site-specific integration conducted by Cre-mediated mutated-*loxP*s recombination. These techniques and mechanisms theoretically supported the study in this thesis.

In Chapter 3, a study on the DNA transfection using minicircle vectors lacking the bacteria-derived plasmid backbone sequences is demonstrated, in which minicircle vectors improved the target gene integration efficiency and the cells viability during cultivation. This study confirmed that bacterial backbone sequences could interfere in the recombination process to decrease the integration efficiency. Target protein productivity was improved when minicircle DNA vectors were used for transgene integration into a predetermined chromosomal site.

In Chapter 4, an improved version of AGIS that facilitates and accelerates the establishment of high-producer Chinese hamster ovary (CHO) cells is described. This system enables speed-up and repeated multiple transgenes integration into the *hprt* locus in mammalian cells. After three rounds of integration, a stable high-producer CHO cell clone with six copies of the scFv-Fc gene was successfully generated, and its productivity reached 44 pg/(cell·day) is closed to the highest level reported previously. These results suggested that the *hprt* locus could be beneficial for high and stable transgene expression. This newly designed AGIS procedure should facilitate the development of producer cells suitable for biopharmaceutical protein production.

In Chapter 5, the thesis study is summarized and prospects for future applications are described.