# 論 文 内 容 の 要 旨

1-Deoxy-D-xylulose-5-phosphatesynthase (DXS1) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR1) proteins that are expected to be key enzymes for isoprene synthesis in the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, while squalene synthase (SQS) protein that catalyzes the first enzymatic step in the central isoprenoid pathway during sterol and triterpenoid biosynthesis. cDNAs for these enzymes were introduced into various *Chlamydomonas reinhardtii* strains including UVM4 and UVM11 strains, which are reported to have a high potency for expressing transgenes in the nuclear genome. The results showed that DXS1, DXR1, and SQS cassettes were not readily overexpressed in the wild-type strains at levels where the products were clearly detectable by Western blotting using a monoclonal antibody. In contrast, Western blot-positive SQS cassette transformants were frequently detected in the UVM4 and UVM11 strains, i.e., at an approximately 4.5 times higher frequency than that in the CC-124 wild-type strain. Moreover, in these strains highly SQS accumulating transformants were detected with 2.2-folds frequently than that in CC-124. Position effect against the integrated expression cassettes was obviously detected not only in the wild-type but also in UVM strains. This suggests that the epigenetic repression mechanism of transgenic genes is not completely knocked out, even in the UVM strains. Thus, further improved *Chlamydomonas* strains are required to easily generate transformants that are expressing the aimed gene robustly.

It was reported that squalene synthase-like (*SSL*) genes are belonging to a family of squalene synthase gene, which encode for key enzymes of botryococcene synthesis in *Botryococcus braunii*. *SSL* genes were also heterologously expressed in various *Chlamydomonas* strains. To improve the expression, codon usage of the open reading frame was optimized to match that of the *C. reinhardtii* nuclear genome. In addition to that, the ble2A expression system was utilized in combined with the UVM4 strain to overcome the poor expression. It resulted in that SSL-3 was more efficiently expressed in the UVM4 strain, i.e., PCR-positive ratio was approximately 1.5-folds higher and Western blot-positive frequency was at an approximately 5-folds higher than that in the CC-1690 strain, respectively. These results show that the strategy, ble2A nuclear

expression vector system combined with UVM4 host strain is the most efficient system for heterologous expression of cDNAs in *Chlamydomonas*.