Preliminary study on improvement of nuclear transformation of marine seaweed, especially brown algae

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In this study, preliminary study on improvement of methods and strategies for efficient nuclear transformation in brown algae, including *Laminaria* and *Undaria*, which grow commonly on the coast of Hokkaido, was carried out. In eukaryotic algae, reproducible and efficient genetic transformation systems have been available for only a part of microalgae such as the green alga *Chlamydomonas* and the diatom *Phaeodactylum*, and endogenous promoters were used in all case. Until now, there were trial experiments for gene transfer in macroalgae for the purpose on significance of ecology and marine resource. In these studies, an expression vector for transformation of land plants was used, and transient expression of GUS gene was reported. However, transformation frequency was low and the stable transformation system in brown algae has not been achieved yet. For establishment of efficient nuclear transformation in brown algae, application of an expression vector using a homologous gene, method of DNA delivery, and stages in the life cycle of the algae for this purpose was examined.

I isolated and analyzed cDNA and genomic DNA coding the polypeptide elongation factor one alpha (EF-1α) from brown alga *Scytosiphon lomentaria*, which is easy to complete the life cycle in laboratory. After that, the promoter and terminator regions were examined by inverse PCR method. As the result, 1.6 kb upstream of the putative translation site and 500 bp downstream of the polyadenylation site were elucidated, and a part of the regions were fused to the green fluorescence protein gene (*Gfp*). Next, the glass beads methods, namely cells are vortexed in the presence of DNA, glass beads and polyethylene glycol (PEG), which has been used for the transformation in *Chlamydomonas*, was tried to introduce genes in reproductive cells of brown algae. But this method was not effective in brown algae. Now, I am examining the standard condition of the particle-gun method in brown algal cells. Also, pharmaceutical sensibility in brown algal cells was examined. As the result, cells of *Scytosiphon* and *Laminaria* were extinct by incubation under the existence of 75–100 μg/ml blastcidine for a week.