氏　　名	濱田　玲
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学位論文題目	The structure and the location of *chlI* genes in algae.
論文審査委員	主　査　教　授　沖野 啓子 教　授　五條堀 孝 教　授　菅原　秀明 助教授　倉田　のり 門脇　光一（農林水産省農業生物資源研究所）

論文内容の要旨

Chloroplasts are intracellular organelles in plants which contain the entire machinery necessary for the process of photosynthesis. They contain their own genetic systems and a number of chloroplast components are encoded in their genomes. The genetic system of the chloroplast has many features in common with prokaryotic organisms and is distinct from the (eukaryotic) nuclear-cytoplasmic system. These features have been invoked in support of the hypothesis that green-plant cells evolved from a symbiosis between a eukaryotic host cell and a photosynthetic prokaryote. Especially, the chloroplasts of green plants are thought to have evolved from cyanobacterial endosymbionts. The size of chloroplast genomes is very small compared with general prokaryotes. The reduction reflects that genes required for free-living existence were lost, most genes useful to the symbiosis were transferred to the nucleus of the host, and some genes were retained within the chloroplast. These remaining genes encoding chloroplast products now comprise plastid genomes. The conservation of gene content among chloroplasts of distantly related taxa suggests that most gene transfer occurred early in organelle evolution. However, evidence of modern gene transfer has been accumulating, suggesting that the process continues, albeit at a greatly reduced rate.

The *chlI* gene encodes a subunit of magnesium chelatase, which catalyzes the insertion of magnesium into protoporphyrin IX. It locates on the nuclear genome of several land plants, although it locates on the chloroplast genome of several algae. During the study of the chloroplast genome structure of the green alga *Chlorella vulgaris*, I found *chlI* on the chloroplast. The amino acid sequence deduced from *C. vulgaris chlI* consists of 354 residues. It showed greater similarity to those of land plants than to those of non-green algae, suggesting that the green alga *C. vulgaris* is more closely related to land plants than non-green algae. Northern hybridization revealed a single band at 1.85 kb which was much longer than that expected from the nucleotide sequence, suggesting that *chlI* would be co-transcribed with the neighboring *trnR* gene. Primer extension revealed a single extension product at position -294 upstream of the translation start site. This matches perfectly with the -35 and -10 regions of the *E. coli* consensus promoter sequence. RNase protection assay revealed the 3' end of the transcripts which showed the existence of the co-transcripts of *chlI* and *trnR*. Although another riboprobe revealed the existence of the tRNA-Arg, the processed *chlI* mRNA could not be detected, suggesting that *trnR* contributed to the stabilization of the transcript, and that the *chlI* transcripts were immediately degraded after the

processing of tRNA.

The chloroplast genome of *C. vulgaris* has ten genes not found in land plants. Many of these genes could have been transferred to the nucleus within the green algal lineage giving rise to land plants. Considering the possibility of the transfer of *chlI* in *C. vulgaris*, the chloroplast and nuclear DNA were analyzed by Southern hybridization. However no *chlI*-like sequence was detected in the nuclear DNA.

It is generally believed that extensive rearrangements occurred within the chloroplast genomes during the evolution of land plants from green algae. At the same time, gene transfer to the nucleus would occur. It has been reported that the *tufA* coding elongation factor Tu (EF-Tu) was transferred within the Charophyceae which was believed to be green algal lineage giving rise to land plants. *chlI* will be one of such genes, because it was detected only in the chloroplast genome of *C. vulgaris* but not in those of any land plants. Therefore, I tried to detect *chlI* from Charophyceae *Nitella* and *Chara*, which were believed to be direct ancestors of land plants. Amplification of *Nitella* chloroplast DNA revealed a *chlI* which showed the highest similarity with that of *Chlorella*. Southern hybridization using the chloroplast gene as a probe revealed the existence of a *chlI*-like structure in the nuclear DNA from *Nitella*. The hybridization pattern was completely different from that observed in the chloroplast DNA. Northern hybridization revealed a single band at 1.4 kb at the poly(A)$^-$ RNA, though the signal at the poly(A)$^+$ RNA is under detectable in this experimental condition. It is suggesting that *Nitella chlI* is still transcribed from the chloroplast DNA. To confirm the existence of a *chlI*-like sequence, amplification of the sequence of nuclear DNA was attempted using a primer pair specific to the chloroplast *chlI*. However, the *chlI*-like sequences could not be amplified, suggesting that the sequence of nuclear *chlI*-like was quite different from that of chloroplast *chlI*. Acquisition of function by a relocated gene requires the gain of compartment-specific regulatory sequences, upstream and downstream, and an amino-terminal transit peptide sequence. The *chlI*-like sequence of *Nitella* will be in this stage which is changing the structure to adapt in the nucleus. The chloroplast encoded *chlI* is well conserved and will retain its function until the nuclear *chlI* will acquire the function. Moreover, the peculiar nucleotide sequences of *chlI* were found from *Chara*. The amino acid sequences deduced from them were the same, though the nucleotide sequences were different at 11 sites, which were found at the third letter of the codons. In connection with these *chlI* structures, gene transfer from the AT-biased chloroplast genome to the GC-biased nuclear genome is discussed.

論文の審査結果の要旨

近年の分子レベルの研究から、植物の葉緑体は進化の過程でラン藻が植物細胞に侵入・共生したもので、その後にいくつかの遺伝子は葉緑体から核へ移行したと考えられるようになった。濱田玲君は修士課程の時にクロレラの葉緑体ゲノム解析チームに参加し、その際に見出された、陸上植物には存在せずクロレラには存在する10の遺伝子のうち3つが濱田君が配列を決定した領域に存在したことに触発されて、葉緑体遺伝子の核への移行の問題に関心を持ち、その中の遺伝子の一つ *chl I* の解析を志した。

chl I は、クロロフィル合成に関わる酵素Mg-chelataseのサブユニットの一つをコードする。この遺伝子は藻類から陸上植物が進化した時期に葉緑体ゲノムから核ゲノムに移ったと考えられている。濱田君は、まずクロレラにおいて *chl I* が葉緑体にはあるが核にはないことを確認した。そしてその構造解析を行って翻訳の開始点を明らかにし、それがバクテリアのプロモーター配列と一致していること、さらにこの遺伝子は近傍にある *trnR* と共転写していることなどを見出し、この遺伝子はクロレラでは葉緑体で機能していることを示した。

次に陸上植物に最も近いと考えられている藻類シャジクモの一種 *Nitella* sp.（フラスコモ）における *chl I* の存在場所と機能を明らかにすることを試みた。*Nitella* では葉緑体ゲノム中にクロレラの *chl I* と高い相同性を持つ配列が見出された。それをプローブとしてSouthern 解析を行ったところ、核ゲノムからもシグナルを得たが、そのシグナルの位置は葉緑体ゲノムのものとはかなり異なっていた。さらにNothern 解析を行ったところ、葉緑体由来RNAにはシグナルが現れたが、核由来RNAにはシグナルが認められなかった。

以上のように、申請者は、葉緑体遺伝子の核ゲノムへの移行がいつ頃どのようにして起こったかという問題に挑戦し、*chl I* の構造と機能および存在場所をクロレラと *Nitella* で解析した。陸上植物に最も近いと考えらる *Nitella* では、葉緑体で *chl I* の配列は完全に転写され機能していることがわかった。一方核ではそれと相同性の高い配列が存在するが、転写は検出できず、かなり構造が変化している機能していない配列と考えられた。本研究は、葉緑体から核へ遺伝子の移行が起こる進化段階の植物と考えられるシャジクモを使って、そのような遺伝子の一つ *chl I* の存在場所と機能を解析した点がユニークであり、未解決の問題を含むものの、将来の研究への糸口となる優れた実験研究であると評価した。

提出論文は英語で書かれており、前半部は国際誌に投稿中とのことであるので、英語の学力は充分あると考えられた。公開発表および審査委員との質疑応答から関連分野の知識にも問題がないと判断した。以上を総合して申請者は遺伝学専攻における学位授与の水準に達していると結論した。