



Title	Characterization of chloroplast protein import in red alga, <i>Cyanidioschyzon merolae</i>
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Abstract of Thesis

Name (Sanghun BAEK)	
Title	Characterization of chloroplast protein import in red alga, <i>Cyanidioschyzon merolae</i> (紅藻シアニディオシゾンにおける葉緑体蛋白質輸送機構の解析)
Abstract of Thesis Chloroplasts are green-colored organelles with complex structures, found only in plants and algae. Energy is essential for growth and reproduction in both animals and plants. While animals consume food to produce energy, plants and algae use chloroplasts to produce the energy they need directly from the sun. This may be a good justification for the study of chloroplasts. The chloroplasts in photosynthetic eukaryotes originated from the cyanobacterium-like endosymbiont and still retain their own genomes. Only about 100 proteins are encoded by the chloroplast genomes, whereas more than 2000 different kinds of chloroplast proteins are encoded by the nuclear genomes, synthesized in the cytosol, and then transported into the chloroplasts. TOC and TIC, translocons at the outer and inner envelope membranes of chloroplasts, respectively, play an essential role in protein transport. Our team previously identified a novel TIC complex consisting of Tic214(Ycf1)/Tic100/Tic56/Tic20 from <i>Arabidopsis thaliana</i> as a main TIC complex and also recently identified a completely novel Ycf2/FtsHi ATP-driven motor complex which associates with the TIC complex. The TIC and the associated motor complex are widely distributed among green lineages including plants and green alga. Red algae diverged quite early from the green lineages during the evolution of photosynthetic eukaryotes. The chloroplast protein transport system of red algae has not yet been studied. Moreover, in the genome of red algae, we could not find orthologs for some of well-known TOC/TIC components except for some central ones. Thus, red alga might retain an ancestral type of chloroplast protein transport system. I aimed to identify and characterize this system in <i>Cyanidioschyzon merolae 10D</i>, a model organism of red algae. In this study, I firstly attempted to establish the in vitro chloroplast protein import system using intact chloroplasts isolated from the red algal cells with purified model pre-proteins, which consist of a chloroplast-targeted preprotein of red algal origin and some tags for detection and purification. Although intact chloroplasts were properly isolated, proteins were not imported into chloroplasts. Therefore, we adopted an in vivo approach to characterize chloroplast protein import in this red alga. To this end, a <i>C. merolae</i> transformant inducibly expressing chloroplast-targeted GFP-fusion protein (Tp-GFP-3xFLAG) was used to purify translocation intermediates. Immunoprecipitation of induced cell lysates using anti-3xFLAG antibody identified Tic20 and FtsH as preprotein-interacting proteins during import. Besides those, a class of GTP-binding proteins, CMI311C and CMQ054C proteins (provisional names), distinct from well-known Toc34/Toc159 preprotein receptor GTPase in green lineages, were found associated with transporting preproteins. Additional experiments suggested that 311C and 054C proteins directly recognize the transit peptides of preproteins. While, in the <i>C. merolae</i> genomic database, CMP284C and CMQ137C were tentatively annotated as Toc34/To159 homologs, the above-mentioned in vivo analysis of preprotein-associated proteins failed to detect them. These data suggest that the Tic20-centered TIC complex and the FtsH import motor likely participate in chloroplast protein import in red algae as in the green lineage. However, TOC complex and cytosolic events related to chloroplast protein import in red algae may be quite different from those of the green lineages, which involve the distinct class of GTP binding proteins. Altogether, the relevance of this study is underscored by the identification of the early chloroplast protein import pathway, it is the first to identify the chloroplast protein import pathway using red algae. Further studies will be needed to elucidate more detailed mechanisms of chloroplast protein import in red alga.	

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

申請者 Sanghun Baek 氏は、紅藻のモデル生物である *Cyanidioschyzon merolae* における葉緑体蛋白質輸送機構の解析を行った。ほぼ全ての光合成真核生物の葉緑体は、シアノバクテリアが内共生した事に由来する。進化の過程で内共生体ゲノムの大部分は核に移り、同時に、これら核コードの葉緑体蛋白質を生合成場所であるサイトゾルから葉緑体へと輸送する輸送機構が確立された。一方、光合成真核生物は、その進化の初期に紅藻、灰色藻と緑藻とが分岐し、その後緑藻から陸上植物へと進化を遂げるが、葉緑体蛋白質輸送機構やそれに関わる因子については、これまで主に陸上植物や緑藻で解析が進められてきた。Baek 氏は、*C. merolae* を用いて *in vivo* で輸送中間体が蓄積する実験系を構築し、陸上植物や緑藻における葉緑体内包膜蛋白質輸送装置の中核に相当する、葉緑体ゲノムコードの Tic20 および FtsH 蛋白質が直接相互作用すること、これらが葉緑体蛋白質輸送に関与する事を証明した。さらに、新奇 GTP 結合蛋白質が、輸送される葉緑体蛋白質の局在化シグナルをサイトゾルで認識する因子として働いている事を見出した。進化的な考察も加え、紅藻では、内包膜蛋白質輸送装置のコア因子は陸上植物や緑藻と共通であるが、サイトゾルから葉緑体への葉緑体蛋白質のターゲティングや外包膜透過のステップについては、分子機構や関わる因子が大きく異なる可能性を提唱した。

以上の成果は、これまで全く手がつけられていなかった紅藻の葉緑体蛋白質輸送機構について、それに関わる因子を特定することのできる実験系を構築し、進化的な観点からも重要な知見を見出した点が高く評価される。よって本論文は博士（理学）の学位論文として十分価値あるものと認める。