Characterization of L-cysteine requiring mutants derived from heavy-ion-beam irradiated cells in the unicellular green alga *Parachlorella kessleri*

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Many microalgae show accumulation of neutral lipids, such as triacylglycerols (TAGs), in oil bodies under conditions in which there is a lack of nutrients, including nitrogen, phosphorus and sulfur, in their environment. Although sulfur starvation in microalgae induces lipid accumulation, little is known about the underlying mechanism. Interest in this phenomenon is divided into two aspects: 1) the connection between recognition of sulfur starvation and lipid synthesis and 2) whether the mechanism of lipid induction under sulfur-starvation conditions is shared with those of other nutrient-starvation conditions. To answer these questions, we obtained heavy-ion-beam irradiated algal mutants that require L-cysteine, which is an amino acid with sulfur.[1)]

Sulfate is the most highly oxidized sulfur compound, is widespread in nature and is the most important supplier of sulfur for green plants, including microalgae (Fig. 1A). Plants incorporate sulfate intracellularly as a sulfur source. In green algae and land plants, sulfate is taken up by the sulfate transporter (SULTR) across the plasma membrane. Sulfate in the cytosol is transported into chloroplasts by chloroplast localized SULTR. In chloroplasts, sulfate is bound to ATP by sulfate adenylyltransferase (ATS) and deoxidized to sulfite by adenylyl-sulfate kinase (APSK) and adenyl sulfate reductase (APR). Sulfide is reduced by sulfide reductase (SiR) and finally assimilated into L-cysteine by exchanging sulfide with the acetyl group of O-acetyl-L-serine (OAS) in a reaction catalyzed by O-acetylserine (thiol) lyase (OAS TL). L-cysteine is metabolized to L-methionine through L-homocysteine in chloroplasts and mitochondria.[2)] Although knowledge of the microalgal sulfate-assimilation pathway is limited, the homologous genes that function in sulfate assimilation in land plants have been identified in *Chlamydomonas*.

Chlorella and Parachlorella species are unicellular immotile green microalgae classified in the Trebouxiophyceae, which have spherical cells less than ~ 10 μm in diameter containing a chloroplast. *Parachlorella kessleri* accumulates TAG under sulfate-limited conditions.[3)] Therefore, this species can be used as a model for investigation of the response to nutrient starvation. We reported an investigation of the regulatory system of lipid and starch synthesis under sulfate-starvation conditions,[1)] and summarize it in this report.

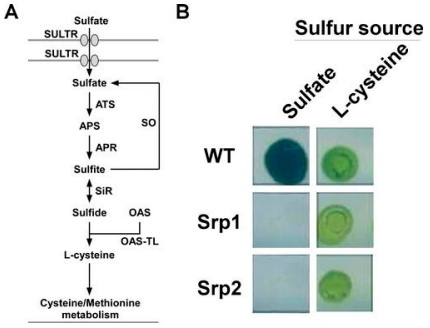

Fig. 1. Sulfur-assimilation pathway and isolated Srp mutants of *P. kessleri*. (A) Schematic of the sulfate assimilation pathway. WT, wild type (B) Phenotype of Srp mutants.

Mutagenesis of *P. kessleri* was performed according to our previous method. For isolation of cysteine requiring *P. kessleri* mutants using Fe-ion treatments, 200 μL aliquots of a 2-day-old *P. kessleri* culture were transferred into tubes. The samples were stored at 4°C prior to being subjected to heavy-ion-beam irradiation. Cells were irradiated in the RIKEN RI-beam factory (Wako, Saitama, Japan) at doses of 25 and 50 Gy. The linear energy transfer value was 790 keVμm^{-1}. The irradiated cells were spread onto L-cysteine medium 1.5% agar plates for isolation of single colonies. Approximately 13,000 colonies were picked as isolates and inoculated onto both L-cysteine medium 1.5% agar plates and sulfate medium 1.5% agar plates. Isolates that grew only in L-cysteine were identified as sulfate repressed proliferation (Srp) mutants (Fig. 1B). The strains were deposited in the National Institute of Technology and Evaluation, Tokyo, Japan, under accession numbers FERM BP-22268 (for Srp 1) and FERM BP-22288 (for Srp 2).

A phenotype of these mutants under sulfate replete conditions exhibited that cell proliferation was suppressed like the phenotype of wild type under sulfur deplete conditions, but starch was highly accumulated. The effects of sulfate and L-cysteine on lipid and starch accumulation in the wild type and mutants were compared in media containing different sulfur compounds as sulfur sources. Our results suggest that a shortage of L-cysteine, which is a metabolite of sulfate, induces lipid accumulation and that sulfate ions promote starch accumulation in chloroplasts. This study was supported by JST, CREST and START (to SK).

References

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