LIVING WELL WITH A SCRAMBLED METABOLISM: CO_2 FIXATION AND CARBOHYDRATE PATHWAYS IN DIATOMS

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Diatoms are responsible for up to 20% of the global carbon fixation, while the mechanisms of CO_2 fixation in these organisms are still unclear. Diatoms have evolved by secondary endosymbiosis, which apparently led to subcellular reorganization of metabolic pathways within the organisms. Studying the genome of the diatom *Phaeodactylum tricornutum* we have identified a number of genes for enzymes which are important for operating a C4-like pathway including several carboxylases. However, we could not yet identify plastid localized decarboxylases. Such enzymes would be important for a putative carbon concentrating mechanism (CCM) by releasing CO_2 from malate or oxaloacetic acid in close proximity to RubisCO. The only decarboxylases identified so far apparently are targeted to the mitochondrial matrix which is separated from the plastid stroma (and thus the RubisCO) by six membranes. Therefore it is unclear yet whether *P. tricornutum* might operate either a biochemical or a biophysical CCM or a combination of both.

To study individual enzymes of the photosynthetic carbon fixation, we are using a “reverse genetics” approach including silencing of genes which are involved in the biochemical CCM via RNAi. Mutants have been designed with sense-antisense constructs for silencing phosphoenolpyruvate carboxylases (PEPC1 & PEPC2), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate carboxylases (PYC1 & PYC2) and NAD malic enzyme (ME1). To control expression of the silencing constructs we have decided to use a nitrate reductase (NR) promoter which can be switched on and off depending on the nitrogen source in the medium. The obtained mutants will be tested for silencing efficiency by Western Blots. Physiological investigations will be conducted under e.g. different CO_2 and light conditions to learn more about their adaptation abilities due to future environmental changes.