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*Letter to the Editor*

# Unique Regulation of the Calvin Cycle in the Ultrasmall Green Alga *Ostreococcus*

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**Abstract.** Glyceraldehyde-3-phosphate dehydrogenase (GapAB) and CP12 are two major players in controlling the inactivation of the Calvin cycle in land plants at night. GapB originated from a *GapA* gene duplication and differs from GapA by the presence of a specific C-terminal extension that was recruited from CP12. While GapA and CP12 are assumed to be generally present in the Plantae (glaucophytes, red and green algae, and plants), up to now GapB was exclusively found in Streptophyta, including the enigmatic green alga *Mesostigma viride*. However, here we show that two closely related prasinophycean green algae, *Ostreococcus tauri* and *Ostreococcus lucimarinus*, also possess a *GapB* gene, while *CP12* is missing. This remarkable finding either antedates the *GapA/B* gene duplication or indicates a lateral recruitment. Moreover, *Ostreococcus* is the first case where the crucial CP12 function may be completely replaced by GapB-mediated GapA/B aggregation.

**Key words:** *Ostreococcus tauri* — *Ostreococcus lucimarinus* — Plant evolution — Glyceraldehyde-3-phosphate dehydrogenase — CP12 — Calvin cycle

**Short Communication:** During photosynthesis, plastids of land plants and algae transform light energy into ATP and NADPH. This chemical energy fuels the Calvin cycle, where carbon dioxide gets fixed to produce sugar compounds that are used for fatty acid, isoprenoid, and amino acid synthesis (Bassham 2003). Following the circadian light/dark rhythm, chloroplasts switch between anabolic and catabolic metabolism exemplified by starch production and degradation. A general metabolic transition in green plants is the inactivation of the reductive Calvin cycle and the activation of the oxidative pentose phosphate pathway (OPPP) for NADPH generation at night (Klein 1986; Schnarrenberger et al. 1995; Martin and Herrmann 1998; Michels et al. 2005). Especially the thioredoxin system is responsible for the reversible redox regulation of the Calvin cycle and it is mediated by a small regulator named CP12 (Wedel et al. 1997). The nuclear-encoded CP12 protein is 75 amino acids long, contains at least two crucial cysteine residues (Pohlmeyer et al. 1996; Petersen et al. 2006), and, together with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GapA) and phosphoribulokinase (PRK), oligomerizes into a stable protein complex (Cerff 1979; Wedel et al. 1997). This mechanism completely blocks the whole cycle at night and is assumed to be generally conserved in cyanobacteria and Plantae, comprising glaucophytes, rhodophytes, chlorophytes, and land plants (Wedel and Soll 1998; Petersen et al. 2006). However, land plants contain an additional inactivation complex. Ordinary GapA is redox-insensitive, but a duplicate named GapB recruited the regulatory redox domain, a characteristic C-terminal extension, from *CP12* by a gene fusion (Pohlmeyer et al. 1996). As a consequence, the Calvin

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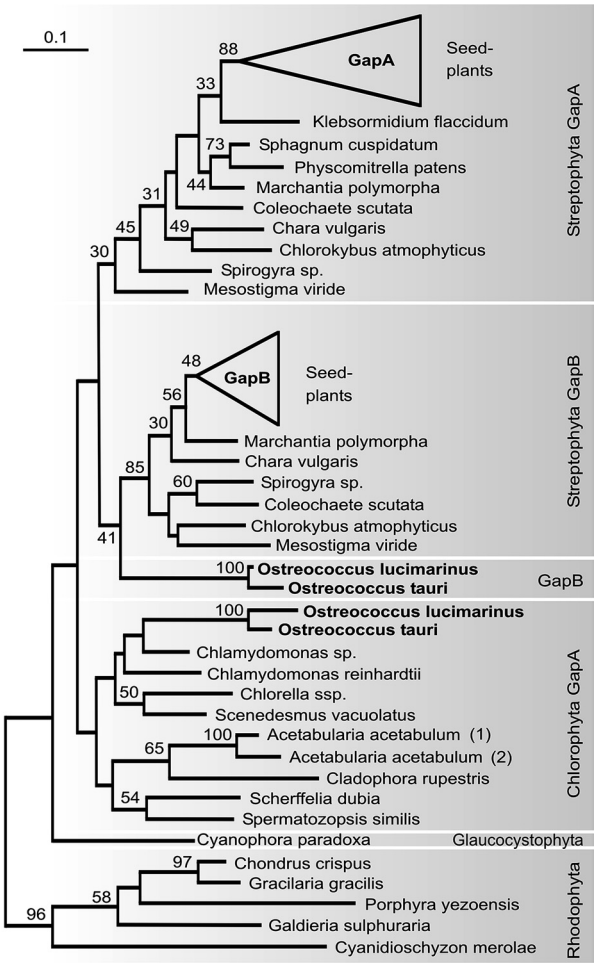


**Fig. 1.** The best maximum likelihood tree based on 71 plastid GAPDH sequences of Plantae and 326 amino acid positions inferred by the program Treefinder under a WAG + Γ4 model. Numbers given at internal nodes correspond to nonparametric bootstrap values (100 replicates). Bootstrap values lower than 30% are not indicated. The new *GapA* and *GapB* sequences from the two *Ostreococcus* strains are shown in boldface.

cycle inactivation is tightened by a second mechanism that is exclusively based on GAPDH association (Scheibe et al. 2002).

Recently, Petersen et al. (2006) determined the sequence for *GapA*, *GapB*, and *CP12* of different green plants. Their analyses revealed that *GapB* sequences can be unequivocally identified by the CTE as well as a specific sequence pattern including two insertions. Petersen and coworkers identified *GapA* and *GapB* sequences from several charophytes, but especially the presence of *GapB* within the unicellular green alga *Mesostigma viride* dates the *GapA*/*B* gene duplication at least to the common ancestor of all Streptophyta, about 700 to 1150 million years ago (mya) (Yoon et al. 2004). Comprehensive analyses of all orders of Chlorophyta, representing prasino-, trebouxio-, ulvo-, and chlorophycean species, including the most prominent and completely sequenced green alga, *Chlamydomonas reinhardtii*, exclusively uncovered GapA sequences. Phylogenetic analyses showed *GapA* sequences of Chlorophyta to form a weakly supported group diverging prior to the distinct *GapA* and *GapB* subtrees of streptophytes, hence allocating the *GapA*/*B* gene duplication to an early stage of streptophycean evolution.

Here we present the distribution of nuclear-encoded plastid *GAPDH* and *CP12* genes from the first completely sequenced prasinophycean green alga, *Ostreococcus tauri* (Derelle et al. 2006), and the closely related strain, *Ostreococcus lucimarinus* (Brian Palenik, personal communication). This unicellular green alga is the smallest free-living eukaryote known to date (Courties et al. 1994, 1998) and has a genome size of 12.56 Mb, distributed over 20 chromosomes (Derelle et al. 2006). Two genes with high sequence similarity to known *GapA*/*B* genes could be detected in both *Ostreococcus* species. A typical *GapA* sequence is located on chromosome 10, shows 72% amino acid identity to the *GapA* sequence of *Chlamydomonas reinhardtii*, another member of the Chlorophyta, and clusters within the *GapA* subtree of Chlorophyta (Fig. 1).

Unexpectedly, the second GAPDH homologue, which is located on chromosome 1, seems to be a genuine *GapB*. It contains the typical C-terminal extension (CTE) with two regulatory cysteine residues (see Supplementary Fig. S1) and exhibits the *GapB* specific sequence pattern including two characteristic insertions (see Supplementary Fig. S2). Even if the statistic support is weak (Fig. 1), in particular, the latter observation authenticates the common origin of *GapB* and rules out CTE recruitment via a second independent gene fusion between *GapA* and *CP12* duplicates. However, the presence of *GapB* in a prasinophyte is surprising, because this gene could previously not be identified in any chlorophyte (prasino-, trebouxio-, ulvo-, or chlorophyceae [Petersen et al. 2006]), and it is definitely absent from the completely sequenced chlorophycean genomes of *Chlamydomonas* and *Volvox* (http://www.jgi.doe.gov/).

At least two scenarios can explain the presence of *GapB* in *Ostreococcus*. First, the *GapA*/*B* gene duplication may have occurred much earlier in green plant evolution than previously assumed (Petersen et al. 2006). Since Chlorophyta and Streptophyta form two deep and distinct green lineages, and prasinophytes represent the most ancient lineage of the former clade (Rodríguez-Ezpeleta et al. 2007), the *GapA*/*B* gene duplication would have occurred in a common ancestor of present-day chloro- and streptophytes (Viridiplantae). This premise would imply secondary losses of *GapB* in chlorophyceae (e.g., *Chlamydomonas*, *Volvox*), but also in ulvo- and trebouxiouphyceae, where this gene has not been detected so far (Petersen et al. 2006). In addition, in its

simpliest version (one duplication event) it would demand the monophyly of all green plant *GapA* sequences, thus suggesting a phylogenetic artifact in the current tree. Second, it cannot be excluded that an ancestor of *Ostreococcus* recruited the *GapB* via horizontal gene transfer (HGT), for instance, from a charophycean green alga. Mixotrophy has been reported for some prasinophytes (Graham and Wilcox 2000) and a certain proportion of *Ostreococcus* genes is closely related to marine algae and not to green plants as one would expect. A striking example is the nuclear-encoded Calvin cycle sedoheptulose-1,7-bisphosphatase (SBP; *O. tauri*, chromosome 3; accession no. CAL53197 [wrongly annotated FBP]), which is closely related to the SBP of the diatom *Phaeodactylum tricornutum* (data not shown). Moreover, a unique finding is the replacement of the cytosolic GAPDH (*GapC*), one of the most prominent housekeeping genes that is otherwise universally present in Plantae, Metaozoa, and Fungi, by a *gap3* gene (*O. tauri*, chromosome 2; accession no. CAL52398), which was previously exclusively identified from bacteria and diplonemids (Figge and Cerff 2001; Qian and Keeling 2001). If the assumption of HGT were also true for *GapB*, the evolutionary rate of the *Ostreococcus GapB* sequences might have been accelerated in the context of recruitment (Fig. 1), resulting in an artifactual basal position (Brinkmann et al. 2005). Taken together, the discovery of additional *GapB* genes within more distantly related chlorophytes would substantiate the former scenario, whereas a sporadic occurrence in *Ostreococcus* would support the HGT explanation in accordance with a mixotrophic lifestyle.

Apart from the presence of *GapB*, the investigation of both *Ostreococcus* species also revealed that the *CP12* genes are absent from their genomes. Comprehensive BLAST analyses yielded two weak hits with the N- and C-terminal domain of CP12, located on chromosomes 17 and 11, respectively. Thus, it can be ruled out that these sequences belong to one *CP12* gene that is separated by introns (see Supplementary Figs. S3 and S4). Since *CP12* was previously assumed to be generally present in cyanobacteria and Plantae (Pohlmeyer et al. 1996; Petersen et al. 2006), its absence from both *Ostreococcus* genomes might have drastic consequences for GAPDH inactivation as well as plastid metabolism. Cyanobacterial *CP12* knockout mutants accordingly show significantly reduced growth rates (Tamoi et al. 2005). The lack of *CP12* in complex algae such as diatoms (Armbrust et al. 2004), which obtained their plastids through secondary endosymbiosis, correlates with the absence of the plastid oxidative pentose phosphate pathway (OPPP), probably due to the missing inactivation of the Calvin cycle at night, which would result in futile cycling (Michels et al. 2005; Petersen et al. 2006). We analyzed the distribution of glucose-6-phosphate dehydrogenase (G6PDH), a key enzyme of the OPPP (Martin and Herrmann 1998), in *Ostreococcus* and identified a single gene for the respective plastid protein (in both species; data not shown). If OPPP and Calvin cycle are present in the chloroplasts of these ultrasmall algae, the presence of GapB is probably essential to ensure GAPDH aggregation at night. Thus, in contrast to streptophytes that harbor two regulatory complexes based on CP12 and GapB, the prasinophyte *Ostreococcus* would be the first example where the Calvin cycle is exclusively inactivated by the formation of GapAB complexes.

**Methods.** Homologous *Ostreococcus* sequences were identified using BLAST (Altschul et al. 1990) and added to the dataset of Petersen et al. (2006). The candidate gene products were manually added to the existing dataset using the EDIT option of the MUST package (Philippe 1993). Manual annotation was performed with Artemis (Rutherford et al. 2000). In the final alignments, HMMer (Eddy 1998) was used to generate specific profiles for each gene family with hidden Markov models.

**Phylogenetic analyses.** All new sequences reported in this letter have been submitted to GenBank under the following accession numbers: *Ostreococcus tauri GapA* and *GapB* (DQ649076 and DQ649078) and *Ostreococcus lucimarinus GapA* and *GapB* (DQ649077 and DQ649079). The final alignment consists of 71 sequences that all belong to the Plantae with the red algae sequences as outgroup. G-blocks was used to eliminate all ambigiously aligned positions resulting in a dataset with 326 amino acid positons (Castresana 2000). The best maximum likelihood (ML) tree was obtained using Treefinder under a WAG + Γ4 model (Jobb et al. 2004). In order to estimate the statistical support of the internal nodes, nonparametric bootstrapping (Felsenstein 1985) on 100 replicates was performed in Treefinder using the same model.

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