

## Unravelling xanthophyll pigment biosynthesis in algae

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**Project Goals:** *Reprogram metabolic networks using in vivo synthetic modules to increase the flux of energy and carbon into biofuel precursors. Goal 1) Profiling the transcriptome, proteome and metabolome to investigate cell responses to physiologically relevant conditions. Goal 2) Identify and manipulate key factors involved in the control of inorganic C assimilation, photosynthetic efficiency and regulation of lipid accumulation. Goal 3) Development of Phaeodactylum genome reconstruction/modeling and our development of novel synthetic genomic tools to achieve our overall goal of increasing productivity.*

Diatoms are the dominant eukaryotic primary producers of the world's oceans. Their versatile peripheral photosynthetic light harvesting antenna, Fucoxanthin Chlorophyll a/c binding Proteins (FCPs), enable diatoms to survive under extreme light conditions through enhanced light absorption and efficient energy dissipation compared to plants. The main accessory xanthophyll pigment, fucoxanthin, gives cells their distinct brown color and an extended spectral range for photosynthesis. Meanwhile, the novel diadinoxanthin (Dd)-diatoxanthin (Dt) cycle xanthophylls contribute to the FCP's switch between a light harvesting and energy dissipation state. Notably, the biosynthetic pathway of these xanthophylls was previously unknown. By applying reverse genetic techniques, we isolated three green *Phaeodactylum tricornutum* mutants, *zep1*, *vdl2* and *criso5* which cannot produce fucoxanthin and exhibit a reduced functional absorption cross-section of PSII. A fucoxanthin biosynthesis pathway is proposed based on the structures of the carotenoids accumulated in the mutants. Another mutant, *zep3*, is unable to convert Dt into Dd after the shift from high light to low light, resulting in irreversible non-photochemical quenching (NPQ). The constitutively accumulated diatoxanthin in *zep3* lines leads to a lower photochemical efficiency of photosystem II ( $\approx 60\%$  of WT). Phylogenies of the VDL, ZEP and CRTISO families from plants and algae, suggest that *zep1*, *zep3*, *vdl2* and *criso5* evolved via multiple duplications and neofunctionalization of their ancestor genes during the process of secondary endosymbiosis that led to Stramenopile algae. These discoveries open the potential for the manufacture of important nutraceutical pigments and can be applied to engineering the efficiency of photosynthesis.

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