Developing episome-based, gene expression modulation by exogenous chemicals in the diatom *Phaeodactylum tricornutum*.

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Project Goals: Overall goal - 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) Forward genetic library generation, screening and genotyping. These approaches complement our development of *Phaeodactylum* genome reconstruction /modeling and our development of novel synthetic genomic tools to achieve our overall goal of increasing productivity.

CRISPR/Cas9 technology has been successfully implemented for targeted genome editing and gene expression changes across a wide range of eukaryotic organisms. This project aims at engineering inducible CRISPRi machinery into an artificial chromosome within the model diatom *Phaeodactylum tricornutum*. The current state of the art for inducible gene expression in diatoms is based on endogenous promoters that respond to different environmental conditions. For instance, in *P. tricornutum*, the most commonly used inducible promoters are the light dependent pLHCF1 and the nitrate dependent pNR promoters. However, the use of these endogenous promoters could have unwanted consequences on cell physiology. This calls for the development of new transcriptional control systems. In this project, we are developing a library of orthogonal, inducible systems which are based on naturally occurring, heterologous chemically responsive transcription factors and cognate promoters. We identified and adapted six synthetic expression systems that have proven effective in other eukaryotic systems for use in *P. tricornutum*. These include the *10xN1/NEV* (4-hydroxytamoxifen inducible) system, the *XVE/OlexA* (β-estradiol inducible) system, the *pOp/LhGR* (dexamethasone inducible) system, the *AlcR/AlcA* (ethanol inducible) system, the *Tet-ON* (tetracycline or doxycycline inducible) system, and *DIG/pUAS* (Digoxin or Digoxigenin inducible) systems. Since the six genetic circuits are modular, genetic parts such as transcription factors, promoters, and terminators can be replaced to create a library of novel inducible systems. We will first evaluate the ability these inducible systems to induce the production of YFP reporter protein and adapt their use to induce gene silencing using the CRISPRi system. Developing externally controlled CRIPSRi technology for efficient and scalable disruption of gene expression in diatoms will provide powerful tools to study gene function, to enable HTP genetic screens and to modify specific pathways to produce high-value metabolites.

This research is supported by the Office of Biological and Environmental Research in the DOE Office of Science – grant # DE-SC0008595