

The ocean/atmosphere interface is the major conduit for the entry of atmospheric CO<sub>2</sub> into oceanic carbon pools that can lead to sequestration or recycled release. The surface layers of the temperate and tropical oceans are often too oligotrophic to result in significant primary production that might lead to carbon sequestration. However, nutrient-rich river plumes can alter the primary production schemes of oligotrophic ocean basins, resulting in increased phytoplankton biomass and carbon fixation. The ultimate goal of this proposal is to understand these carbon cycling processes in major river plumes from the molecular processes involved in biological DIC uptake to contribution to basin-wide production and potential sequestration. Our research efforts include a field component to answer the questions raised concerning DIC in plumes entering ocean basins and an intensive genomics approach to understanding these processes on the cellular level using genomic fragments obtained from plume biota. This project is actually composed of 3 separate PI-initiated projects, including projects at the University of South Florida (USF) College of Marine Science, the University of Puerto Rico, and The Ohio State University. This report concerns research conducted at The Ohio State University and studies performed in collaboration with USF.

In order to understand what might occur in the field, two model systems were studied in the laboratory. Carbon fixation in the unicellular cyanobacterium *Synechococcus* sp Strain PCC 7002 took place mainly through the CBB pathway. Nitrogen nutrition in cyanobacteria is regulated by NtcA, a transcriptional regulatory protein. We show that the rubisco activity and gene (*rbcL*) expression were not affected when cells were exposed to prolonged periods of nitrogen stress, however cells appear to use intracellular nitrogen reserves during nitrogen starvation. Transcripts of the global transcriptional regulator NtcA are expressed under nitrogen starved and nitrogen replete (nitrate or ammonia) growth conditions, with slight decrease in transcription in the presence of ammonia. These results suggest that intracellular levels of NtcA do not directly affect carbon metabolism. Gene expression of the other nitrogen regulatory signal transducer, encoded by *glnB* was also studied. The *glnB* gene was highly transcribed in nitrogen-limited cells compared to nitrogen depleted growth conditions. Therefore in the cyanobacterium *Synechococcus* sp PCC 7002, nitrogen does not affect the metabolic potential and carbon fixation. The NtcA regulator behaved differently and studies indicate that the product of the *ntcA* gene (NtcA) has an indirect effect on carbon assimilation and the genes involved in the carbon concentrating mechanism of strain 7002. The product of the *ccmM* gene plays an important role in carboxysome assembly and inorganic carbon transport within the cell. We hypothesized that under nitrogen limiting conditions the transcriptional regulator NtcA binds at the region upstream of *ccmM*, near the transcription start site, and blocks the transcription of *ccmM*. This hypothesis was experimentally proven.

In another study, with USF researchers, we performed experiments in situ on RubisCO expression. To determine the relationship between expression of the major gene in carbon fixation, we evaluated *rbcL* mRNA abundance using novel quantitative PCR assays, phytoplankton cell analyses, photophysiological parameters, and pCO<sub>2</sub> in and around the Mississippi River plume (MRP) in the Gulf of Mexico. Lower salinity (30–32) stations were dominated by *rbcL* mRNA concentrations from heterokonts; i.e., diatoms and pelagophytes, which were at least an order of magnitude greater than haptophytes, *Synechococcus* or high-light *Prochlorococcus*. However, *rbcL* transcript abundances were similar among these groups at oligotrophic stations (salinity 34–36). Diatom cell counts and heterokont *rbcL* RNA showed a

strong negative correlation to seawater  $p\text{CO}_2$ . While *Prochlorococcus* cells did not exhibit a large difference between low and high  $p\text{CO}_2$  water, *Prochlorococcus* *rbcL* RNA concentrations had a strong positive correlation to  $p\text{CO}_2$ , suggesting a very low level of RuBisCO RNA transcription among *Prochlorococcus* in the plume waters, possibly due to their relatively poor carbon concentrating mechanisms (CCMs). These results provide molecular evidence that diatom/pelagophyte productivity is largely responsible for the large  $\text{CO}_2$  drawdown occurring in the MRP, based on the cooccurrence of elevated RuBisCO gene transcript concentrations from this group and reduced seawater  $p\text{CO}_2$  levels. This may partly be due to efficient CCMs that enable heterokont eukaryotes such as diatoms to continue fixing  $\text{CO}_2$  in the face of strong  $\text{CO}_2$  drawdown. This work represents the first attempt to relate in situ microbial gene expression to contemporaneous  $\text{CO}_2$  flux measurements in the ocean.