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Calcification and inorganic carbon uptake in the coccolithophore Emiliania huxleyi

L.C.M. Mackinder (1,2), L.T. Bach (1), K.G. Schulz (1), G Wheeler (2,3), D.C. Schroeder (2), C Brownlee (2), and U Riebesell (1)

(1) Helmholtz-Zentrum für Ozeanforschung (GEOMAR), Kiel, Germany, (2) Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, UK, (3) Plymouth Marine Laboratory, Prospect Place, Plymouth, UK

Calcification in the coccolithophore *Emiliania huxleyi* is a tightly regulated process requiring the intracellular transport of Ca^{2+} and inorganic carbon. The presented work focuses on the mechanisms of calcification in *E. huxleyi* identifying key genes involved in Ca^{2+} and dissolved inorganic carbon (DIC) transport. An initial experiment involving the removal of Ca^{2+} from the culture medium to stop calcite formation supports previous data that photosynthesis has no mechanistic dependence on calcification with organic carbon fixation rates maintained in the absence of Ca^{2+}. Monitoring gene expression identified several key genes putatively involved in calcification with Ca^{2+} removal resulting in a "non-calcifying" gene expression profile. In a series of separate experiments the importance of the individual components of the carbonate system (CO_2, HCO_3^-, CO_3^{2-} and pH) on coccolithophore calcification and photosynthesis were investigated. To disentangle the carbonate system *E. huxleyi* was cultured at constant CO_2 and constant pH and various physiological parameters including calcification, organic carbon fixation and growth rates were measured. In conjunction the transcriptional response of *E. huxleyi* was also analysed with the gene expression of multiple genes putatively involved in inorganic carbon transport and pH homeostasis profiled. The data strongly supports that HCO_3^- is the principle substrate for calcification and growth and organic carbon fixation rates are primarily influenced by $CO_2$with pH also playing a key role at lower values. The transcriptional analyses of multiple genes show that a putative HCO_3^- transporter, four putative H^+ transporters, and three carbonic anhydrases remained largely unaffected at high DIC concentrations but are significantly up-regulated at low concentrations. This transcriptional profile supports the presence of a carbon concentrating mechanism (CCM) in *E. huxleyi* and provides, for the first time, the genetic basis of a CCM in a haptophyte algae. Results presented here indicate that the removal of calcification does not reduce photosynthesis and that potentially at very low DIC availability calcification is sacrificed to allow the reallocation of intracellular inorganic carbon from calcite to organic carbon. The understanding of the calcification process and the influence of changing carbonate chemistry on *E. huxleyi* are needed to comprehend how this species could respond to environmental change.