**Control of light saturated photosynthesis: concentration
and activity of ribulose biphosphate carboxylase.**

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Control of light saturated photosynthesis: concentration and activity of ribulose biphosphate carboxylase.

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INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is one of the most abundant enzymes on the planet and is responsible for catalysing the net fixation of CO_2 into organic matter. It is central, therefore, to primary productivity in marine and terrestrial ecosystems. Rubisco is a large enzyme with low substrate affinity and low catalytic efficiency and is considered to limit the rate of light-saturated photosynthesis.

This report summarises research into the molecular basis of the regulation of phytoplankton photosynthesis. It describes experimental and theoretical studies of the role of Rubisco in regulating the photosynthetic rate of phytoplankton. It also describes the integration of a mechanistically based phytoplankton growth model into a description of primary productivity in the sea. This work was conducted as part of the Ocean Margins Program.

Summary and Significance of Results

The light environment experienced by phytoplankton is highly variable. The photosynthetic apparatus of phytoplankton responds to variations of irradiance on a range of time scales from seconds to days. In describing the photosynthetic characteristics of phytoplankton, it is useful to distinguish between photoacclimation, which responds slowly in comparison to changes of irradiance, and other characteristics that respond more rapidly. These exert ultimate and proximate controls on photosynthesis, respectively (i.e., the photosynthesis rates achieved under steady-state and transient conditions). Ultimate control will be determined by factors that vary in response to changes of irradiance on a time scale of hours to days. Proximate control will be determined by factors that respond on much shorter time scales. Over periods of hours to days, the abundance of Rubisco relative to light-harvesting pigments is regulated by irradiance and nutrient availability through effects on the rate of synthesis and turnover of photosynthetic proteins and pigments. Over periods of minutes, the activity of Rubisco is modulated in response to short term transients of irradiance. We considered these two aspects of regulation of photosynthesis under the categories of (1) photoacclimation and (2) Rubisco activation state.

Photoacclimation

Phytoplankton adjust their chemical composition in response to cues received from their environment, an adjustment that is termed acclimation. Acclimation serves to increase the growth rate under sub-optimal conditions over the value that would be achieved if cellular chemical composition were static. The importance of acclimation to phytoplankton growth rate in the sea is indicated by variations of pigment:biomass observed in vertical profiles and along horizontal transects. Variability of chlorophyll:carbon has long been recognised as a major source of uncertainty in investigations of phytoplankton growth in the sea.

To address the importance of acclimation, dynamic models of phytoplankton growth were developed. During the project, the models evolved through three iterations. The first iteration (Geider et al., 1996) described photoacclimation of the chlorophyll:carbon ratio under nutrient-replete conditions. A basic premise of this model was the invariance of the biomass-specific light-saturated photosynthesis rate. Photoacclimation under nutrient-replete conditions involves adjustment of the pigment:biomass ratio, with Rubisco remaining a constant proportion of cell biomass. The second iteration (Geider et al., 1997), accounted for modulation of the light-saturated rate of photosynthesis, and hence of Rubisco activity, by nutrient supply and temperature through multiplicative effects on the maximum growth rate. We embedded this model within a simple model of pelagic primary production to obtain estimates of the seasonal and latitudinal variability of chlorophyll:carbon in the North Atlantic Ocean (Taylor et al. 1997). The most advanced model (Geider, MacIntyre, Kana and Taylor, submitted) describes growth, pigment content and the carbon:nitrogen ratio in response to light intensity and nitrogen availability. As such, it provides a flexible and realistic link between the fields of biogeochemistry and bio-optics.

Our most advanced model (Geider, MacIntyre, Kana and Taylor, submitted) considers phytoplankton growth and chemical composition to be affected by irradiance (I), nutrient concentration (N) and temperature (T). It treats nutrient uptake and photosynthesis rates as functions of both environmental factors and cellular chemical composition. The environmental variables (I , N , T) determine the instantaneous rates of light utilisation, carbon assimilation, chlorophyll a synthesis and nutrient assimilation. The intracellular variables (e.g., chlorophyll a :carbon and nitrogen:carbon) integrate the effects of past environmental conditions on cell physiology. Thus, the model accounts for the effects of past and present environmental conditions on nutrient and carbon assimilation. We designated this new formulation of phytoplankton growth and acclimation the IQ model, where I stands for irradiance and Q for the weight of internal nutrient per unit biomass designated by Michael Droop as the cell quota of the limiting nutrient. The IQ model is based on three features of phytoplankton acclimation. First, it includes down-regulation of pigment content at high irradiance or when growth rate is limited by nutrient availability or temperature. Second, it includes the accumulation of energy storage polymers when growth rate is light saturated and/or nutrient-limited, and the subsequent mobilisation of these polymers when light is limiting or nutrients are re-supplied. Third, it includes feedback between nitrogen and carbon metabolism. The IQ model differs from previous steady-state models of balanced growth by

explicitly considering the time-dependence of biomass and pigment accumulation under non-steady-state conditions of unbalanced growth and by parameterizing regulation in terms of chemical composition ratios.

The basis for the IQ model lies in experimental observations on responses of the photosynthetic apparatus to variations of irradiance and nutrient-limited growth rate obtained as part of this research (Geider, MacIntyre, Graziano and McKay, submitted) and from the literature. We examined the changes in photosynthesis rates, pigment content, Rubisco content and light-harvesting complex protein content of *Dunaliella tertiolecta* (Geider, MacIntyre, Graziano and McKay, submitted) and *Monochrysis lutheri* (unpublished). Changes in light-saturated photosynthesis of *D. tertiolecta* in response to nitrogen- or phosphorus-limitation were associated with changes in the abundance of Rubisco. The initial slope of the photosynthesis-light curve showed less variability than the light-saturated rate, primarily because of compensatory changes in its determinants, the efficiency of light absorption per unit chlorophyll *a* and the quantum efficiency of photosynthesis.

Regulation of Rubisco Activation State

In coastal and estuarine waters, phytoplankton circulating through the mixed layer can be exposed to a low mean irradiance that is punctuated by brief exposures to saturating light. Previous models that relate photosynthesis to changes in irradiance that result from mixing have focused on the time-dependence of light-saturated rates of photosynthesis. However, the time-constants that are incorporated are characteristic of photoacclimation, photoinhibition or both. These processes are associated with the breakdown and synthesis of cellular components, and occur over a period of hours. Consequently, the time constants embedded in previous empirical models match the time-scales of mixing in nearshore systems, where the rate of change of irradiance is comparatively slow, but may not describe the responses in estuarine environments, where the rate of change can be very rapid. Research on photosynthetic responses to variable light fields in vascular plants has shown that one of the limiting steps during changes in the photosynthetic response is the light-sensitive activation and deactivation of Rubisco. In contrast to photoacclimation, this level of regulation depends on the activity of the pool of enzyme rather than the size of the pool and proceeds at rates that may match the rate of change in irradiance for a cell mixed through an estuarine water column.

The proximate regulation of light-saturated photosynthesis by Rubisco activation status was examined in representative microalgae. We optimised assays for Rubisco carboxylase activity (MacIntyre et al. 1997) and applied these assays to examine the rates of activation and inactivation of enzyme activity in laboratory cultures (MacIntyre et al. 1997) and a diatom-dominated natural assemblage (MacIntyre et al. 1996). These studies provide the first measurements of the rates of activation and deactivation of Rubisco in marine microalgae, the first demonstration of variations of activation state *in situ*, and the first evidence for the regulation of phytoplankton photosynthesis during dark-light transitions by Rubisco activation. The time-constants for activation and deactivation in response to step changes in light intensity were used as the basis for a model of photosynthetic response to mixing in a turbid estuary (MacIntyre & Geider, 1997). Because of the short duration of

transitions through the photic zone, the model considers these changes explicitly in terms of the proximate regulation (the activity of Rubisco) and not in terms of the ultimate regulation (changes in the pool size of Rubisco through photoacclimation). The model predicts that in turbid waters (photic zones 0.5 - 1 m in depth), conventional estimates of primary production by *in situ* or simulated *in situ* incubations may overestimate productivity by more than 20%. This prediction compares favourably with published data.

EDUCATION AND TRAINING

Contract DE-FG02-95ER62092 provided partial support for PhD research of Hugh L. MacIntyre.

MacIntyre, H.L. (1996) Photosynthesis in variable light fields: regulation of Rubisco activity. PhD. Dissertation. University of Delaware. 151 pp.

PUBLICATIONS

The results of this research are summarised in eight manuscripts that have been published (or submitted for publication) in peer-reviewed professional journals. The results fall into two categories: (1) experimental studies of the abundance and activity of Rubisco in phytoplankton cultures and coastal waters and (2) mechanistic models of phytoplankton acclimation to variations of irradiance, nutrient availability and temperature. Publications acknowledging support from DOE contracts DE-FG02-95ER62092 and DE-FG02-93ER61695 are listed below and reprints or page proofs of manuscripts that have been published or are in press are included as an appendix to this document.

1. Geider, R.J., H.L MacIntyre and T.M. Kana (1996) "A dynamic model of photoadaptation in phytoplankton." *Limnology and Oceanography*, 41: 1-15
2. MacIntyre, H.L. and Geider, R.J. (1996) "Regulation of Rubisco activity and its potential effect on photosynthesis during mixing in a turbid estuary." *Marine Ecology Progress Series*. 144: 247-264
3. MacIntyre, H.L., Geider, R.J. and McKay, R.M. (1996) "Photosynthetic characteristics of net phytoplankton from Delaware Bay: efficiency and regulation." *Journal of Phycology*. 32: 718-732
4. Geider, R.J., H.L MacIntyre and T.M. Kana (1997) "A dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and chlorophyll a:carbon ratio to light, nutrient-limitation and temperature." *Marine Ecology Progress Series*. 148: 187-200.
5. MacIntyre, H.L., T.D. Sharkey & R.J. Geider (1997) "Activation and deactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in three marine microalgae." *Photosynthesis Research* 51:93-106
6. Taylor, A.H., Geider, R.J. and Gilbert, F. (1997) Latitudinal and depth dependence of the carbon:chlorophyll *a* ratio: results of a modeling study. Submitted to *Marine Ecology Progress Series*. *Marine Ecology Progress Series*. In Press.
7. Geider, R.J., H.L MacIntyre, T.M. Kana and A.H. Taylor (in revision) Cybernetic phytoplankton: a new regulatory model of physiological acclimation of phytoplankton to light, nutrients and temperature.
8. Geider, R.J., MacIntyre, H.L., Graziano, L.,M. and R.M.L. McKay (in revision) Responses of the photosynthetic apparatus of *Dunaliella tertiolecta* to nitrogen- and phosphorus limitation.

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PRESENTATIONS

The results of this research have also been presented at professional meetings of the American Society of Limnology and Oceanography, the British Phycological Society and a NATO Advanced Study Institute on "Molecular ecology of aquatic microbes."

1. MacIntyre, H.L. and R.J. Geider (1994) "Variation in the activity and activation state of the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the diatom *Phaeodactylum tricornutum*." Presented at the February 21-25 1994 Ocean Sciences Meeting in San Diego, California.
2. MacIntyre, H.L. and R.J. Geider (1994) "Primary production and turbulent mixing: time scales of the limiting physiological processes." Presented at the June 12-16 American Society of Limnology and Oceanography/Phycological Society of America Joint Meeting in Miami, Florida.
3. Geider, R.J. (1995) "A dynamic model of algal photoadaptation." Presented at the January 4-6 Annual Meeting of the British Phycological Society. Portsmouth University.
4. Geider, R.J., Kana, T.M. and MacIntyre, H.L. (1996) "A Mechanistic Description of the Temperature, Irradiance and Nutrient Dependence of the Chlorophyll *a*:Carbon Ratio." Presented at the 12-16 February Ocean Science Meeting in San Diego, California. Eos, Transaction American Geophysical Union, Supplement, 76 (3) OS138
5. Geider, R.J. and MacIntyre, H.L. (1996) "Photosynthesis in fluctuating light: role of Rubisco activation state." Presented at the 3-5 January 1996 British Phycological Society Annual Meeting. Lancaster University.
6. MacIntyre, H.L. and Geider, R.J. (1996) The role of Rubisco in photosynthetic regulation. Presented at the 12-16 February Ocean Science Meeting in San Diego, California. Eos, Transaction American Geophysical Union, Supplement, 76 (3) OS113
7. Geider, R.J. (1997) Cybernetic cells: acclimation to light and nutrient limitation. Presented at the January 2-5 1997 British Phycological Society Winter Meeting. University of Sheffield, Sheffield, England.
8. Geider, R.J. and Taylor, A.H. (1997) Variability of the carbon:nitrogen and carbon:chlorophyll ratios in the sea: results of a modeling study using a new description of phytoplankton growth. Presented at the February 10-14 1997 Annual Meeting of the American Society of Limnology and Oceanography, Santa Fe.
9. Kana, T.M., Geider, R.J. and Critchley, C. (1997) A dynamic balance theory of photosynthetic pigment regulation in microalgae by multiple environmental factors. Presented at the February 10-14 1997 Annual Meeting of the American Society of Limnology and Oceanography, Santa Fe.
10. MacIntyre, H.L. and Geider, R.J. (1997) Regulation of Rubisco activity and its potential effect on photosynthesis during mixing in a turbid estuary. Presented at the February 10-14 1997 Annual Meeting of the American Society of Limnology and Oceanography, Santa Fe