Phytoplankton Division Rates in Light-Limited Environments: Two Adaptations

Abstract. *Red tide–forming dinoflagellates maximize cell numbers during periods of low light intensities in two ways. For short-term exposures to suboptimal light intensities such as might occur during recirculation in frontal convergences, cell division rates can be maintained at the expense of stored carbon for up to two generation times. During longer periods, corresponding to subsurface transport below a pycnocline, cell division rates eventually decrease as a portion of the fixed carbon is diverted to replenishing stored carbon. As a result, maximum rates of cell division can be resumed rapidly upon advection into surface waters where light intensities are optimal for growth.*

When light is absorbed by the photopigment system of plants, it provides the free energy required for the assimilation of inorganic carbon. In this way, rates of carbon uptake are dependent on intensities of light. In well-mixed waters, phytoplankton are exposed to changes in light intensities that vary exponentially; in stratified waters, they are exposed to extended periods of suboptimal light intensities. In these environments of transient light, species that can store photosynthetically fixed carbon have higher rates of reproduction during periods when intensities of light are low than do closely related species that lack this ability. A decoupling of division rates and rates of photosynthesis indicates a storage pool of organic carbon in addition to that required for cellular integrity.

The marine dinoflagellate *Gonyaulax polyedra* can retain its maximum rate of cell division for up to two generation times during exposure to suboptimal light intensities, and this rate is independent of the light intensity. During the transition period (phase 1), the total amount of carbon per cell decreases. If the carbon requirement for the maximum division rate, μ_{max}, which is light-independent, is less than the carbon uptake rate, $P(I)$, which is light-dependent (*1*), the stored cell carbon, C_s, is used. Thus for phase 1

$$\frac{dC_s}{dt} = -\mu_{max}C + P(I);$$

$$\mu_{max} > \frac{P(I)}{C}; \qquad C_s \rightarrow 0 \qquad (1)$$

If low light continues after phase 1, the division rate decreases to the light-dependent rate, $\mu(I)$, and a portion of the photosynthetically fixed carbon is used to replenish storage pools at a rate (*2*)

$$\frac{dC_s}{dt} = -\mu(I)C + P(I);$$

$$\frac{P(I)}{C} > \mu(I); \qquad C_s \rightarrow C_s^{max} \qquad (2)$$

where C_s^{max} is the maximum amount of carbon that can be stored. Dinoflagellates migrating between high light intensity, low nutrient surface waters and low light intensity, high nutrient waters at a nutricline (*3, 4*) and dinoflagellates participating in recirculation mechanisms within frontal convergences with time periods of tidal cycles (*5, 6*) maintain their maximum cell division rates. In bloom-forming dinoflagellates that undergo prolonged (weeks) subsurface transport below a pycnocline at light intensities significantly below saturation levels for photosynthesis (*3, 7*), there is a maximum number of cells in the seed population that can rapidly resume their maximum division rates upon vertical advection into higher light intensities in surface waters.

Gonyaulax polyedra cultures were initially adapted to maximum growth rates at light intensities that were saturating for photosynthesis (*8*) and then were exposed to light of eight lower intensities. The rates of photosynthesis measured by short-term (1 to 2 hours) radiocarbon uptake were significantly below those obtained during the period of light saturation. For about 6 days, the specific cell division rates remained at the maximum values (indicated by the constant slopes of the curves for the logarithm of cell concentration plotted against time) (Fig. 1). During phase 1 (Table 1), the division rate for all replicates at 13 light intensities had a mean ± standard error of the mean (S.E.M.) of 0.28 ± 0.01 per day. After day 5, the value of μ decreased, and at low light intensities (for example, for I = 25 μE/m^2-sec), some cell death occurred. After day 10, cultures were reexposed to saturating light intensities (Fig. 1B). Within 1 day (*9*),

Table 1. Specific division rate (per day) and net photosynthesis (in picograms of carbon per cell per hour) of *Gonyaulax polyedra* adapted to saturating light intensities (210 or 180 μE/m^2-sec) for several generations and then transferred to nine lower light intensities. Phase 1 is the duration of light-saturated growth at limiting light intensities. Phase 2 begins on reexposure to saturating light intensities (180 μE/m^2-sec) after the division rate had adapted to the lowered light intensities.

Light intensity during phase 1 (μE/m^2-sec)	Specific division rate (slope ± S.E.)		Net photosynthesis (pg/cell-hour)		Δt, duration of phase 1 (days)	Δt/mini-doubling time*	Specific division rate (slope ± S.E.) during phase 2†
	During phase 1	After light adaptation	During phase 1	After light adaptation			
210	0.28 ± 0.01	0.28 ± 0.01	130	134			
210	0.30 ± 0.01	0.30 ± 0.01					
180	0.26 ± 0.02	0.26 ± 0.02					0.26 ± 0.02
135	0.27 ± 0.01	0.27 ± 0.01	128	132			
88	0.33 ± 0.02	0.17 ± 0.01			4.5	2.0	
85	0.28 ± 0.01	0.14 ± 0.01	94	96	6	2.4	
75	0.27 ± 0.03	0.10 ± 0.01					0.28 ± 0.03
60	0.28 ± 0.01	0.094 ± 0.01	54	58	5	2.0	
47	0.34 ± 0.01	0.048 ± 0.01			4.5	2.0	
41	0.25 ± 0.01	0.03 ± 0.01					0.26 ± 0.02
30	0.25 ± 0.01	0.0	0	0	4	1.6	
25	0.25 ± 0.04	0.0	−10	−10	4	1.6	
25	0.27 ± 0.03	0.0					0.23 ± 0.04

*Number of cell generations required to adapt to the lowered light intensities. †Reexposed to 180 μE/m^2-sec at day 10.

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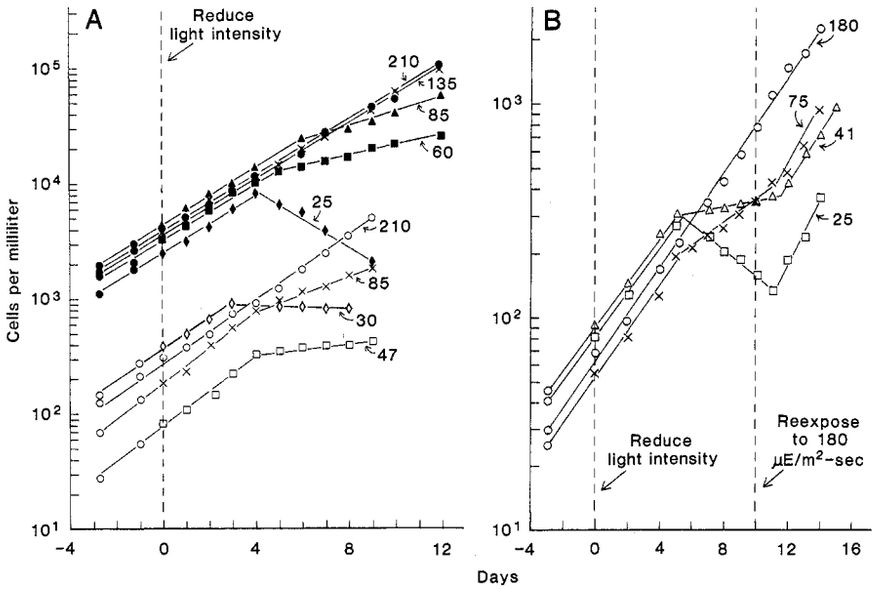

Fig. 1. Growth of *Gonyaulax polyedra* as a function of time at various light intensities. Light intensities in microeinsteins per square meter per second during phase 1 are indicated alongside each curve. (A) On day 0, cultures originally growing at 210 μE/m²-sec (control) were exposed to control and lower light intensities. (B) On day 0, cultures originally growing at 180 μE/m²-sec (control) were exposed to control and lower light intensities. On day 10, cultures were transferred back to 180 μE/m²-sec.

the value of μ for all cultures increased to that of the control cultures maintained at a light intensity greater than that required for saturation (I = 180 μE/m²-sec). The amounts of chlorophyll a and carbon were measured before and during phase 1 for the cultures maintained at light intensities greater than saturating and for cultures maintained at lower light intensities (*10*) (Fig. 2A). In continued saturating light (210 μE/m²-sec), the ratio (*1*) $P_{max}/\mu_{max}C = 1$ (columns 3 and 4 in Table 1 and Fig. 2B). For both reexposure to saturating light and continued exposure to low light after phase 1, $P(I)/\mu(I)C > 1$ (*11*); this ratio decreases to 1 at the rate that storage carbon is replenished (*2*).

The values of $\mu(I)$ and $P(I)$ are similarly dependent on light intensity for long-term steady-state growth (as usually measured in laboratory cultures) (Fig. 2B) (*12*).

The organic carbon requirement for maintenance of respiration as well as for cell division apparently imposes a stringent requirement for maintained stored carbon, even at light intensities that limit growth (*13*). From the shapes of the $\mu(I)$ and $P(I)$ curves (Fig. 2B) the ratio $P(I)/\mu(I)$ for steady-state growth appears to be a constant and $C(I) = C_{max}$ for all values of I (*13–15*). Therefore, after phase 1, the carbon-specific uptake (λ) will temporarily exceed the requirements for cell division, not only at saturating light intensities, corresponding to the observations for N and P uptake (*11*), but also for growth at light intensities that are less than saturating (Table 1),

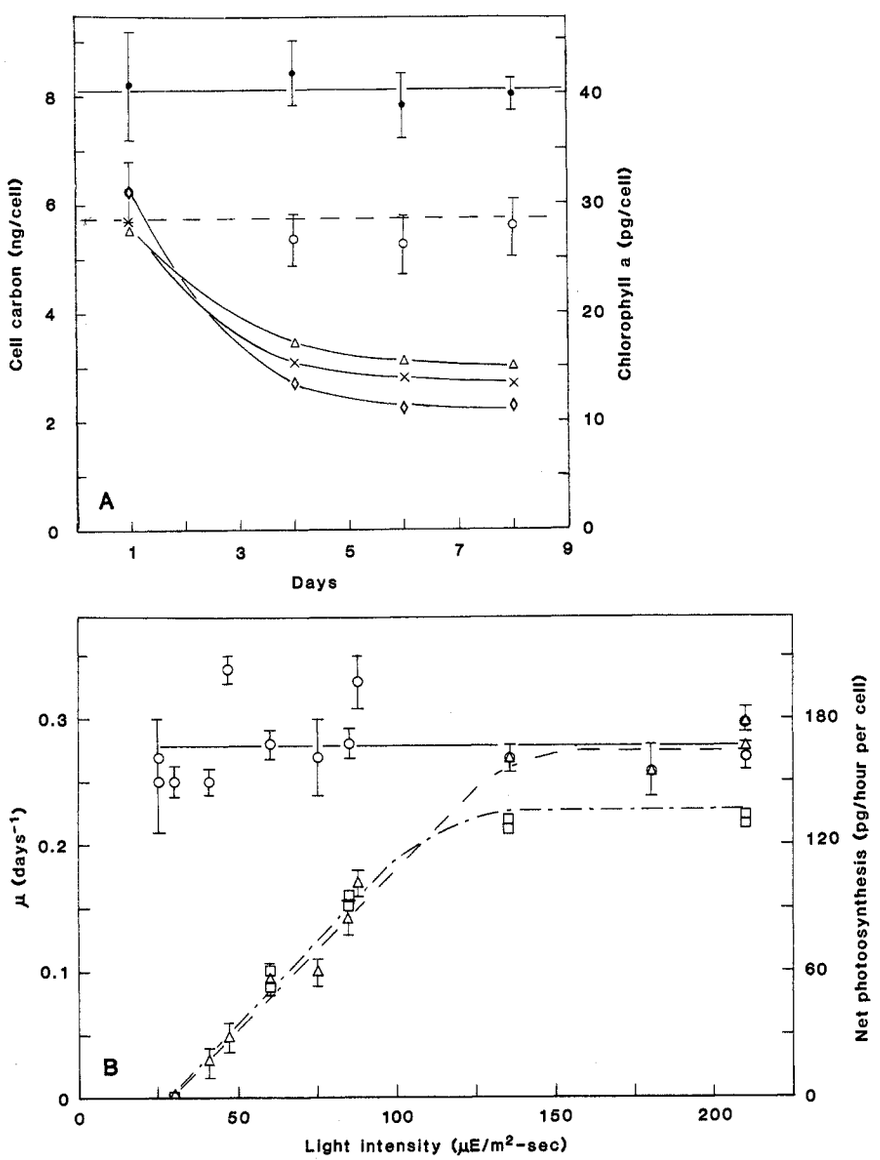

Fig. 2. (A) Chlorophyll a (in picograms per cell) and total cell carbon in nanograms per cell for *Gonyaulax polyedra* during phase 1 (days 0 to 5) and after phase I. Cell carbon (open symbols) is the mean of duplicate determinations (~ 10 percent relative standard error) at (○) 210, (△) 88, (x) 47, and (◇) 30 μE/m²-sec. Chlorophyll a (●) is the mean + S.E.M. (N = 8) for all light intensities. (B) Rectangular hyperbolic curves showing (□) $P(I)$ and (△) $\mu(I)$ as a function of light intensity after adaptation to steady-state growth; (○) $\mu(I)$ as a function of light intensity during phase 1. Vertical bars represent S.E.M.

until the storage pools of carbon are replenished (Eq. 2).

The uncoupling of μ and λ has implications for models of phytoplankton population dynamics. Usually λ in these models is estimated from measurement of $P(I)$ and integration of the $P(I)$ curve over the photic zone, the photophase variation in light intensities, the chlorophyll a concentrations, and an assumed ratio of carbon to chlorophyll a; it is then assumed that $\lambda = \mu$. Usually homogeneity is assumed and therefore phototaxis, recirculation mechanisms, and transport are neglected. The possible diurnal periodicity of photosynthetic capacity in the phytoplankton can introduce a significant error in the estimation of λ (*16*). Finally, our results imply that even though correction of $P(I)$ for diurnal periodicity may provide better estimates of λ, the assumption that $\lambda = \mu$ is not necessarily valid in the natural system. Rather there would be, in changing photic environments, a selection for individuals or species characterized by a flexible metabolism (*17*), capable of allocating cell carbon to maximize this rate of reproduction (*15, 18*) in changing photic environments and thus optimize survival (*19*).

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References and Notes

1. The specific cell division rate, μ, is defined as $\mu = 1/N(dN/dt)$, where N is the cell concentration. The carbon-specific growth rate, $\lambda = P(I)/C$, where $P(I)$ is the rate of photosynthetic carbon uptake per cell and C, total cell carbon, is the sum of storage carbon, C_s, and structural or constitutive cell carbon, C_c. Under steady-state conditions of growth at any light intensity, I, $P(I) = \mu(I)(C_c + C_s^{max})$.
2. The solution of this linear differential equation is $C_s = C_s^{max}\{1 - \exp[-P(I)t/C_{max}]\}$.
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7. H. H. Seliger, M. A. Tyler, K. R. McKinley, in *Toxic Dinoflagellate Blooms*, D. L. Taylor and H. H. Seliger, Eds. (Elsevier, New York, 1979), p. 239.
8. A series of replicate 500-ml Erlenmeyer flasks containing 250 ml of seawater enriched with F/2 [R. R. L. Guillard and J. H. Ryther, *Can. J. Microbiol.* **8**, 229 (1962)] were inoculated with *Gonyaulax polyedra* adapted to high light (210 $\mu E/m^2$-sec) and were incubated at 23° ± 1°C in a photoperiodic cycle of 12 hours of light and 12 hours of darkness with cool white fluorescent illumination of 210 (or 180) $\mu E/m^2$-sec until the specific cell division rates (determined by linear regression of the natural logarithm of the daily cell counts plotted against time) in all the flasks were the same. Replicate pairs of flasks were distributed among eight lower light intensities. Cells were counted in a Palmer-Maloney chamber. As an example of the precision of these division rate measurements, the regression slopes ± S.E. (N = 18) for four replicate flasks at 120 $\mu E/m^2$-sec were 0.22 ± 0.01, 0.22 ± 0.01, 0.22 ± 0.02, and 0.23 ± 0.01 per day. Chlorophyll a, extracted in 90 percent acetone, was measured fluorometrically. Cell carbon was measured in a Perkin-Elmer elemental analyzer (model 240). Photosynthetic carbon uptake was measured by a standard radiocarbon technique [M. E. Loftus, A. R. Place, H. H. Seliger, *Estuaries* **2**, 236 (1979)].
9. The lag appears to be real and may be related to the necessity for replenishment of stored carbon pools.
10. The constancy in the amount of chlorophyll a per cell throughout phase 1 (Fig. 2A) and the parallel shapes of the $\mu(I)$ and $P(I)$ curves (Fig. 2B) contrast with previously published results for *G. polyedra* [B. B. Prezelin and B. M. Sweeney, *Mar. Biol.* **48**, 27 (1978)]. These authors report a μ_{max} of 0.3 per day, chlorophyll a of 36 to 72 pg per cell, and maximum rates of photosynthesis of 150 ng of carbon per cell per hour (O_2 evolution corrected to carbon uptake by the factor 1.2) [B. B. Prezelin and R. S. Alberte, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1801 (1978)] that are almost identical with those in this report. The differences may be due in part to a longer period of adaptation to steady-state conditions for which our experiments were not designed.
11. This pattern of carbon uptake parallels that observed for the uptake of nitrogen and phosphorus [P. M. Glibert and J. C. Goldman, *Mar. Biol. Lett.* **2**, 25 (1981); R. W. Eppley and E. H. Renger, *J. Phycol.* **10**, 15 (1974); G. W. Fuhs, S. D. Demmerle, E. Canelli, M. Chen, in *Nutrients and Eutrophication*; G. E. Likens, Ed. (American Society of Limnology and Oceanography, Lawrence, Kans., 1972), p. 113; B. H. Ketchum, *J. Cell. Comp. Physiol.* **13**, 373 (1939); E. J. Ketchum and B. H. Ketchum *Biol. Bull.* **123**, 134 (1962); R. B. Rivkin, thesis, University of Rhode Island (1979)]. The condition analogous to $P(I)/\mu(I)C > 1$, the uptake of nutrients at rates higher than that required for cell division, has been reported for N- and P-specific uptake in situations where cells are nutrient-limited and exposed to high nutrient concentrations. [J. C. Goldman, J. J. McCarthy, D. G. Peavey, *Nature (London)* **279**, 210 (1979); J. J. McCarthy and J. C. Goldman, *Science* **203**, 670 (1979); R. B. Rivkin and E. Swift, *J. Phycol.*, in press.]
12. The parallel shapes of $\mu(I)$ and $P(I)$ for steady-state growth require that the total amount of carbon per cell be essentially the same for all light intensities.
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14. This constancy in the amount of carbon per cell may be species-specific; the cellular concentrations of total and storage carbon may be light-dependent in some other algae [B. Shuter (*15*); E. A. Laws and T. T. Bannister, *Limnol. Oceanogr.* **25**, 457 (1980)].
15. B. Shuter, *J. Theor. Biol.* **78**, 519 (1979).
16. L. W. Harding, B. W. Messon, B. B. Prezlin, B. M. Sweeney, *Mar. Biol.* **61**, 95 (1981).
17. Selection pressure is the major determinant of metabolism among competing organisms, and the effect of this selection pressure is an optimization of metabolism [R. Rosen, *Optimality Principles in Biology* (Plenum, New York, 1967)].
18. B. C. Goodwin, *Analytical Physiology of Cells and Developing Organisms* (Academic Press, New York, 1976); A. L. Koch, *Adv. Microb. Physiol.* **6**, 147 (1971); R. A. Levins, *Evolution in Changing Environments* (Princeton Univ. Press, Princeton, N.J., 1968); R. H. McArthur and E. R. Pianka, *Am. Nat.* **100**, 603 (1966). The retention of maximum rates of cell division during phase 1 has also been found for the red tide dinoflagellates *Prorocentrum mariae lebouriae* and *Gymnodinium nelsoni* and the chlorophyte *Dunaliella tertiolecta* but not for diatoms *Skeletonema costatum* and *Thalossiosira pseudonana*.
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N-Acetylation Regulates the Behavioral Activity of α-Melanotropin in a Multineurotransmitter Neuron

Abstract. *A multineurotransmitter neuronal system that synthesizes and secretes both acetylated and deacetylated forms of α-melantropin and β-endorphin is present in rat and human brain. The* N*-acetylated form of α-melanotropin had more potent behavioral effects than the deacetylated α-melanotropin. In the case of β-endorphin, however, the deacetylated form has been shown to be more potent than the acetylated form. Enzymatic* N*-acetylation appears to be an important regulatory process for modulating the behavioral activity of peptides secreted from the opiomelanotropinergic multineurotransmitter neuron.*

Neurons have usually been thought to contain and release only one neurotransmitter substance. Recently, however, a number of neurons have been shown to contain more than one putative neurotransmitter. The opiomelanotropinergic neuron is one such multineurotransmitter neuron; this neuron synthesizes and releases at least five neuropeptides—β-endorphin, *N*-acetylated β-endorphin, γ-melanotropin (γ-MSH), and *N*-acetylated and deacetylated α-melanotropin (α-MSH) (*1–3*). All of these peptides are biosynthetically derived from a single precursor protein, pro-opiomelanocortin (*4*). Because α-MSH is formed by endopeptidase cleavage from pro-opiomelanocortin, a deacetylated form of α-MSH is also likely to be present in these neurons and may be one of the α-MSH-related peptides found in brain; deacetylated α-MSH is present in the pituitary glands of a number of species that also synthesize pro-opiomelanocortin (*2, 5*).

To determine if deacetylated α-MSH was present in brain we used high-pressure liquid chromatographic fractionation combined with radioimmunoassays for α-MSH. Fractionation of α-MSH immunoreactive material in rat and human brain demonstrated that deacetylated α-MSH is present in the central nervous

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