The Loss of the Circadian Rhythm in Photosynthesis in an Old Strain of *Gonyaulax polyedra*¹

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ABSTRACT

Cultures of *Gonyaulax polyedra* Stein maintained in the laboratory for 15 to 20 years, including an axenic strain isolated in 1960, have gradually lost the ability to survive in darkness. *G. polyedra* (70A), isolated in 1970 and maintained in a 12:12 light-dark cycle, now tolerates continuous darkness for a much shorter time than a strain isolated in 1981. I have compared the properties of strain 70A with those of this newer strain (81N), to investigate changes in *Gonyaulax* with length of time in culture, which may account for poor survival in darkness. When grown in continuous light (13, 12, or 4.5 watts per square meter), strains 70A and 81N have similar growth rates, yields, cell diameters, protein contents, C/N ratios, respiration rates, pigment complements, and photosynthetic rates. When entrained by a light-dark cycle (12L:12D), 70A showed no photosynthesis rhythm, although such a rhythm was formerly present. However, the circadian rhythms in bioluminescence and cell division were normal in both strains. Thus, the circadian clock is apparently still intact in 70A as in 81N. The rate of photosynthesis in strain 70A was constant at a low level, the consequent smaller accumulation of photosynthetic products probably accounting for the limited survival in darkness. The defect in strain 70A may be the loss of a component either directly affecting *F*ₘₐₓ or necessary for transduction from the circadian clock to photosynthesis.

When cells have been in culture for a long time, the question arises whether they have the same properties as when they were originally isolated from nature. There is some evidence that dinoflagellates such as *Pychodiscus brevis* may become polyploid over years (8). We have maintained cultures of dinoflagellates, particularly *Gonyaulax polyedra*, for many years. One strain, isolated in 1960 and maintained axenically, gradually lost the ability to survive even a short time in darkness, although growing normally under continuous light. This strain was lost during a short electrical failure after more than 20 years in laboratory culture in f/2 medium. Accumulation of harmful bacterial strains was obviously not responsible, since tests for the presence of bacteria made at every transfer were negative. The strain behaved as if photosynthesis was defective in some way. Recently a strain (70A) of *G. polyedra* isolated from the Santa Barbara Channel in 1970, has begun to show the same symptoms. We have investigated this deficiency by comparing the composition and physiological properties of strain 70A with those of a more recently isolated strain of *G. polyedra* first cultured in 1981, also from the Santa Barbara Channel. While most physiological properties were identical in both strains, we report here our finding that photosynthesis in the 70A strain is no longer rhythmic, although the circadian rhythms of bioluminescence and cell division are normal in both strains, showing that a circadian clock is intact in strain 70A. There is evidence that a single clock controls all the circadian rhythms in *Gonyaulax* (5, 9). We discuss the nature of the defect in strain 70A and the possibility that the absence of a circadian rhythm in photosynthesis may account for the poor survival of old strains in darkness.

MATERIALS AND METHODS

Organisms and Conditions of Culture. The marine dinoflagellate, *Gonyaulax polyedra* Stein, strains 70A (UCSB Culture Collection No. 5M20) isolated by the author in 1970 and strain 81N isolated in 1981 by Nan Sterman, both strains from the Santa Barbara Channel, were grown in f/2 (1), with the addition of soil extract (1%) and the omission of silicate. Cultures were illuminated in a plant growth chamber with cool-white fluorescent lamps (GE F20T12CW) on a 12:12 LD ² at 20 to 21°C and 8 to 10 W m⁻² (40X optimizer, United Detector Technology Inc.). For some experiments cultures were grown in constant light (12, 13, or 4.5 W m⁻²) at 21°C.

Physiological Parameters. Photosynthesis and respiration were measured with a Rank O₂ electrode as described previously (6). Cells from 80 ml of a late-log culture (5,000–10,000 cells ml⁻¹) were sedimented by centrifugation for 30 s at 400 g in an International tabletop centrifuge, washed once with an equal volume of filtered sea water, and resuspended in 5 ml f/2 containing 10 mM NAHCO₃. This concentrated cell suspension was placed on the Teflon membrane-covered electrode, which was polarized to 0.7 V. The output from the electrode was recorded on a Soltec strip chart recorder. After the O₂ measurements, the chloroplast pigments were extracted in acetone, and the Chl a and c concentrations were calculated by the equations of Jeffrey et al. (3). The acetone extract containing all the chloroplast pigments was transferred to ether, and the pigments were separated on thin layer cellulose plates (20 × 20 cm. No. 07511, Anateck Inc.), using as solvent 1.5% n-propanol in ligroine. Rₚ values were determined for the principal colored bands. Electron flow from water to methyl viologen was measured in chloroplast-enriched cell extracts as described previously (7), using the O₂ electrode.

Growth rates and yields were determined from cell counts made with a calibrated Palmer-Malloy chamber (1 ml volume). The circadian rhythm in cell division was measured by counting the number of paired cells in a known volume of cell suspension over the end of the dark period and the beginning of the light period as described previously (11). The diameter of cells was measured with a calibrated ocular micrometer and microscope.

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² Abbreviations: LD, light-dark cycle; CT, circadian time, one cycle divided into 24 h, 0 h being taken as the beginning of the light period or the corresponding phase; LL, continuous light; DD, continuous darkness.
PHOTOSYNTHESIS RHYTHM LOST IN AN OLD STRAIN OF GONYAULAX

Table I. Survival of Gonyaulax polyedra Strains 70A and 81N in Continuous Darkness, as Measured (A) by the Ability to Emit Spontaneous Flashes of Bioluminescence, or (B) by Loss of Motility Cells in DD, 21°C.

<table>
<thead>
<tr>
<th>Culture Conditions before DD</th>
<th>Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain 70A</td>
</tr>
<tr>
<td>A. LL (4.5 W m⁻²)</td>
<td>29.3 ± 6.6* (n = 3) 114.5 ± 42.6 (n = 3)</td>
</tr>
<tr>
<td>LD 12:12 (4.5 W m⁻²)</td>
<td>36.5</td>
</tr>
<tr>
<td>B. LL (13 W m⁻²)</td>
<td>69</td>
</tr>
</tbody>
</table>

* sd of the mean.

Table II. Comparison of Physiological Properties of the Old Strain of G. polyedra, Strain 70A, with a More Recently Isolated Strain, 81N

<table>
<thead>
<tr>
<th>Irradiance in Culture</th>
<th>Strain of Gonyaulax</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70A</td>
</tr>
<tr>
<td>W·m⁻² Generation time (days div⁻¹)</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>Yield (cells ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Cell diameter (µm)</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>13</td>
</tr>
<tr>
<td>Protein content (mg cell⁻¹ × 10⁶)</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>Chl a (µg cell⁻¹ × 10⁵)</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>4.5</td>
</tr>
<tr>
<td>Respiration (µM O₂ mg⁻¹ Chl a h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>LD or LL</td>
<td>0</td>
</tr>
</tbody>
</table>

Table III. Chloroplast Pigment Composition of G. polyedra, Strains 70A and 81N

Rₛ values of pigments separated on cellulose thin-layer plates, solvent 1.5% n-propanol in ligroin.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Rₛ</th>
<th>Rₛ 70A</th>
<th>Rₛ 81N</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene</td>
<td>0.93</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Chl a</td>
<td>0.52</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Upper yellow xanthophyll</td>
<td>0.34</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Lower yellow xanthophyll</td>
<td>0.27</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Peridinin</td>
<td>0.13</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Chl c</td>
<td>0.07</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

Protein content was determined by the Lowry method (4) on cells disrupted by vortexing in the presence of glass beads (0.45-0.5 mm diameter from B. Braun Melsungen), as described in Samuelsson et al. (7). For the determination of the C/N ratio, whole cells were collected on glass-fiber filters incinerated prior to use. The determination of relative hydrogen:carbon:nitrogen (H:C:N) was done by Robert Petty in the analytical laboratory of the Marine Science Institute, University of California, Santa Barbara.

Survival time in continuous darkness was determined using two criteria for cell death, inability to emit spontaneous flashes of bioluminescence and lack of motility. Bioluminescence of 1-ml aliquots of cell suspension was measured with a photomultiplier photometer, with or without stimulation by the addition of 0.05 m acetic acid (0.5 ml), as described previously (10). Values for light emission integrated over 30 s were converted to photons per cell, using a ¹⁴C scintillation standard (2).

RESULTS

When we noticed that the 70A strain of Gonyaulax polyedra survived for an unusually short time in darkness, we compared the survival of strain 70A with the more recently isolated strain 81N, as indicated by the ability of cells to emit spontaneous flashes during long-continued darkness. The results (Table IA) confirmed that 70A tolerated continuous darkness for a much shorter time than did 81N. When motility was the criterion (Table IB), times of survival were longer than when the ability to flash was the test for viability, but a definite end point was difficult to determine since cells of both strains moved more and more slowly as the time in darkness increased and more and more cells were immotile.

Cells of both strains were grown in continuous light and compared with respect to a number of physiological properties (Table II). No differences were discovered that could account for the difference in survival in darkness between the two strains. The composition of the cells of both strains was similar (Tables II and III), as shown by their protein and Chl content, C/N ratio, pigment composition, and size. The rates of respiration, photosynthesis, bioluminescence, and growth were also similar.

The first striking difference between the 70A and 81N strains of G. polyedra was apparent when the photosynthesis of cells from a light-dark cycle (LD 12:12) was measured at different times, either in LD or in LL soon after transfer from LD conditions. While 81N showed the expected circadian rhythm in photosynthesis manifested both in α and Pₘₑₓ, the photosynthesis of 70A was low and constant with time (Fig. 1). The rate of electron flow from water to methyl viologen was also constant with cycle time, as measured in LL following entrainment with an LD cycle (Table IV). There was no trace of a circadian rhythm when photosynthetic carbon fixation was measured at different times in one circadian cycle in LL (Table V). A circadian rhythm in both O₂ evolution in whole cells and electron flow through PSII was observed in the 70A strain as late as the end of 1982 (7).

The presence or absence of the circadian rhythms in cell division and bioluminescence in G. polyedra strains 70A and 81N was then investigated. Both strains proved to have normal circadian rhythms in both cell division (Fig. 2) and bioluminescence (Fig. 3). Thus, the circadian clock appeared to be intact and functioning normally in 70A as in 81N.

It is understandable that the level of bioluminescence in strain 70A should be lower than that of strain 81N, because the level of bioluminescence at night is known to depend on the total photosynthesis of the preceding d (12).

DISCUSSION

Previous measurements of O₂ (6, 7) and electron flow (7) provide evidence that photosynthesis in G. polyedra strain 70A was formerly rhythmic. The loss of this rhythm during the years in culture can probably be attributed to the accumulation of...
The deleterious effects served as a reminder that the site of the mutation eliminating the rhythm in photosynthesis in the 70A strain could be in the photosynthetic machinery itself, imposing a limitation on the value of $P_{\text{max}}$. Whole cells and cell-free extracts were able to photosynthesize normally during the night phase and in constant light but were unable to attain the usual midday maximum in both $P_{\text{max}}$ or $\alpha$. The rhythmic increase in photosynthesis during the day phase appears to be required for survival during extended dark periods. This implies that in nature the circadian rhythm in photosynthesis...
nature of the defect seen in strain 70A and responsible for the loss of the rhythm in photosynthesis is that a step in the transduction chain of events by which time information from the clock is transferred to photosynthesis is altered. We are currently comparing the peptides in extracts of strains 70A and 81N prepared at different times in the circadian cycle by SDS gel electrophoresis. It would be of considerable interest to identify a component in transduction between the clock and an overt rhythm, about which nothing is known at present. The existence of a strain without a photosynthetic rhythm but having two other overt rhythms intact may provide an opportunity to identify a step in the transduction from the circadian clock to photosynthesis.

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LITERATURE CITED