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Green light-mediated photomorphogenesis in a dinoflagellate resting cyst

Brian J. Binder & Donald M. Anderson

Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Developmental responses to light are well known and ubiquitous among higher plants[1], but examples of such responses among the algae are less common. Those responses which have been reported in this group are generally photoperiodic or require prolonged light exposures; most involve macrophytes[2]. We now report a non-photosynthetic, low-threshold, photomorphogenic response in a unicellular alga, the dinoflagellate *Scrippsiella trochoidea* (Stein) Loeblich. In contrast to the reported behaviour of most other dinoflagellate species, resting cysts of *S. trochoidea* require light to germinate. This requirement is satisfied to a large extent by low photon fluences delivered in exposures as short as 1 second. Green light is most effective in eliciting the response. Given the importance of dinoflagellates as primary producers in many aquatic ecosystems and the potential role of resting cysts in controlling the dynamics of natural dinoflagellate populations, the present observations are of obvious ecological significance. The primitive phylogenetic standing of dinoflagellates[3], and the relative rarity of green light-mediated photomorphogenic responses in eukaryotes generally[4,5], suggest that this phenomenon may also hold considerable evolutionary and photophysiological interest.

Temperature has been almost universally cited as the environmental factor exerting primary control over germination in dinoflagellate resting cysts[6,7]. When considered, light conditions have generally been found to exert little effect on cyst germination[8-11]. In the two studies to date which have demonstrated a light effect, germination was delayed but not prevented by darkness[12,13]. Preliminary studies with *S. trochoidea* have confirmed that temperature is important in controlling germination in cysts of this species, but have also demonstrated that germination may be significantly reduced in the absence of light[14]. The present study was undertaken to elucidate the influence of light on germination in cysts of *S. trochoidea*.

Scrippsiella trochoidea is a small photosynthetic marine dinoflagellate of widespread neritic distribution. Cysts for the present study were produced in axenic clonal cultures (clone SA10, from Perch Pond, Falmouth, Massachusetts) under a 14:10 h daily light:dark (L:D) cycle at 18 °C[14]. Within a week of the appearance of cysts, the cultures were enriched with inorganic nutrients at $f/2$ levels[15] and placed in darkness at 3 or 18 °C. Every precaution was taken to insure that no further light reached these cultures. Sampling and manipulations, when necessary, were carried out in total darkness.

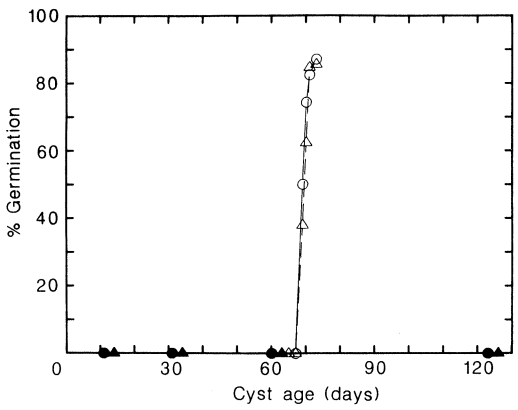

Fig. 1 Germination of *S. trochoidea* cysts placed in the dark at 3 °C (●) or 18 °C (▲) starting at age 7 days. On day 65, some cysts from both the 3 °C and 18 °C dark storage (○, △, respectively) were placed under a continuing 14:10 h L:D cycle at 18 °C.

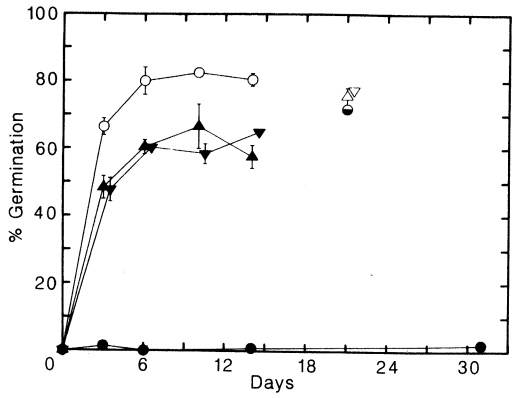

Fig. 2 Time course of germination by cysts (stored at 3 °C in the dark) on transfer to 18 °C and exposure to a continuing 14:10 h L:D cycle (○), to 60 min of light only (▼), to 2 min of light (▲), and to no light (●). On day 14, cysts from the three latter treatments were exposed to the continuing 14:10 h L:D cycle and assessed 6 days later (▽, △, ◒, respectively); means ±1 s.e. ($n = 2$).

Under these strictly dark conditions, cysts stored at 18 °C failed to germinate over the 120 days of the experiment, although conditions should otherwise have been optimal for excystment (Fig. 1). Exposure of these cysts to the standard 14:10 h L:D cycle (~650 μmol photons m^{-2} s^{-1}), at the same temperature, resulted in rapid germination.

An upward shift in temperature commonly results in rapid and complete germination of dinoflagellate cysts which have been stored at low temperature (Fig. 1)[6,7]. In the present case, however, no germination occurred among cysts so treated if they were deprived of all light (Fig. 2). In contrast, cysts exposed to as little as 2 min of light germinated rapidly and only slightly less successfully (though significantly so) than those exposed to the same level of illumination for 14 h daily ($P < 0.001$). The response to 60 min of light was indistinguishable from that in the 2-min treatment.

The relationship between total 'white light' photon fluence and germination underscores the sensitivity of *S. trochoidea* cysts to low levels of light (Fig. 3). A 50% response (based on a maximum achieved germination frequency of 60%) occurs in these cysts at approximately 0.2 μmol m^{-2} photon fluence. This photon fluence corresponds to an exposure time at standard

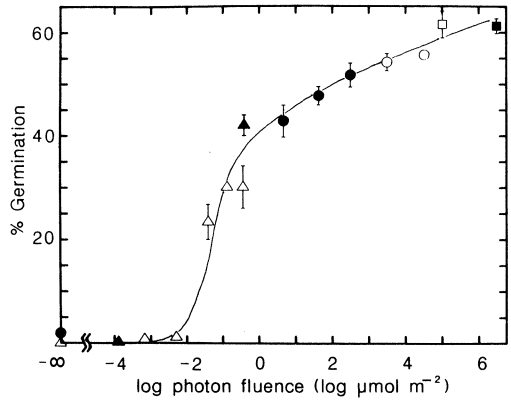

Fig. 3 Effect of 'white light' photon fluence (μmol m^{-2}) on germination frequency. Incandescent source, except □ and ■ (which are taken from Fig. 2), illuminated with a cool white fluorescent source. Photon fluence is the product of exposure time and fluence rate [measured for photosynthetically active wavelengths only (400 nm < λ < 700 nm) with a scalar irradiance meter (Biospherical Instruments, Inc.) and adjusted, as necessary, with neutral density filters]. Exposure times are ▲, 1 s; △, 5 s; ●, 12 s; □, ○, 120 s; ■, 3,600 s; means ± 1 s.e. ($n = 3$, except $n = 6$ for □ and ■). Line drawn by eye.

culturing irradiances (see above) of far less than 1 s.

The germination response appears to be dependent upon photon fluence (μmol m^{-2}), rather than fluence rate (μmol m^{-2} s^{-1}) or exposure time separately. Thus, equivalent germination is elicited by equal photon fluences, whether applied over 10 or 1,000 s (Fig. 4).

These results clearly demonstrate that light is required for germination in *S. trochoidea* cysts. Furthermore, the fact that a low-irradiance exposure lasting 1 s is sufficient to stimulate germination raises the possibility that previous studies with dinoflagellate cysts (including our own), designed primarily with longer-term light effects in mind, may have been unable to distinguish between such a low threshold response and true 'dark' germination.

The response by *S. trochoidea* cysts to the same photon fluence (0.12 μmol m^{-2}) at different wavelengths is shown in Fig. 4. Germination is maximal in the 550-nm (yellow-green) band, with wavelengths above 620 nm being ineffective at this fluence level. The response drops more slowly on the low side of 550 nm, and is still apparent in the blue (450 nm) band. There was no evidence of modification of the 550-nm band response by subsequent exposure to red or far-red light (650 ± 20 nm or 750 ± 20 nm) at equivalent fluence levels (data not shown). The relatively low germination frequency among all the wavelength-band treatments is consistent with the low response achieved in the white-light controls of this experiment (Fig. 4) and therefore cannot be taken to indicate a low response to monochromatic light generally. The cause of the reduced germination in this experiment is not known, but the pattern of response to different wavelength bands was confirmed by preliminary experiments which, however, involved less complete coverage of the spectrum.

Overall, our data indicate that the response to light in *S. trochoidea* cysts is not photosynthetic. This conclusion is based on the low photon fluence requirement of the response and its relative sensitivity to green light as compared with blue or red. The conclusion is further supported by the inability of dark-stored *S. trochoidea* cysts to photosynthesize immediately after their transfer to light[14].

Other non-photosynthetic responses to light which have been reported in dinoflagellates include phototaxis[16–18] and growth inhibition by far-red exposures[19]. Generally, green wavelengths are not active in these phenomena; the photoreceptor systems responsible are therefore probably different from that involved in the light-triggered germination of *S. trochoidea* cysts.

Among algal resting stages generally, germination of cyanobacterial akinetes[20–22] and diatom resting spores[23,24] has been widely found to be light-dependent. In contrast to the present case, the response in akinetes is maximal in the red wavelengths and generally requires extended exposures (hours to days) and high photon fluences[21,25]. Similarly, diatom resting spores are responsive only to relatively high fluence rates[23]. In neither of these cases can photosynthetic involvement be confidently discounted.

The extent to which the unique light requirement described here could control the germination of *S. trochoidea* cysts in the natural system depends on several factors, including the optical properties of overlying waters (note that coastal waters generally transmit maximally in the green band), the extent to which reciprocity in the response holds (that is, the maximum time over which 'photon counting' can occur), and the influence of cyst age and environmental parameters on the response itself. Preliminary calculations, based on conservative assumptions about these factors (including a maximum photon counting time of 1,000 s), indicate that in average coastal waters of moderate depth (~40–80 m) significant germination will occur, although the germination frequency ultimately achieved could be light limited[14]. On the other hand, in particularly deep or turbid waters, light could be sufficiently attenuated to prevent germination completely. Furthermore, the burial of cysts in the sediment

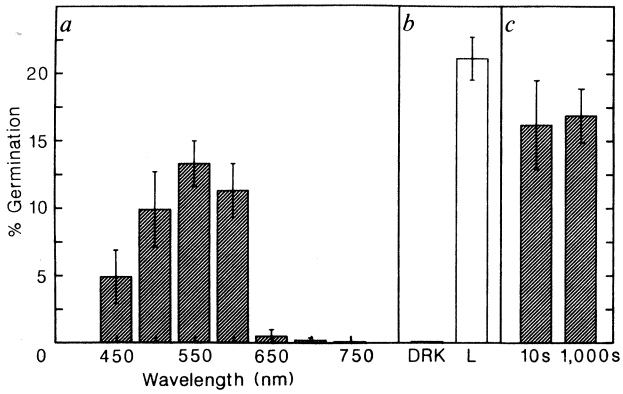

Fig. 4 Germination response of *S. trochoidea* cysts to wavelength. *a*, Bars show the frequency of germination achieved in cysts exposed to equal photon fluences (~0.12 μmol m^{-2}) in seven different wavelength bands. Incandescent source, in combination with wide-band interference filters (40 nm half-power bandwidth, blocking outside of band better than 0.1% between low ultraviolet and 1,000 nm; Ditric Optics Inc.). Exposure time was 10 s in all cases; fluence rate was adjusted with neutral density filters and by varying source voltage; means ± 1 s.e. ($n = 3$); bar width represents half-power bandwidth, on wavelength scale shown. *b*, Open bar is the 'white light' control; unfiltered incandescent source, 4.2 μmol m^{-2} (corresponding to ~0.5 μmol m^{-2} in the 550 ± 20-nm band). DRK bar , the dark control. *c*, Germination response to approximately equivalent photon fluences (~1 μmol m^{-2}, $\lambda = 550 \pm 20$ nm) administered over 10 s or 1,000 s, as indicated.

would probably result in prohibitively low light levels regardless of the depth of the overlying water column. In these latter cases, resuspension from the sediment surface and/or advection to shallower depths would presumably be required before significant germination could occur.

Although the ecological consequences of light-triggered germination in *S. trochoidea* cannot be fully appraised without further information on the physiology of the response, it is clear that light can no longer be ignored in considerations of the behaviour of dinoflagellate cysts in the natural system.

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