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SHORT COMMUNICATION

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Photoperiodic control of germination in the unicell *Chlamydomonas*

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Abstract Photoperiodic time measurement is a well-documented adaptation of multicellular plants and animals to seasonal changes in the environment, but it is unclear whether unicellular organisms can exhibit bona fide photoperiodic responses. We demonstrate that the occurrence of zygospore germination of the unicellular alga *Chlamydomonas* is a genuine photoperiodic response. Germination efficiency is enhanced in long days as compared with short days. While the total amount of light exposure influences the efficiency of germination, the photoperiod is a significant cue for germination. The developmental stage that senses the photoperiod is just prior to mating and during the first days of zygospore development, so there may be a critical window of zygospore maturation that is regulated by photoperiod. Because zygospores are resistant to freezing injury, whereas vegetative cells are not, it is likely that the suppression of germination by short days is an adaptive response for overwintering of *Chlamydomonas*. Therefore, *Chlamydomonas* is a single-celled organism that is capable of photoperiodic responses.

Introduction

Photoperiodic time measurement (PTM) is the ability of plants and animals to sense the season of the year by measuring the duration of the day and/or night in the natural environment and responding appropriately so as to adapt to seasonal changes in their environment (Reppert 1989; Thomas and Vince-Prue 1997). This phenomenon is a fundamental adaptation of organisms to their environment, especially in the cases of reproduction and development. The first demonstration that organisms sense the changing season by measuring the duration of the day or night was in higher plants, where the seasonal flowering of plants was shown by Garner and Allard to be controlled by photoperiod (Garner and Allard 1920). The model for PTM that has become generally accepted is that a circadian (daily) clock is the timer that somehow measures the length of the night and triggers developmental events, as first proposed by Erwin Bünning (1936). When Bünning first proposed this idea, the model seemed too fantastic to be realistic, but subsequent studies supported his idea (Hamner and Takimoto 1964; Thomas and Vince-Prue 1997). A wide range of physiological data in plants and animals supports the hypothesis that circadian clocks and PTM are intrinsically linked, as do more recent genetic analyses (Hamner and Takimoto 1964; Thomas and Vince-Prue 1997; Carré 2001; Johnson 2001).

When considering the effects of day- and night-length on organisms, it is important to distinguish a simple "response to photoperiod" from a bona fide "photoperiodic response." The former is any differential response of an organism to different day- and/or night-lengths, whereas the latter is a response to different day- and/or night-lengths that confers adaptive responses to seasonal changes in their environment. For example, most plants grow more rapidly on long-day photoperiods because they receive more light than on short-day cycles, but this result may have nothing to do with adaptation to season. On the other hand, as first reported by Garner and Allard (1920), the flowering of many plants is determined by photoperiod as an adaptation to season. In particular, there are "short-day" plants that will grow more robustly in long days ("response to photoperiod"), but flower only in short days (bona fide "photoperiodic response").

To date, PTM is well documented in multicellular eukaryotes, but it seems logical that unicellular organisms might also benefit from being able to anticipate and respond to seasonal changes in their environment. Nevertheless, there has been only one report of a photoperiodic response in an unicell, the dinoflagellate alga *Gonyaulax polyedra*. Balzer and Hardeland (1991) reported that *Gonyaulax* cells rapidly form "temporary cysts" in response to cool temperatures and short photoperiods,

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and they hypothesized that these cysts were the first step towards differentiation into a permanent dormant cyst for overwintering. This was an attractive hypothesis because an annual cycle of *Gonyaulax* "permanent cysts" in ocean sediments had been reported by a different group (Anderson and Keafer 1987). However, Balzer and Hardeland (1991) were not able to demonstrate that the temporary cysts could develop into permanent cysts, and *Gonyaulax* cells frequently form temporary cysts in response to stress. Usually permanent cysts that resist poor environmental conditions develop after sexual reproduction, but it has not been possible to induce sexual reproduction in *Gonyaulax* under laboratory conditions. It is therefore possible that the observations of Balzer and Hardeland (1991) were of a stress response (a "response to photoperiod") that is unrelated to an adaptation to seasonal changes of ecological relevance.

From the perspective of the evolution of PTM and responses to season, the question of whether unicellular organisms can accomplish photoperiodic timing is important. Must PTM be an emergent property determined by intercellular interactions within a multicellular organism, or are single cells capable of this complicated timing process? We decided to examine this issue using the well studied unicellular green alga, *Chlamydomonas reinhardtii*. In the life cycle of *Chlamydomonas*, non-optimal conditions (e.g., nitrogen deprivation) provoke the differentiation of haploid vegetative cells into gametes, which mate and form diploid zygospores that are dormant cells which cannot divide until after they undergo meiosis and germinate into four haploid vegetative cells. Zygospores survive stressful environmental conditions, such as darkness, desiccation, and starvation, better than vegetative or gametic cells (Harris 1989), and are therefore a stage of the life cycle in which overwintering might be more successful.

Materials and methods

Strains

This study used a variety of wild-type strains of *C. reinhardtii*. Most of the data reported herein used a 137c strain (mating types mt^+ and mt^-) that had originally been derived from CC-125 (mt^+) and CC-124 (mt^-) and crossed repeatedly by S. Nakamura (University of the Ryukyus, Japan) and C. Sato (Kagawa Medical School, Japan). Wild-type strains that have recently been isolated from the field were CC-2935, CC-2936, CC-2937 and CC-2938 (Sack et al. 1994) and these were obtained from the *Chlamydomonas* Genetics Center (Duke University, N.C., USA).

Culture conditions for mating and germination

Cells were maintained on Tris-acetate-phosphate (TAP; Harris 1989) medium containing 1.5% agar under constant light (LL) at 22°C. To induce gametes, cells of both mating types were cultured on 1/4 NH_4 TAP agar medium (this is TAP medium in which the NH_4Cl concentration is reduced to 0.1 g/l) for 3 days under each experimental light/dark condition (LD = light/dark; LD 12:12 means 12 h light/12 h dark, LD 9:15 means 9 h light/15 h dark, and so on). The cells were harvested into mating buffer (0.6 mM $MgCl_2$, 1.2 mM HEPES, pH 6.8), and the cell density was adjusted to $1–5\times10^7$ cells/ml. The cells from both mating types were incubated in mating buffer for 3 h, then the two mating types were mixed to allow mating. One hour after mating, zygospores were plated on 4% agar TAP medium and incubated for 2 days under the various light conditions described below. To evaluate germination efficiency, plates of 2-day-old zygospores were scraped with a razor blade to remove the vegetative cells that had failed to mate (zygospores stick to 4% agar, but vegetative cells do not). Then zygospores were transferred using a glass needle to plates of TAP medium (2% agar) under a binocular microscope (125 zygospores per plate). For several days after mating (if not otherwise specified, up to the tenth day), the number of zygospores that had germinated were counted using the binocular microscope, and the mean percentage of germination was calculated based on data from 7–18 plates, depending upon the particular experiment. To confirm that the germination rate had reached a plateau level, we counted cells for several days before and after the standard evaluation day (day 10 after mating).

Freezing resistance

Vegetative cells (mt^+ and mt^-) were spread on TAP plates to a density of 1×10^8, 1×10^7, and 1×10^6 cells per plate. Some plates were inoculated to a density of ~10^7 cells per plate and then cultured for 4 days in LL (22°C) until a density of >5×10^8 cells per plate had been reached. Zygospores were spread on TAP plates 90 min after mating to a density of 5×10^6 zygospores per plate (these zygospores were produced from gametes grown and mated in LL with a mating efficiency of 94%). Zygospores were allowed to mature for 2 days in LL. Then both vegetative plates and zygospore plates were frozen at –3°C. After 5 days at –3°C, plates were placed at 22°C in LL for 10 days.

Light and temperature conditions

White fluorescent lamps were used as a light source at an intensity of 50 $\mu E\ m^{-2}\ s^{-1}$ (except for the experiments depicted in Fig. 1E where the intensity was modulated by changing the distance between the fluorescent lamp and the plates). Unless otherwise specified, temperature was 21–22°C.

Statistical tests

All of the germination efficiency data are plotted with standard error (SEM) bars calculated from the germination rate on 7–18 plates. Statistical tests (ANOVA followed by Tukey-Kramer's all-pairs comparison) were calculated with the computer program, JMP IN, version 3.

Results

Germination efficiency in different photoperiods

Induction of sexual reproduction is an indispensable tool for genetic analysis in *Chlamydomonas* (Harris 1989). A typical standard germination protocol (SGP) for inducing hatching of zygospores is to expose newly mated zygospores to constant light (LL) for one day, followed by constant darkness (DD) for several days (e.g. 6 days), and then to return the zygospores to LL (Harris 1989). About 12–16 h after the final transfer to LL, zygospores initiate meiosis and germination from the thick zygotic cell wall. This protocol allows germination to occur synchronously and with high efficiency (Fig. 1A, C, "SGP"). Exposure to LL continuously after mating also

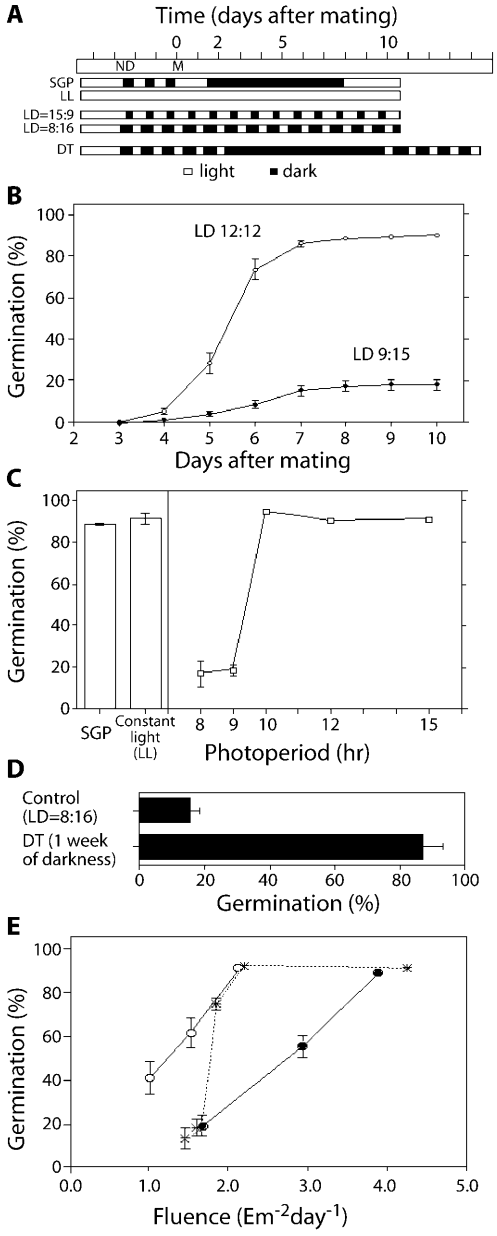

Fig. 1A–E Germination efficiency in different photoperiods. **A** Different photoperiodic conditions: *Chlamydomonas* cells were cultured on TAP medium (Harris 1989). To induce gametes, cells of both mating types were transferred to TAP agar medium in which the ammonium concentration was reduced to 1/4 of the original level (nitrogen deprivation). The two mating types were mixed to allow mating and subsequent production of zygospores (mating). Standard germination protocol (*SGP*), a four-step standard laboratory protocol for inducing synchronous germination for genetic analysis, was used: (1) gametes were produced in LD 12:12 up to the time of mating, (2) zygospores were placed in LL for one day after mating, (3) transferred to constant darkness (*DD*) for six days, and (4) placed in LL for germination. Constant light (*LL*) represents cells exposed to light for 24 h a day instead of LD cycles. *Black bars* indicate darkness, *light bars* indicate light. *ND* time of nitrogen deprivation, *M* time of mating, *DT* dark treatment protocol. **B** Germination kinetics: germination of zygospores of strain 137c (mt^+/mt^-) was monitored in long-day conditions (LD 12:12, *open circles*) and in short-day conditions (LD 9:15, *filled circles*). Zygospores were plated on agar medium and the number of zygospores that had germinated were counted every day for 10 days using a binocular microscope. The SEMs were calculated from 10 (LD 12:12) and 18 (LD 9:15) plates. The intensity of light during the illuminated intervals was constant at 50 µE m^{-2} s^{-1}, and the temperature was maintained at 21–22°C. These data are the same as those plotted in panel **C** for 12- and 9-h photoperiods. **C** Photoperiodic response curve: the germination efficiencies 10 days after mating are shown as function of different conditions. For the photoperiodic response curve (*right part of panel C*), the variable condition is the light duration (photoperiod) in light/dark cycles of 24 h total duration. The data shown in panel **C** are the percentage germination at 10 days after mating (for SGP zygospores, this is 3 days after the final transfer to LL). All data are shown with SEM bars calculated from 8–18 plates; for data without apparent error bars, the errors were too small to appear on this graph. Light intensity 50 µE m^{-2} s^{-1}, temperature 21–22°C. **D** Release from short-day suppression of germination: "*Control*" is the germination efficiency of zygospores under short-day conditions (LD 8:16). "Dark treatment" (*DT*) is the germination efficiency of cells grown and mated under LD 8:16, transferred to constant darkness (*DD*) 2 days after mating, maintained in DD for 1 week, then returned to LD 8:16. The control cells were counted 10 days after mating and the dark treatment cells were counted 5 days after the dark treatment ended (14 days after mating). *Error bars* are SEM (n=10 plates each). **E** Germination efficiencies in different photoperiods and different light fluences: the *dashed line* (with *asterisks*) is a photoperiodic response curve experiment performed as in **C**, but plotted as a function of fluence [photoperiods from left to right: LD 8:16, 9:15, 10:14, 12:12, and 24:0 (LL)]. Those photoperiodic response curve data are compared with measurements in which the light intensity was varied in either a long-day versus a short-day light/dark cycle. In long-day conditions (LD 12:12), the light intensities were 23, 35, and 50 µE m^{-2} s^{-1} (total fluences are 0.99, 1.5, and 2.1 E m^{-2} per day, *open circles*). In short-day conditions (LD 9:15), the light intensities were 50, 90, and 120 µE m^{-2} s^{-1} (total fluences are 1.6, 2.9, and 3.9 E m^{-2} per day, *filled circles*). All data are plotted as the light fluence in a day, calculated as irradiance (light intensity, µE m^{-2} s^{-1}) × (duration of light treatment in seconds), and are shown with SEM error bars (n=7–9 plates each). Temperature 21–22°C

allowed a high percentage of germination (Fig. 1A, C, "LL"). However, neither LL nor SGP are conditions that *Chlamydomonas* would encounter in the natural environment. We wanted to determine whether the germination efficiency of *Chlamydomonas* zygospores varied as a function of the different photoperiods that the cells might experience in nature. Therefore, the germination efficiency was compared in various day and night length conditions (always with a 24 h period) at constant temperature.

In all the conditions we tested, germination began 4 or 5 days after mating, reaching a maximum at approximately 7 days after mating (LD 12:12 and LD 9:15 conditions are shown in Fig. 1B). In a wild-type strain (137c; see Materials and methods), the germination efficiency at 10 days after mating was as high for zygospores exposed to long-day LD cycles, i.e., LD 15:9, 12:12, 11:13, or 10:14, as for zygospores exposed to constant light conditions or the standard germination protocol used for genetic analysis (Fig. 1C). However, the germination rate plummeted in short-day conditions, i.e., LD 9:15 and 8:16. The overall means of the data depicted in Fig. 1C were significantly heterogeneous as assessed by an ANOVA (P<0.0001, F ratio = 208.2, df = 6, 69). A subsequent Tukey-Kramer's all-pairs comparison indicated that the germination rates in the long-day

group [LD 15:9, 12:12, 11:13, and 10:14 (including LL and SGP)] were statistically equivalent ($P>0.05$), and the rates in the short-day group (LD 9:15 and 8:16) were also equivalent ($P>0.05$), but that all long-day group means were significantly different from all short-day group means ($P<0.05$). To ascertain that these differences were not due to a peculiar response of a particular strain, we tested other wild type strains – CC-2935, CC-2936, CC-2937, and CC-2938 – all of which had been recently isolated from the field (Sack et al. 1994). These other four strains exhibited the same pattern of responses to various photoperiods (unpublished data). These data are consistent with the interpretation that germination and/or zygospore maturation in *Chlamydomonas* might be a "long-day" photoperiodic response.

Zygospores could be maintained for at least 30 days in short-day conditions without an increase in the germination frequency (unpublished data). Zygospores that were induced and maintained for 10 days in short-day conditions did not increase their germination efficiency if they were subsequently transferred to either long days or LL. These short-day zygospores were not dead, however – if they were transferred to DD for 7 days, then placed in LD conditions, they germinated with high efficiency (Fig. 1D).

Germination efficiency is a function of photoperiod and fluence

Although the germination efficiency showed differential responses to different photoperiods, we cannot yet conclude that germination and/or maturation of *Chlamydomonas* zygospores is a true photoperiodic response. Because *Chlamydomonas* is photosynthetic and therefore depends upon light energy, it is possible that germination was merely inhibited by a shortage of photosynthetic energy in short days, and not specifically by differences in the daily durations of light or darkness. Therefore, to distinguish between the complementary effects of light dosage versus photoperiod, we varied the light intensity on a long-day photoperiod and on a short-day photoperiod (from 23 to 120 $\mu E\ m^{-2}\ s^{-1}$; see Fig. 1E). The germination efficiency was then compared as a function of fluence per day versus the duration of the photoperiod.

The germination rate of zygospores from both long-day (LD 12:12) and short-day (LD 9:15) conditions exhibited a direct response to light intensity – the brighter the light, the higher the germination efficiency (Fig. 1E). Therefore, bright light can overcome the suppressing effect of short days. The fluence curves for LD 12:12 (open circles) and LD 9:15 (filled circles) were parallel, but displaced from each other. These data indicate that the germination efficiency is sensitive to fluence *and* to photoperiod (if *Chlamydomonas* zygospores did not respond to photoperiod differentially, the LD 12:12 and LD 9:15 curves should have superimposed when plotted as a function of fluence, as in Fig. 1E). Moreover, when the photoperiodic response curve (using a constant light intensity of 50 $\mu E\ m^{-2}\ s^{-1}$, a repeat of the experiment depicted in Fig. 1C) is plotted as a function of fluence (dashed line), there is a clear discontinuity between the short-day conditions that plot with the LD 9:15 data and the long-day conditions that plot with the LD 12:12 data; the discontinuity occurs at a fluence of about 1.7 (1.62–1.80) $E\ m^{-2}$ in a single day.

Night break experiments

One of the common protocols for characterizing photoperiodism in plants is a "night-break," or night interruption experiment (Thomas and Vince-Prue 1997). The design of night-break experiments is to give a short duration photoperiod in which the long night is interrupted with a light pulse. Short-day responding plants often perceive the pulse as dawn, ignore the dark interval between pulse and photoperiod, and thereby evoke a long-day response to a night-break protocol. In long-day responders (as appears to be the case for *Chlamydomonas* germination), the response to a night-break can be either a long-day response (a so-called "dark-dominant" response) or a short-day response (a so-called "light-dominant" response) (Thomas and Vince-Prue 1997). When we tested *Chlamydomonas* germination under such a protocol, we observed that the cells responded to night-breaks and to short days similarly (i.e., low germination; unpublished data). Therefore, *Chlamydomonas* germination appears to fall into the "light-dominant, long-day" response category (Thomas and Vince-Prue 1997).

A developmental window of photoperiodic sensitivity

From gametogenesis through zygospore development and germination, *Chlamydomonas* cells undergo significant morphological and physiological changes, including the switching of expression of specific sets of genes on and off (Ferris and Goodenough 1987; Wegener and Beck 1991; von Gromoff and Beck 1993; Uchida et al. 1993; Kuvari et al. 1998). In defining the ecological significance of photoperiodic control over germination, it is important to clarify which developmental stage perceives photoperiodic differences that influence germination efficiency. To answer this question, photoperiods were exchanged between short-day and long-day conditions at various times before and after mating.

If zygospores that had been raised in LD 8:16 were placed in LD 15:9 two days after mating, they exhibited a short-day response (Fig. 2). If the transfer to long days occurred earlier, the zygospores germinated in a long-day pattern. The opposite protocol also suggested that the developmental interval between 1-day-premating to 2-days-postmating was a window in which the photoperiodic exposure determined the germination response (Fig. 2). The long-day data at the bottom of Fig. 2 ("2 or 3 long days") support this conclusion, but the short-day data at the very bottom of the Fig. 2 ("2 or 3 short days")

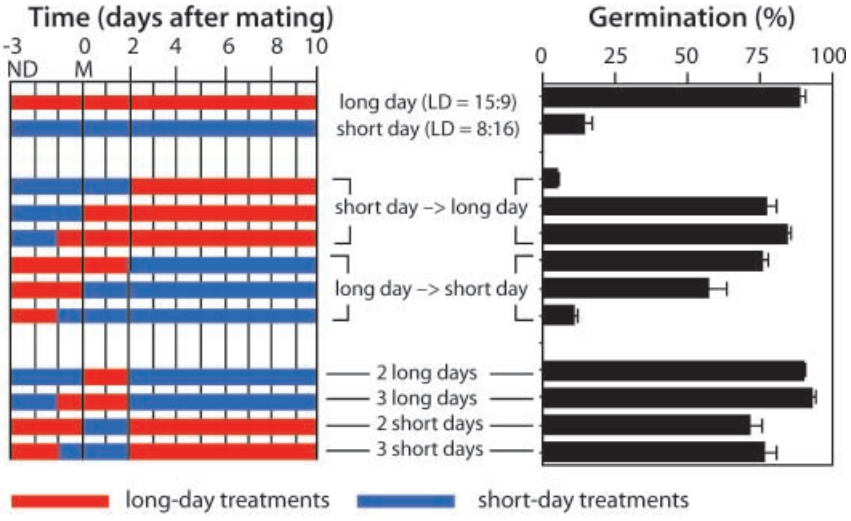

Fig. 2 Assessment of photoperiod-sensitive stage. The *left panel* represents the photoperiodic conditions from 3 days before to 10 days after mating. *Vertical lines* indicate days (*ND* time of nitrogen deprivation, *M* time of mating). *Blue hatched boxes* represent short-day conditions (LD 8:16) and *red shaded boxes* represent long-day conditions (LD 15:9). The *right panel* shows the germination efficiencies for the corresponding photoperiodic conditions shown in the left panel. All germination data are shown with SEM calculated from 10 plates each. Light intensity 50 µE m^{-2} s^{-1}, temperature 21–22°C

indicates that the window is not absolute. The data of Fig. 2 suggest that there is a critical window for photoperiodic perception in the early phase of zygospore development, but that this window is conditional, and that sufficient long-day exposure can overcome a brief 2- or 3-day short-day exposure that is centered on this window. The data also indicate that the stage(s) of the *Chlamydomonas* life cycle that is(are) regulated by photoperiod might not be germination per se, but some earlier event(s) in zygospore maturation.

Zygospores are resistant to freezing

The data shown in Fig. 1 implied that photoperiodic control of germination efficiency could be a response to distinguish fall/winter seasons from spring/summer seasons, suggesting that zygospores might exhibit improved survival over the winter. It is well known that zygospores resist stressful conditions such as desiccation and starvation (Harris 1989); however, to our knowledge there is no definitive evidence that indicates that zygospores are better able to withstand cold temperatures than are vegetative cells. At 4°C, the viability of vegetative cells in liquid medium and the germination efficiency of zygospores on plates did not change for at least 1 month in

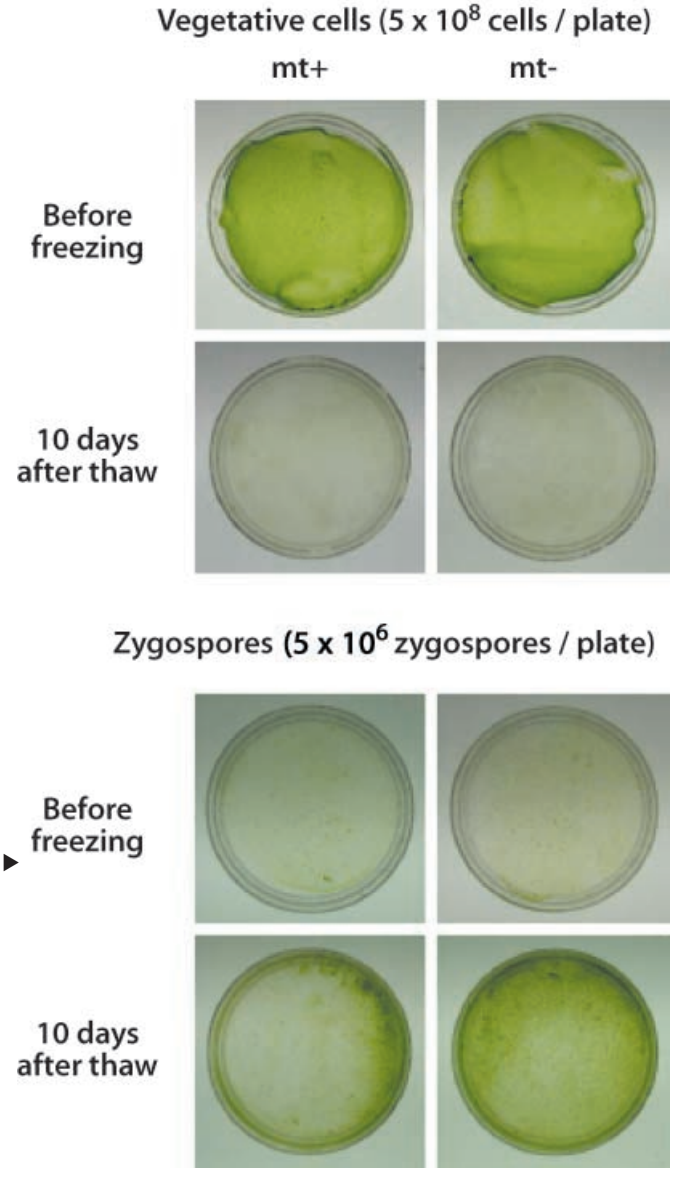

Fig. 3 Zygospores can survive freezing conditions better than vegetative cells. *Upper panels*, vegetative cells; *lower panels*, zygospores. *Upper two plates* of each panel: plates of cells and/or zygospores prior to freezing (plating densities: >5×10^8 vegetative cells per plate, 5×10^6 zygospores per plate). *Lower two plates* of each panel: the same plates after 5 days at –3°C, followed by 10 days at 22°C in LL. Dying cells first turn white and then lyse, whereas healthy cells remain green. Duplicate plates are shown, and the experiment was repeated once with equivalent results (for vegetative cells, results are from a total of 24 plates: Expt 1: four mt^+ plates and four mt^- plates; Expt 2: eight mt^+ plates and eight mt^- plates; for zygospores, results are from a total of 12 plates: Expt 1: four plates; Expt 2: eight plates)

darkness (unpublished data). However, when we tested the resistance to freezing of zygospores versus vegetative cells, a clear difference emerged. In this test, more than 5×10^8 vegetative cells or 5×10^6 zygospores were frozen at –3°C for 5 days on plates, then thawed and cultured for 10 days at 22°C in LL (Fig. 3). The vegetative cells appeared to die immediately after the thaw (or died during the freeze), because the color of the thawed plates soon changed from green to white and growth on the plates never recovered. The results with smaller inoculum sizes of vegetative cells (10^8, 10^7, and 10^6 cells/plate) were equivalent to those shown in Fig. 3 (unpublished data). Therefore, vegetative cells cannot survive this freezing protocol, and this observation was not caused by high cell density or deprivation of nutrients.

On the other hand, zygospore plates were also white in the first few days after the thaw (perhaps due to the death of unmated vegetative cells), but the zygospores subsequently germinated and green cells grew up during the 10-day incubation at 22°C (Fig. 3). The percentage of zygotic germination was somewhat variable. On vegetative cell plates, there were no detectable colonies after freezing on plates inoculated with 5×10^8 cells. This means that the survival was less than one cell in 5×10^8 cells (and possibly much less than that). On zygospore plates, we estimate that roughly 10–1,000 zygospores survive freezing and germinate on plates that have been inoculated with 5×10^6 zygospores (it is difficult to estimate this number exactly because the frozen/thawed plates collect liquid medium on the agar in which the germinated cells swim and consequently do not form discrete colonies). Therefore, on average one zygospore out of approximately 10^4 zygospores survives freezing and germinates. Therefore, zygospores survive this freezing protocol at least 10^4 times better than vegetative cells. All of the zygotic plates had some growth, whereas there was no growth on any of the vegetative plates subjected to this protocol. These results indicate that zygospores can survive freezing, but vegetative cells cannot.

Discussion

We provide here evidence for a photoperiodic response in an unicellular organism that has clear potential for adaptation to seasonal changes. Zygotic development is a well-characterized stage of the *Chlamydomonas* life cycle from which germination restarts the vegetative stage. Even zygospores produced under short-day conditions can germinate with high efficiency under appropriate conditions (Fig. 1D). Furthermore, although germination efficiency can be influenced by the intensity of light to which *Chlamydomonas* is exposed, it is clear that cells in low-to-medium intensities of light germinate in response to the duration of the photoperiod (Fig. 1E). Because *Chlamydomonas* cells are frequently found in dim environments (soil or ponds), these intensities are ecologically relevant. Finally, there appears to be a critical, but not absolute, developmental window for the perception of short-day versus long-day photoperiod that determines the likelihood of rapid versus suppressed germination. This window is approximately from the time of mating through the second day of zygotic development, implying that some step(s) in the maturation of zygospores is(are) regulated by photoperiod (Fig. 2). Therefore, even though germination is the endpoint we measured, it is probable that a step earlier in the zygospore maturation process is the time at which photoperiod is perceived and the commitment is made for either rapid germination (long days) or suppression of germination (short days).

Our hypothesis is that the photoperiodism we describe herein is a light-dominant, long-day response that facilitates the survival of *Chlamydomonas* cells through stressful conditions. For example, zygospores survive freezing much better than vegetative cells (Fig. 3), and there may be other stress conditions that zygospores may resist more effectively (for instance, desiccation). In the case of cold temperatures, the response we report may help *Chlamydomonas* to overwinter. For example, if the cells encounter starvation conditions in the fall, the mating of gametes will produce zygospores that are exposed to short-day conditions during the critical "decision" window. The strain of *Chlamydomonas* that we have primarily used was originally isolated from Amherst, Massachusetts, which is at a latitude of 42°N (Harris 1989). This locale first experiences LD 10:14 in early November. Therefore, in Massachusetts young zygospores will experience a photoperiod in early November that suppresses germination in 75% of the zygospores. The suppression of germination by photoperiod, probably coupled with lower temperatures, means that *Chlamydomonas* cells are likely to overwinter as zygospores, which are better able to survive low temperatures (Fig. 3). The germination abilities of these zygospores can be rescued (as in Fig. 1D), so they are not terminal. After extended winter exposure to low temperatures or dark (Fig. 1D), the wintering zygospores germinate in response to warmer springtime temperatures and longer photoperiods.

We have not tested the interactions between photoperiod and temperature (other than those shown in Fig. 3), but the above hypothesis is consistent with the known flowering responses of plants (Thomas and Vince-Prue 1997). In any case, we have demonstrated that the unicell *Chlamydomonas* is capable of bona fide photoperiodic responses that are relevant developmentally and ecologically. Further research may uncover other examples of unicells besides *Chlamydomonas* (and *Gonyaulax*) that have true photoperiodic responses.

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References

Anderson DM, Keafer BA (1987) An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. Nature 325:616–617

Balzer I, Hardeland R (1991) Photoperiodism and effects of indoleamines in a unicellular alga, *Gonyaulax polyedra*. Science 253:795–797

Bünning E (1936) Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. Ber Dtsch Bot Ges 54:590–607

Carré IA (2001) Day-length perception and the photoperiodic regulation of flowering in *Arabidopsis*. J Biol Rhythms 16:415–423

Ferris PJ, Goodenough UW (1987) Transcription of novel genes, including a gene linked to the mating-type locus, induced by *Chlamydomonas* fertilization. Mol Cell Biol 7:2360–2366

Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment of growth and reproduction in plants. J Agric Res 18:553–606

Gromoff ED von, Beck CF (1993) Genes expressed during sexual differentiation of *Chlamydomonas reinhardtii*. Mol Gen Genet 241:415–421

Hamner KC, Takimoto A (1964) Circadian rhythms and plant photoperiodism. Am Nat 98:295–322

Harris E (1989) The *Chlamydomonas* sourcebook. Academic Press, San Diego, Calif.

Johnson CH (2001) Endogenous timekeepers in photosynthetic organisms. Annu Rev Physiol 63:695–728

Kuvari V, Grishin NV, Snell WJ (1998) A gamete-specific, sex-limited homeodomain protein in *Chlamydomonas*. J Cell Biol 143:1971–1980

Reppert SM (1989) Development of circadian rhythmicity and photoperiodism in mammals. Perinatology Press, Ithaca, N.Y.

Sack L, Zeyl C, Bell G, Sharbel T, Reboud X, Bernhardt T, Koelewyn H (1994) Isolation of four new strains of *Chlamydomonas reinhardtii* (Chlorophyta) from soil samples. J Phycol 30:770–773

Thomas B, Vince-Prue D (1997) Photoperiodism in plants, 2nd edn. Academic Press, San Diego, Calif.

Uchida H, Kawano S, Sato N, Kuroiwa T (1993) Isolation and characterization of novel genes which are expressed during the very early stage of zygote formation in *Chlamydomonas reinhardtii*. Curr Genet 24:296–300

Wegener D, Beck CF (1991) Identification of novel genes specifically expressed in *Chlamydomonas reinhardtii*. Plant Mol Biol 16:937–946