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Gametic Differentiation of *Chlamydomonas reinhardtii*[1]

Control by Nitrogen and Light

Christoph F. Beck* and Axel Acker

Institut für Biologie III, Albert-Ludwigs-Universität, Schänzlestraße 1, D-7800 Freiburg, Federal Republic of Germany

ABSTRACT

Gametic differentiation of the unicellular green alga *Chlamydomonas reinhardtii* proceeds in two steps controlled by the extrinsic signals nitrogen deficiency and light. Nitrogen deprivation induces the differentiation of vegetative cells to sexually immature pregametes. A light signal is required to convert the pregametes to gametes. Both signals are also required for the maintenance of mating competence. Two converging signal transduction chains are proposed to control gamete formation. For the differentiation of pregametes to gametes, a fluence rate-dependent reaction, requiring continuous irradiation, is suggested by photobiological experiments.

By gametic differentiation, *Chlamydomonas reinhardtii* vegetative cells are transformed into mating-competent cells. This differentiation has been shown to be associated with changes in the cell's biochemistry and subcellular morphology. The most prominent changes at the molecular level have been demonstrated for the ribosomes. Cytoplasmic and chloroplast ribosomes decrease in number (7, 8, 13) and new ribosomes appear that exhibit decreased translational accuracy (2), altered susceptibility to antibiotics (9), and ribosomal proteins with different electrophoretic mobilities (9). Morphologically, the acquisition of two new organelles, a mating structure and a special type of Golgi-derived vesicle are most prominent (3, 7). These and other changes, *e.g.* the appearance of sexual agglutinins (1, 11, 19) and the synthesis of gametic autolysin (12, 16) suggest that gametic differentiation is based on changes in the pattern of gene expression. Characteristic differences in the rates of synthesis of individual proteins (15) and the accumulation of mRNA species specific for gametes (von Gromoff, C.F. Beck, unpublished observations) nitrogen have been observed.

Gametogenesis of vegetative cells is induced by the removal of a utilizable nitrogen source (usually NH_4^+) from the culture medium (4, 10). We have shown previously that light is the second essential signal required for gametic differentiation (14). Cells could be converted to mature gametes by irradiation with light only after extended incubation in nitrogen-free medium in the dark (14). The first step in gametic differentiation, triggered by nitrogen starvation, may thus be uncoupled temporally from the second step, which is induced by light. The light-competent intermediates in gamete formation were termed pregametes. Inhibitors of cytoplasmic protein synthesis and an inhibitor of RNA synthesis prevented the differentiation of pregametes to gametes (14). This indicates an effect of light at the level of gene expression during the conversion of pregametes to gametes. From the action spectrum, we deduced that a blue light/UV-A photoreceptor is the sensor for light required for the differentiation of pregametes to gametes (18).

Here we report that mating competence is generated and maintained only in the presence of both extrinsic signals—lack of a utilizable nitrogen source and light. Photobiological studies show that a quantitative conversion of pregametes to gametes requires continuous stimulation of the photoreceptor. The kinetics of this conversion is shown to be controlled by the fluence rate.

MATERIALS AND METHODS

Strains and Culture Conditions

Chlamydomonas reinhardtii wild-type strains 137c of mating types (mt) + and − were used. The experiments were performed with 137c mt− as tester strain, whereas 137c mt+ served as mating partner. The growth conditions were as described previously (14, 17).

Procedures for Gametogenesis

Gametic differentiation was induced as described (14) with the following alteration: vegetative cells of 137c + and 137c − were resuspended in nitrogen-free medium to densities of 1×10^7 and 4×10^6 cells/mL, respectively; 10-mL portions of cells in 100-mL glass beakers were used in all experiments. Pregametes were generated by incubation of the cells with shaking (120 rpm) in the dark for 19 h.

Illumination Protocol

During illumination cells were continuously agitated at 120 rpm. White light at 73 μmol m^{-2} s^{-1} was provided by fluorescent tubes (Osram L 36 W/25, Germany). As sources of blue

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light we used either a 1200-W xenon arc lamp (model XBO 1200; Osram) in combination with a 452-nm interference filter, bandwidth 19.4 nm (Jenaer Glaswerke; Schott und Gen., Mainz, Germany), or a 600-W slide projector in combination with a 459.5-nm interference filter, bandwidth 60.2 nm (Balzers AG, Liechtenstein). When individual cultures during a single experiment were irradiated with different fluence rates, copper screens of fine mesh were used for a reduction in fluence rates as described by Weissig and Beck (18). Applied fluence rates were measured with a digital photometer (model J16; Tektronix Inc., Beaverton, OR).

Determination of the Percentage of Mating

The percentage of gametes in a test culture was assayed by mixing the cells (mt−) with a threefold excess of mature gametes of mt+. Mating was allowed to proceed for 60 min in the dark. Cells in the mating mixture were then fixed with glutaraldehyde (final concentration, 1%) and microscopically scored for quadriflagellate cells (*Qfc*) and biflagellate cells (*Bfc*). The mating efficiency was calculated using the following equation:

$$\% \text{ mating} = \frac{Qfc \times 1/a}{Bfc + 2 \times Qfc} \times 100$$

$$\text{where } a = \frac{\text{No. of cells to be tested for mating competence in the mating mixture}}{\text{total No. of cells in the mating mixture}}$$

The cell numbers were determined with a Coulter counter. In some experiments, the results from the mating reactions were normalized by taking the highest values of each individual experiment as 100%. For the presentation of these data, average values were calculated.

RESULTS

In a standard protocol for the generation of gametes, vegetative cells are suspended in nitrogen-free medium and incubated with continuous illumination (4, 10). To test the influence of each extrinsic signal independently, a two-step protocol for gametogenesis was devised (14). In a first step, initiated by the removal of a utilizable nitrogen source, pregametes are generated from vegetative cells by incubation in the dark for approximately 19 h. In a second step, these pregametes are converted into sexually competent gametes by irradiation with white or blue light (14, 18). The number of sexually mature gametes can be assayed by their ability to form zygotes, *i.e.* quadriflagellate cells, after addition of a sexually competent partner.

Mating Competence Is Lost upon Addition of a Nitrogen Source or a Shift from Light to Dark

To test whether the conversion of pregametes to gametes may occur in the presence of a nitrogen source, NH_4Cl (final concentration, 7.5 mM) was added to pregametes at the start of illumination. Only 2% of the pregametes differentiated into gametes during a 1-h light pulse. This result suggested that both extrinsic signals—nitrogen deprivation and light—are required for the differentiation of pregametes to gametes. Experiments in which either nitrogen was added to gametes or gametes were shifted from light to dark were performed to gain insight into processes regulating the differentiation of vegetative cells to gametic cells. Addition of a nitrogen source to gametes (generated from pregametes) resulted in a rapid loss in their mating competence (Fig. 1A). Similarly, shifting gametes into the dark caused a dramatic loss in mating competence (Fig. 1B). These results suggest that the mating competence of gametes is unstable. Gametes generated by nitrogen deprivation with simultaneous illumination for 19 h behaved differently. Upon addition of a nitrogen source or shift from light to dark they lost their ability to mate at much

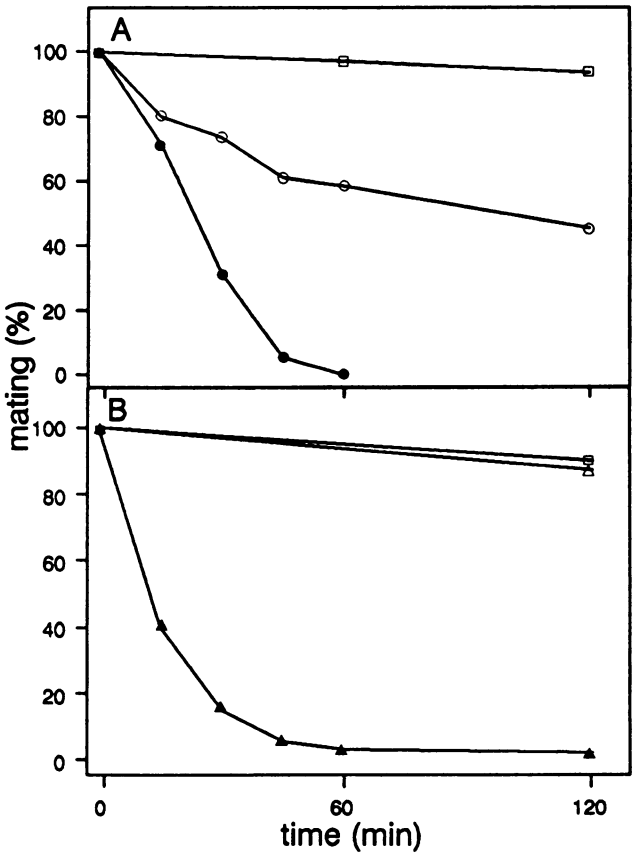

Figure 1. Loss of mating competence after re-addition of a nitrogen source or shift from light to dark. Cultures were illuminated with white light (73 μmol m^{-2} s^{-1}). The ordinate gives the normalized values for the percentage of mating as defined in "Materials and Methods." A, Pregametes were irradiated for 120 min before addition of NH_4Cl to a final concentration of 7.5 mM at time 0 (●). To cells irradiated continuously throughout gametogenesis, NH_4Cl was also added at time 0 (○). To control cultures, no nitrogen source was added (□). The results represent the data of two independent experiments. B, Pregametes were irradiated for 120 min before shift into dark at time 0 (▲). Cells shifted to dark after continuous irradiation throughout gametogenesis at time 0 (△). Control, no dark-shift (□). The average result from six independent experiments is presented.

slower rates (Fig. 1, A and B). The differences of these cells to gametes generated from pregametes will be discussed.

Gametes Inactivated by Dark Incubation Rapidly Recover Mating Competence upon Reillumination

Gametes generated from pregametes that have lost their mating competence during a 45-min dark incubation regained their ability to mate without a lag phase when re-exposed to light (Fig. 2). The same result was observed with gametes incubated in the dark for 2 h before re-exposure to light (data not shown). For a comparison, the kinetics for the conversion of pregametes to gametes are shown. This conversion proceeded only after a lag phase and at a significantly slower rate. We conclude that gametes inactivated by incubation in the dark are physiologically different from pregametes.

Conversion of Pregametes to Gametes Depends on the Duration of Irradiation

Pregametes can differentiate into gametes only after prolonged irradiation. To test the dependence of this differentiation on duration of stimulus, pregametes were irradiated with a constant fluence at reciprocal combinations of fluence rates and irradiation times. A fluence of 10.8 mmol m^{-2} was chosen because irradiation with a fluence rate of 1.5 μmol m^{-2} s^{-1} for 120 min induced a quantitative conversion of pregametes to gametes. The results suggest that between 12 and 75 min, the degree of conversion of pregametes to gametes is approximately a linear function of irradiation duration (Fig. 3). Thus, illumination for extended time periods is required for a quantitative differentiation of pregametes to gametes.

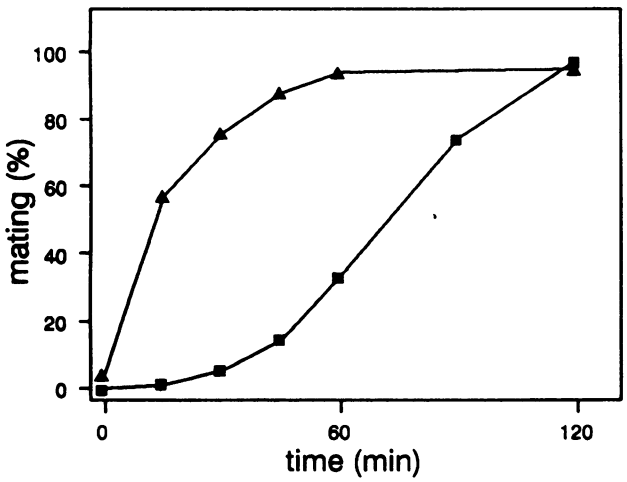

Figure 2. Recovery of mating competence in the light after a light-dark shift. Pregametes were irradiated with white light (73 μmol m^{-2} s^{-1}) for 2 h, incubated in the dark for 0.75 h, and then shifted back into white light at time 0 (▲). Pregametes generated by incubation in the dark for 21.75 h were also shifted into white light at time 0 (■). The ordinate gives the normalized values for the percentage of mating as defined in "Materials and Methods." The average of five independent experiments is presented.

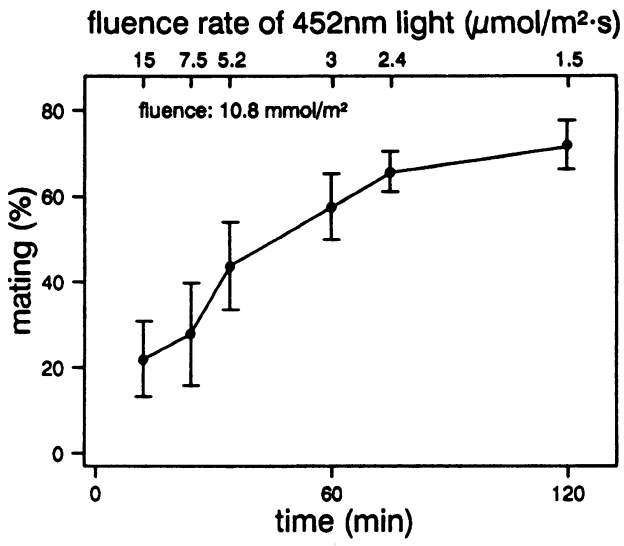

Figure 3. Dependency of the differentiation of pregametes to gametes, induced by a constant fluence, on duration of irradiation. A fluence of 10.8 mmol m^{-2} of 452-nm light was applied by the indicated combinations of fluence rates and duration of light stimulus.

Fluence Rates Determine the Kinetics and Extent of Differentiation from Pregametes to Gametes

The kinetics of conversion of pregametes to gametes were assayed at fluence rates of blue light (λ = 459 nm) between 0.098 μmol m^{-2} s^{-1} and 5.7 μmol m^{-2} s^{-1}. High fluence rates resulted in a rapid differentiation of pregametes, reaching a plateau level around 90 min after start of irradiation (Fig. 4). When fluence rates were reduced, we observed slower kinetics for the conversion of pregametes to gametes. These results suggest that the fluence rate controls the kinetics of differentiation.

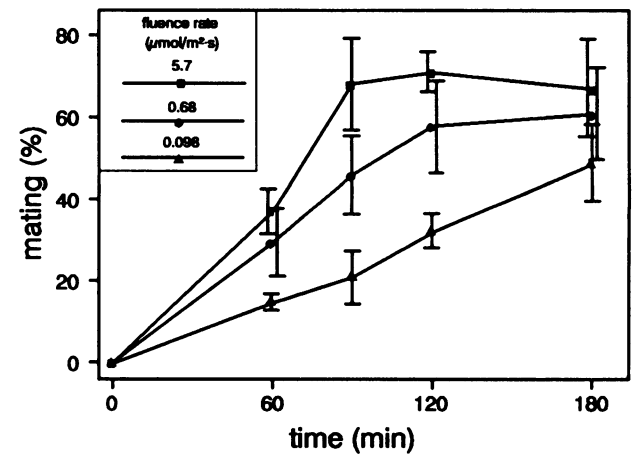

Figure 4. Differentiation of pregametes to gametes induced by different fluence rates of blue light. Pregametes were irradiated with light of 459 nm at the fluence rates indicated. The data of four independent experiments are presented.

DISCUSSION

Gametic differentiation of *C. reinhardtii* may proceed in two steps: pregametes generated by extended nitrogen starvation in the dark can differentiate into sexually mature gametes upon exposure to light (Fig. 5A). From physiological studies, we have devised a model for the interaction of two signal transduction chains activated by the two extrinsic signals (Fig. 5B). This model predicts that the differentiation of pregametes to gametes is dependent on the interaction of end products from both signal transduction chains. The basis for this model was the observation that both signals are required for the light-dependent step in gametogenesis.

Gametes generated from pregametes by irradiation for 120 min rapidly lost their mating competence upon re-addition of a nitrogen source (Fig. 1A) or upon incubation in the dark (Fig. 1B). This observation implies that in the absence of either signal the mating competence is unstable. Since absence of the extrinsic signals caused this loss in mating competence, we also infer that intermediates of the signal transduction chains are subject to decay or to reversible inactivation. Gametes generated by simultaneous irradiation after nitrogen removal behaved differently. They lost their ability to mate at a much slower rate. We suspect that the physiology and the program of gametic differentiation with simultaneous irradiation are different from the system in which both extrinsic signals were applied sequentially. The increased stability of the mating competence of these gametes may be due to an enhanced stability or an increased pool size of the molecules responsible for the mating reaction, *e.g.* the agglutinins. Alternatively, the pool size and/or stability of molecules that mediate the transduction of the extrinsic signals may be elevated.

Gametes inactivated by incubation in the dark upon re-illumination regained their mating competence rapidly and without a lag phase (Fig. 2). The slower kinetics of gamete formation from pregametes may indicate that light controls more than one step in the conversion of pregametes to gametes. Possibly in dark-inactivated gametes only a final step in the establishment of mating competence requires light. A candidate for this latter step is the activation of agglutinin molecules by light as has been observed for the related alga *Chlamydomonas eugametos* (5, 6). Alternatively, dark-inactivated gametes may maintain elevated pool levels of product(s) from the signal transduction chain for light. A minor increase in these pools, achieved by short durations of irradiation, may then be sufficient to reach a critical level required for the establishment of mating competence.

Fluence rates determine the kinetics of pregamete to gamete differentiation (Fig. 4). The slower kinetics at reduced fluence rates suggest a fluence rate-dependent accumulation of the endproduct(s) and/or intermediates of the signal transduction chain for light to a level required for the activation of a program of differentiation mediated by the two converged signal chains (Fig. 5B).

Conversion of pregametes to gametes, when induced by a constant fluence between 12 and 75 min was approximately linearly dependent on duration of irradiation (Fig. 3). Continuous stimulation of the photoreceptor appears to be required. Experiments with repeated short pulses of light (1–7 min) intermitted by short periods of darkness (2–15 min) did not result in a quantitative differentiation of pregametes to gametes. Thus, reciprocity is not valid for light controlled differentiation of pregametes to gametes. Since short pulses of light are not sufficient to trigger differentiation of pregametes to gametes, a light dependent reaction, *e.g.* the accumulation of the endproduct of a signal chain to a threshold level, may limit the conversion. However, the data are also consistent with a program of differentiation where more than one step is controlled by light.

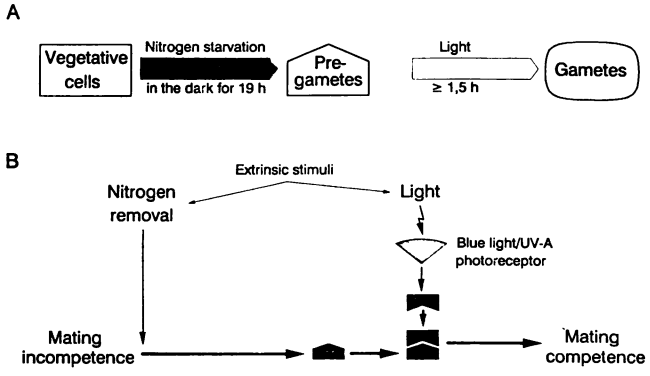

Figure 5. Differentiation of vegetative cells to gametic cells (A) and (B) a model to explain the interaction of intracellular signals generated by the two extrinsic signals required for the attainment and maintenance of mating competence.

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LITERATURE CITED

1. **Adair WS** (1985) Characterization of *Chlamydomonas* sexual agglutinins. J Cell Sci Suppl **2:** 233–260
2. **Bulté L, Bennoun P** (1990) Translational accuracy and sexual differentiation in *Chlamydomonas reinhardtii*. Curr Genet **18:** 155–160
3. **Friedmann I, Colwin AL, Colwin LH** (1968) Fine structural aspects of fertilization in *Chlamydomonas reinhardtii*. J Cell Sci **3:** 115–128
4. **Kates JR, Jones RF** (1964) The control of gametic differentiation in liquid cultures of *Chlamydomonas*. J Cell Comp Physiol **63:** 157–164
5. **Kooijman R, Elzenga TJM, de Wildt P, Musgrave A, Schuiring F, van den Ende H** (1986) Light dependence of sexual agglutinability in *Chlamydomonas eugametos*. Planta **169:** 370–378
6. **Kooijman R, de Wildt P, Homan WL, Musgrave A, van den Ende H** (1988) Light affects flagellar agglutinability in *Chlamydomonas eugametos* by modification of agglutinin molecules. Plant Physiol **86:** 216–223
7. **Martin NC, Goodenough UW** (1975) Gametic differentiation in *Chlamydomonas reinhardtii*. I. Production of gametes and their fine structure. J Cell Biol **67:** 587–605
8. **Martin NC, Chiang K-S, Goodenough UW** (1976) Turnover of chloroplast and cytoplasmic ribosomes during gametogenesis in *Chlamydomonas reinhardtii*. Dev Biol **51:** 190–201
9. **Picard-Bennoun M, Bennoun P** (1985) Change in the cytoplasmic ribosome properties during gametogenesis in the alga *Chlamydomonas reinhardtii*. Curr Genet **9:** 239–243

10. **Sager R, Granick S** (1954) Nutritional control of sexuality in *Chlamydomonas reinhardtii*. J Gen Physiol **37:** 729–742
11. **Saito T, Tsubo Y, Matsuda Y** (1985) Synthesis and turnover of cell body-agglutinin as a pool of flagellar surface-agglutinin in *Chlamydomonas reinhardtii* gamete. Arch Microbiol **142:** 207–210
12. **Schlösser UG** (1976) Entwicklungsstadien-und sippenspezifische Zellwand-Autolysine bei der Freisetzung von Fortpflanzungszellen in der Gattung *Chlamydomonas*. Ber Dtsch Bot Ges **89:** 1–56
13. **Siersma PW, Chiang K-S** (1971) Conservation and degradation of cytoplasmic and chloroplast ribosomes in *Chlamydomonas reinhardtii*. J Mol Biol **58:** 167–185
14. **Treier U, Fuchs S, Weber M, Wakarchuck WW and Beck CF** (1989) Gametic differentiation in *Chlamydomonas reinhardtii:* light dependency and gene expression patterns. Arch Microbiol **152:** 572–577
15. **Treier U, Beck CF** (1991) Changes in gene expression patterns during the sexual life cycle of *Chlamydomonas reinhardtii*. Physiol Plant (in press)
16. **Waffenschmidt S, Kuhne W, Jaenicke L** (1989) Immunological characterization of gamete autolysins in *Chlamydomonas reinhardtii*. Bot Acta **102:** 73–79
17. **Wegener D, Treier U, Beck CF** (1989) Procedures for the generation of mature *Chlamydomonas reinhardtii* zygotes for molecular and biochemical analysis. Plant Physiol **90:** 512–515
18. **Weissig H, Beck CF** (1991) Action spectrum for the light-dependent step in gametic differentiation of *Chlamydomonas reinhardtii*. Plant Physiol **97:** 118–121
19. **Wiese L** (1965) On sexual agglutination and mating-type substances (gamones) in isogamous heterothallic Chlamydomonads. 1. Evidence of the identity of the gamones with the surface components responsible for sexual flagellar contact. J Phycol **1:** 46–54