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ORIGINAL PAPER

H. van den Ende

Sexual development in the homothallic green alga *Chlamydomonas monoica* Strehlow

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Abstract In *Chlamydomonas monoica*, cell division and mating are interdependent processes, since under gametogenic conditions only newly born cells are mating competent. By refeeding nitrogen-starved cells with nitrate or ammonium ions, cell division and mating were synchronized. The mating competence of the progeny cells was dependent on the amount of the nitrogen source parent cells were refed, with an optimum around $0.1 \mu\text{mol} \cdot 10^5$ cells. A second treatment with nitrate inhibited gametogenesis, but only when applied during the first part of the cell cycle, suggesting that an essential part of sexual development takes place during this period. During the latter part of the cell cycle, cells required light to acquire mating competence.

Key words *Chlamydomonas monoica* · Green algae
Cell cycle · Gamete

Introduction

Gametogenesis in the unicellular green alga *Chlamydomonas* is generally triggered by nitrogen stress. For example, when proliferating cells of *C. reinhardtii* are transferred to a nitrogen-free medium and incubated in continuous light, they produce mating-competent but non-dividing cells several hours later (Treier et al. 1989). Gametes can be induced to divide by providing them with a nitrogen source, but then the daughter cells are no longer mating competent (Kates and Jones 1964).

Matsuda et al. (1990) found that mating competence in *C. reinhardtii* is expressed only during a specific period in the G1 phase of the cell cycle. They showed that synchronized cells transferred to N-free conditions in early G1 became mating competent directly, but cells transferred 6 or 12 h later needed a cell division before

producing gametes. Also, synchronous cells of *C. eugametos* showed mating competence only during the first part of the cell cycle (Zachleder et al. 1991).

The clearest manifestation of cell-cycle-dependent mating competence is seen in cells of *C. monoica*, which mate directly following their release from sporangia (van den Ende and VanWinkle-Swift 1994). This implies that by synchronizing cell proliferation using the appropriate conditions, the mating process can also be synchronized. Such conditions are described in this paper.

Materials and methods

Cultures and media

Chlamydomonas monoica Strehlow (UTEX 220) was obtained from the collection of algae at the University of Texas, Austin, USA. It was cultivated in nitrate-limited chemostat cultures at 20°C in 1-l culture vessels containing 500 ml culture medium. Illumination from one side was provided by an HPI/T 400-W daylight lamp (Philips, Eindhoven, The Netherlands) at an average fluence rate of $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in the center of the vessel, as measured with a Li-cor model LI-185B photometer with an LI-190 SB quantum sensor. The cultures were grown in a 16 h light:8 h dark regime. The cells were grown in Bold's basal medium (van den Ende et al. 1992). Nitrate limitation was imposed by reducing the nitrate concentration to 10% (0.3 mM) of the normal value and by adjusting the influx rate of the culture medium between 0.3 and $0.6 \text{ ml} \cdot \text{min}^{-1}$, corresponding with specific growth rates of 0.035 and 0.07 h^{-1} , respectively. The cell density at these growth rates was 2×10^5 to $5 \times 10^5 \cdot \text{ml}^{-1}$. The cultures were aerated with 2% CO_2 in air.

Induction and assay of zygote formation

The development of mating competence was studied in cell batches derived from chemostat-grown cells. They were incubated under various conditions for various periods while being aerated with 2% CO_2 in air. Subsequently, aliquots were placed in light at $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, provided by a fluorescent tube (TLD32W/84HF; Philips, Eindhoven, The Netherlands), and various amounts of NaNO_3 or $(\text{NH}_4)_2\text{HPO}_4$ were added as a nitrogen source, which induced entry into mitosis. After approximately 15 h, the cell suspensions were transferred to plastic petri dishes (5 cm diameter), which were illuminated from below with a fluorescent tube (as

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above). This facilitated the synchronous germination of sporangia and subsequent mating of the released daughter cells. After 24 h, the suspensions were placed in the dark for 3–5 days, allowing newly formed zygotes to round off and to develop a thick wall by which they could be distinguished from non-fused cells. Cell and zygote numbers were obtained by hemocytometer countings.

Results and Discussion

Sexual reproduction in *C. monoica* resembles that of other *Chlamydomonas* species: gametes of opposite mating type agglutinate via their flagella and fuse, subsequently forming quadriflagellated zygotes (VanWinkle-Swift and Aubert 1983). In heterothallic species, synchronous sexual interaction takes place when gamete suspensions of opposite mating type are mixed. In a homothallic species such as *C. monoica*, cells of opposite mating type are

always present together; this results in asynchronous mating events.

This report describes a method of synchronizing cell division and mating. When nitrogen-starved cells, most of which were arrested in the early part of G1 as judged by their size (not shown), were given a nitrogen source (nitrate or ammonium ions), they initiated cell division and produced sporangia with some synchrony (Fig. 1). These sporangia contained 4–16 daughter cells (the result of 2–4 consecutive cell divisions). When released, these cells mated directly with each other and formed zygotes. The zygote yield is considered a measure of the mating competence of the cells.

The effect of nitrate addition on zygote formation in a population of starved cells is shown in Figs. 2 and 3. While the cell number resulting from one round of cell divisions increased continuously with nitrogen concentration, zygote formation exhibited an optimum at approximately 0.1 μmol per 10^5 cells (Figs. 2, 3). At slightly higher concentrations the zygote yield was lower,

Fig. 1 Cell division in N-starved cells of *C. monoica* in response to different nitrate concentrations. Five-milliliter samples taken from a continuous culture were incubated in glass tubes, bubbled with CO_2 -enriched air and illuminated (fluence rate $150 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Samples were treated with different concentrations of NaNO_3 . At various times after nitrate addition, the densities of cells (A) and sporangia (B) were determined by hemocytometer countings. Mating was observed approximately 16 h after nitrate addition. The data are averages of two independent sample sets

Fig. 2 The effect of different nitrate concentrations on zygote production at various light intensities. Samples were incubated as in Fig. 1 and treated with different concentrations of NaNO_3 . After 15 h, they were transferred to small petri dishes which were illuminated but not agitated. Approximately 6 h after the onset of germination of the sporangia, the samples were placed in the dark for 3 days, after which cell (A) and zygote (B) numbers were obtained by hemocytometer counting

Fig. 3 The effect of duration of illumination on zygote production. Samples were incubated as in Fig. 1 and treated with different concentrations of NaNO_3 . They were illuminated continuously up to 13.5 or 15 h after nitrate addition. After 15 h, they were transferred to small petri dishes which were illuminated but not agitated. Approximately 6 h after the onset of germination of the sporangia, the samples were placed in the dark for 3 days, after which cell (A) and zygote (B) numbers were measured by hemocytometer counting

showing that sexual development is very sensitive to excess nitrogen. Using ammonium ions as nitrogen source, the same optimal concentration for gametogenesis was observed, but the production of zygotes was at least 50% lower (not shown).

The following experiment shows that an essential part of sexual development occurs during progression through the cell cycle. After presenting cells with $0.1 \mu\text{mol}$ nitrate per 10^5 cells to generate mating competent progeny (Table 1), a second, but excess, amount of nitrate was added. When the time interval between the two additions was 11 h, no mating competent cells were formed. However, when the interval was longer the inhibitory effect of the second addition decreased. After 18.5 h, when most of the sporangia had germinated, the addition of supplementary nitrate was no longer inhibitory.

Table 1 Effect of supplementary additions of nitrate on zygote production. Ten-milliliter aliquots of N-starved cells (5×10^5 cells·ml $^{-1}$) were incubated for 11 h in light after which gametogenesis was induced by adding $4.2 \mu\text{mol}$ NaNO_3 per batch. At various times an additional $8.4 \mu\text{mol}$ NaNO_3 was given. Samples for cell counts were taken at 22 h, after which the cells were placed in the dark. Zygote densities were determined after 3 days. Data are averages of duplicate measurements. Standard deviations are indicated

Time of NO_3^- addition after induction (h)	Cells·ml $^{-1}$ ($\times 10^{-5}$)	Zygotes·ml $^{-1}$ ($\times 10^{-5}$)
Non-induced control	5.3 ± 0	0
Induced control	11.9 ± 0.1	1.64 ± 0.4
11	11.8 ± 0.1	0
13	11.0 ± 0.1	0.47 ± 0.05
14.5	9.7 ± 0	0.53 ± 0.06
16.5	9.0 ± 0.06	0.88 ± 0.1
18.5	8.6 ± 0.04	1.14 ± 0.23

Fig. 4 The effect of a dark period during a late stage of cell division on zygote production. Aliquots of N-stressed cells were incubated with $0.1 \mu\text{mol}$ NaNO_3 per 10^5 cells and after 15 h placed in the dark for varying lengths of time. After 29 h, the cells were kept in the dark. Zygote and cell numbers were assessed after 3 days by hemocytometer counting

Under optimal nitrate additions, mating competence increased considerably with higher light intensities, without affecting cell proliferation (Fig. 2). This dependency of sexual development on light was also shown by imposing a dark period at various times during the cell cycle. During the first 10 h, darkness inhibited cell division, in agreement with the fact that this stage is energy-dependent (Spudich and Sager 1980) and is delimited by the so-called commitment point, after which cell division proceeds even in the dark. However, a dark period at a later stage of the cell cycle impeded the development of mating competence, as shown in Fig. 3. Figure 4 shows

Table 2 Zygote yields (zygotes·ml⁻¹×10⁻³) in cell suspensions transferred from aerated tubes to non-agitated petri dishes at various times after addition of NaNO₃. The sporangia germinated at 16–18 h. Data are averages of at least three measurements. Standard deviations are indicated

Nitrate added (μmol per 10 ⁵ cells)	Time of transfer after nitrate addition (h)			
	15	18	20	23
0	0	0	0	0
0.05	43±7	34±9	28±5	19±4
0.08	152±23	143±6	138±1	67±17
0.13	133±13	127±6	91±20	67±10
0.18	26±7	40±4	6±3	9±3

Table 3 Effect of light/dark conditions of N-starved cells prior to refeeding with NaNO₃ on zygote yield. Ten-milliliter aliquots of N-starved cells were incubated for 13 h in the light or dark after which cell division was induced by the addition of various amounts of NaNO₃. The cells were incubated in the light for 24 h. Zygote yields were determined after 3 days. Data are averages of duplicate measurements. Standard deviation are indicated

Nitrate concentration (μmol per 10 ⁵ cells)	Zygotes·ml ⁻¹ (×10 ⁻³)		Cells·ml ⁻¹ (×10 ⁻⁵)	
	Dark	Light	Dark	Light
0	0	0	5.4±0.1	5.4±0.9
0.05	0	6±0	8.3±0.1	9.1±0.5
0.09	27±4	32±4	11.1±0.3	11.8±0
0.13	21±2	33±2	18.1±0.6	17.4±0.1

that even in a late stage of the cell cycle, when sporangia had developed (cf. Fig. 1), the mating competence of the daughter cells was inhibited by darkness.

The effect of light on the attainment of mating competence in *C. monoica* is reminiscent of that in *C. reinhardtii*. Treier et al. (1989) found that in this species a light treatment was only effective after prolonged incubation in a nitrogen-free medium. These authors proposed that N-starvation in the dark initiates a program of gametic differentiation resulting in cells they called "pregametes." These pregametes were unable to mate but could be converted to competent gametes by light; in *C. monoica*, the conversion might take place while the cells are still within the sporangial cell wall.

The timing of cell division and the percentage of cells that entered division were independent of the amount of nitrate added to the cells, within the range studied. Apparently cell numbers increased with increasing nitrate concentration mainly because, on average, more daughter cells were produced per sporangium.

Proper aeration was essential for synchronous cell division, but disturbed the sexual interaction between gametes. When aeration was continued during germination of the sporangia, the zygote yield was considerably decreased, as shown in Table 2. In order to optimize zy-

gote production, aeration was discontinued before the sporangia germinated.

A dark period prior to refeeding the cells with nitrate had no detectable effect on the zygote yield, suggesting that the induction of sexual development by N-stress does not require light (Table 3). Likewise, the length and stringency of N-starvation had no clear effect on the development of mating competence. For example, incubating cells without nitrogen for periods between 4 and 13 h did not affect later zygote yields. Longer starvation periods resulted in decreased viability. A shorter period affected the synchrony of the process. Thus, by refeeding N-starved cells with nitrate, the induction of sexual development can be separated from a growth phase in which sexual development takes place. Whether the expression of gamete-specific genes is confined to the latter phase can only be established using gamete-specific molecular probes.

What is the molecular signal eliciting gametogenesis? Is it nitrate or a product of its assimilation? Matsuda et al. (1992) observed that in *C. reinhardtii* storage forms of nitrogen, such as arginine and glutamine, did not interfere with sexual differentiation or de-differentiation. They concluded that the NH₄⁺ ion per se, not a metabolite, is responsible for sexual induction. They assume that there is an intracellular threshold level of NH₄⁺ ions above and below which gametogenesis is repressed or stimulated, respectively. This point needs more investigation in the light of our finding that refeeding N-starved cells with NH₄⁺ rather than nitrate ions resulted in lower gamete yields.

References

- Ende H van den, Briel ML van den, Lingeman R, Gulik P van der, Munnik T (1992) Zygote formation in the homothallic green alga *Chlamydomonas monoica* Strehlow. *Planta* 188: 551–558
- Ende H van den, VanWinkle-Swift KP (1994) Mating-type differentiation and mate selection in the homothallic *Chlamydomonas monoica*. *Curr Genetics* 25:209–216
- Kates JR, Jones RF (1964) The control of gametic differentiation in liquid cultures of *Chlamydomonas*. *J Cell Comp Physiol* 63: 157–163
- Matsuda Y, Saito T, Koseki M, Shimada T (1990) The *Chlamydomonas* non-synchronous and synchronous gametogenesis as analyzed by the activities of the cell body-agglutinin and cell wall lytic enzyme. *Plant Physiol* 9:1–6
- Matsuda Y, Shimada T, Sakamoto Y (1992) Ammonium ions control gametic differentiation and dedifferentiation in *Chlamydomonas reinhardtii*. *Plant Cell Physiol* 33:909–914
- Spudich JL, Sager R (1980) Regulation of the *Chlamydomonas* cell cycle by light and dark. *J Cell Biol* 85:136–145
- Treier U, Fuchs S, Weber M, Wakarchuk WW, Beck CF (1989) Gametic differentiation in *Chlamydomonas reinhardtii*. *Arch Microbiol* 152:572–577
- VanWinkle-Swift KP, Aubert B (1983) Uniparental inheritance in a homothallic alga. *Nature* 303:167–169
- Zachleder V, Jakobs M, Ende H van den (1991) Relationship between gametic differentiation and the cell cycle in the green alga *Chlamydomonas eugametos*. *J Gen Microbiol* 137:1333–1339