

Effect of heat shock on the cell cycle duration of algae-containing and algae-free ciliates *Climacostomum virens*

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Summary

Cells of algae-containing (“green”) and alga-free (“white”) strains of the ciliate *Climacostomum virens* were compared for their responses towards a 1 h pulse of temperature stress. Although the upper heat shock temperature limits for the two strains were different (40–41 °C for “green”, and 37–38 °C for “white” cells), the responses to high temperature were similar. The delay in the completion of cell cycle depended on the cell cycle stage at which heat shock was given. Cells exposed to heat shocks at G₁ stage or at the late dividing stage and returned back to 22 °C did not show any noticeable cell cycle lengthening. The most prominent effect was observed when the cells were heat-shock treated at the early dividing stage. In this case, heat shock resulted in up to 4 h delay of the cell cycle completion. It was found that in all experiments, when heat shock was given at different stages of division, the cell cycle duration of the progeny cells was significantly longer (42–50 h) than that of the control cells (21–25 h).

Key words: ciliates, cell cycle, heat shock, macronucleus, *Climacostomum virens*

Introduction

In response to an increase of temperature, eukaryotic cells display significant changes in their pattern of protein synthesis. It has been shown that in a wide variety of cells from yeast to mammalian cells heat stress induces the synthesis of heat shock proteins (HSP/chaperones), which are thought to protect cells from the toxic effect of the stress. It has been observed that transcription of most of the previously active genes diminishes during heat

shock, while previously processed mRNA remains stable (Fragkostefanakis et al., 2015; Jacob et al., 2017; Garbuz, 2017). Heat shock proteins are represented by a number of families differing in molecular weight, sequences and functions. Heat shock proteins in ciliates are rather poorly studied. There are several studies of 70kDA family heat shock proteins in ciliates (Goodkov et al., 2010). It was also shown that in the ciliate *Tetrahymena thermophila* the heat shock protein Ssa5 is abundantly expressed in response to heat stress as well as during sexual

reproduction. A specific involvement of a heat shock protein in pronuclei fusion during conjugation was suggested (Fukuda et al., 2015).

Due to the properties of both an entire organism and a single cell, ciliates cannot be synchronized by standard reagents (e.g. nocodazole, colchicine), which prevent mitotic spindle formation, and this obstacle for a long time had been hampering the experimental studies of ciliates in mass cultures. In the middle of the XX century, a system for synchronizing mass cultures of *Tetrahymena* based on eight heat shocks was developed (Zeuthen, 1964). Initially asynchronous but uniformly growing cultures now can become synchronized with regard to cell division and the associated morphogenetic processes. However, the effects of heat-shock stress on the ciliates' cell cycle stages and their duration remain ambiguous.

The ciliates *Climacostomum virens* from natural populations usually contain algae in their cytoplasm. The association of *C. virens* and symbiotic *Chlorella* sp. has all characteristics of stable endosymbiotic units: algae are retained throughout cell division and sexual reproduction of the ciliates (Reisser et al., 1984). However, it was shown that an artificially produced algae-free *C. virens* strain can be obtained by prolonged (about 5 months) cultivation of "green" algae-containing clone in the dark (Karajan et al., 2007).

The aim of this work was to study an effect of heat shock on the cell cycle duration of algae-containing and algae-free ciliates *C. virens*. As shown earlier, the development of cells treated by heat shock at the stage closer to division is delayed for a longer period of time than of those cells treated not as close to division (Berger, 2001). For this reason, we exposed *C. virens* cells to heat shock at different stages of the cell cycle and division which could be easily identified cytologically by morphologic criteria. During division, a horseshoe-shape interphase macronucleus of the ciliate *C. virens* condenses into a compact body 40–60 min before separation of daughter cells; thereafter, the macronucleus is stretched and pinched in two more or less equal parts in the daughter cells. In this study, we designated the cells with condensed macronucleus as D_0 , the cells with stretched macronucleus and early signs of cytokinetic furrow as D_1 , the cells at advanced state of the cytokinetic furrow – as D_2 , and the young cells just after division – as G_1 .

Material and methods

CULTURES

The algae-containing ("green") and the algae-free ("white") strains of the ciliate *Climacostomum virens* were used in this study. The "green" strain W24 was collected from a pond at the Valaam Island (Lake Ladoga), Russia. An artificially produced "white" strain B1S was obtained as the result of a long (ca. 5 months) cultivation of "green" cells of the clone W24 in darkness (Karajan et al., 2007). Cells were kept at constant temperature (22 °C) in boiled tap water and fed 3 times a week with *Tetrahymena pyriformis* that were cultivated separately.

CELLS SYNCHRONIZATION

Synchronous cells were selected from the log-phase mass culture by picking up with a micropipette 80–100 dividing cells with an advanced division furrow. The cells maintained excellent synchrony, and approximately 80–85% of these cells divided synchronously during the next growth cycle.

OPTICAL MICROSCOPY

The cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 20 min, rinsed in phosphate buffered saline (pH 7.3) two times for 5 min, and stained either according to standard Feulgen staining protocol or with 0.5 µg/ml DAPI for 15 min. The specimens were photographed using Leica DMI4000B or Leica TCS SP5 microscopes.

HEAT SHOCK SELECTION AND TREATMENT

The heat shock intensity was chosen in such a way as to ensure 90–100% ciliates survival when the cells were returned to the cultivation medium at 22 °C after heat stress. Four points of the cell cycle were chosen for heat shocks: newly separated daughter cells (G_1), cells with condensed macronucleus (D_0), cells with stretched macronucleus and early signs of cytokinetic furrow (D_1), and cells at the advanced state of the cytokinetic furrow (D_2). The microphotographs of the G_1 , D_0 , D_1 and D_2 cells of *C. virens* are given in Fig. 1.

For heat shock experiments, 20–30 cells at the G_1 , D_0 , D_1 and D_2 stages of cell cycle were

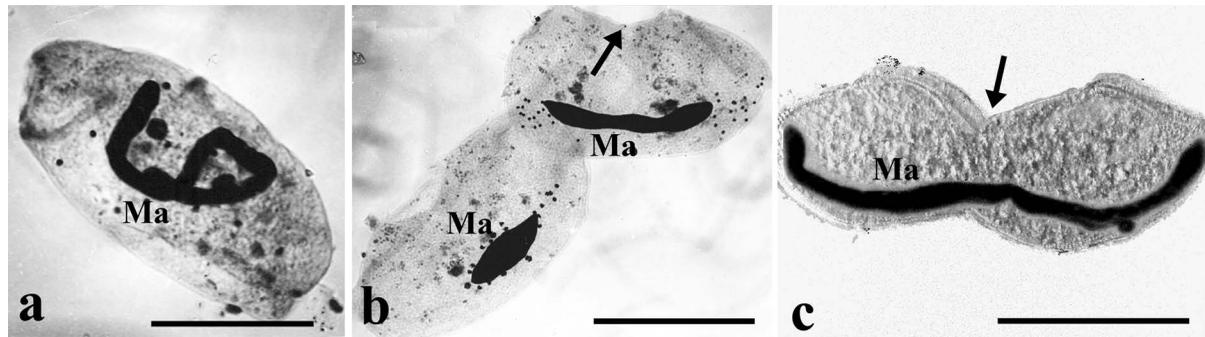


Fig. 1. Optical microscopy of *Climacostomum virens* cells. a – Young cells after division (G_1); b – D_0 cells with condensed macronucleus (bottom left), and D_1 cells with stretched macronucleus and early signs of cytokinetic furrow (top right); c – cells at advanced state of the cytokinetic furrow. a, b – Feulgen staining, c – DAPI. Abbreviation: Ma – macronucleus; arrows show cytokinetic furrows. Scale bars: 100 μm .

transferred to 37.5 °C (algae-free cells) or 41 °C (algae-containing ciliates). After 60 min, the shocked ciliates were returned to the medium at 22 °C, and the time interval between the beginning of heat shock and separation of the daughter cells after the division was determined.

The experiments were run in triplicates. Statistical analyses were conducted using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). The Student's t-test was used for comparing the results; differences between the parameter values were considered significant at $p < 0.05$.

Results

CELL CYCLE DURATION AND THE HIGHEST HEAT SHOCK TOLERABLE TEMPERATURES

The duration of cell cycle and the highest heat shock temperatures that could be tolerated by both studied strains of the ciliate *Climacostomum virens* were determined. It was found that the artificially produced algae-free (“white”) strain differed in cell cycle duration and response to temperature stress from the “green” strain. The duration of the cell cycle for “white” ciliates was 24–25 h, for algae-containing “green” ciliates – 21–22 h. The algae-containing ciliates were shown to be more resistant to temperature stress: the highest tolerated temperature for “green” cells and for algae-free “white” ciliates was 40–41 °C, and 37–38 °C, respectively.

EFFECT OF HEAT SHOCK ON G_1 CELLS

Cells exposed to heat shock at the G_1 stage completed division almost simultaneously with the control cells. Four independent experiments showed that after heat shock the newly separated daughter cells (G_1) showed no statistically significant ($p < 0.05$) delay in completion of the division cycle in both investigated strains (Tables 1, 2; Fig. 2). The treated cells had the same or even ca. 1 h shorter duration of the cell cycle than that of the control cells (20–21 h vs. 21–22 h in the “green” strain, and 23–24 h vs. 24–25 h in the “white” strain). The G_1 ciliates exposed to heat shock did not increase in their size during the treatment. After returning to standard 22 °C conditions, they were inactive and did not consume food, but after an hour these cells did not differ from the control cells kept at 22 °C. In the next division cycle, the G_1 progeny cells in both investigated strains showed the similar duration of cell cycle as the control cells (Tables 1, 2; Fig. 3).

EFFECT OF HEAT SHOCK ON THE D_0 CELLS

The D_0 cells exposed to heat shock showed a significantly ($p < 0.05$) increased time interval between the beginning of heat shock and separation of the daughter cells in both investigated strains (Fig. 2). The delay in completing the cell division was 80–150 min for the “green” (Table 1) and 130–180 min for the “white” ciliates (Table 2). The treated D_0 cells were inactive within 1.5–2 h after returning to 22 °C. A small portion of the cells (2–3 from 30

Table 1. The time interval between the beginning of heat shock and separation of the daughter cells after the division, and duration of the first cell cycle of algae-containing (“green”) *C. virens* progeny cells.

Cell cycle stage	Time interval between the beginning of heat shock and separation of daughter cells, h		Progeny cell cycle duration, h
	Control	Heat shocked cells	
G ₁	21–22	20–21	21–23
D ₀	0.67–1.0	2.0–3.5	40–45
D ₁	0.5–0.67	0.67–1.0	44–50
D ₂	0.17–0.25	0.17–0.25	42–50

in each experiment) rounded and later died. The unexpected results were obtained when we measured the cell cycle duration of the progeny cells from the cells treated at the stage D₀: it was much longer ($p < 0.05$) than in the control cells and ranged from 40 to 45 h in both investigated strains (Tables 1, 2).

EFFECT OF HEAT SHOCK ON THE D₁ AND D₂ CELLS

Cells at the early (D₁) and late (D₂) phases of cell division were significantly ($p < 0.05$) more resistant to heat shock than those at the D₀ stage. The heat shocked D₁ cells showed 10 to 20 min and 30 to 45 min delays in completing the cell division for “green” and for “white” ciliates, respectively (Tables 1, 2). Interestingly, in each experiment, 2–4 cells completed division during the heat shock period at high temperature, i.e. before the cells were transferred to 22 °C. At the late stage of division (D₂), heat shock did not affect the cells – they completed the division at the scheduled time. However, the progeny of those cells treated at the stages D₁ and D₂ showed significantly ($p < 0.05$) longer cell cycle duration than the control cells (Fig. 3). It turned out to be in the range from 42 to 50 h, similar to the progeny of the D₀ treated cells (Tables 1, 2).

Discussion

The data obtained show that the effect of heat shock on algae-containing and algae-free ciliates *C. virens* depends on the stage of cell cycle, and little depends on the presence of algae symbionts. Despite the fact that the upper temperature limits were different for the two strains studied (40–41 °C for the “green” and 37–38 °C for the “white” cells), the responses to high temperature were basically the same, with only slight differences in details. The

Table 2. The time interval between the beginning of heat shock and separation of the daughter cells after the division, and duration of the first cell cycle of algae-free (“white”) *C. virens* progeny cells.

Cell cycle stage	Time interval between the beginning of heat shock and separation of daughter cells, h		Progeny cell cycle duration, h
	Control	Heat shocked cells	
G ₁	24–25	23–24	24–26
D ₀	0.83–1.0	3.0–4.0	42–45
D ₁	0.5–0.75	1.0–1.5	45–50
D ₂	0.17–0.25	0.17–0.25	42–50

cells from both investigated strains divided when temperature shock was given at the G₁ stage of the cell cycle. Cells treated at the G₁ stage of the cell cycle showed no delay in completing the division in the following division cycle, too. The progeny cells showed the same duration of cell cycle as the control cells. These data correlate well with the results published by Rasmussen with co-authors (1985) who studied the growth of *Paramecium tetraurelia* at restrictive temperature during the G₁ stage of the cell cycle. The authors showed that the duration of the G₁ interval in *P. tetraurelia* had been established during the preceding cycle, but not in the present cell cycle. No specific arrest point of growth at the G₁ stage has been reported for other investigated ciliates as well. It was also shown in *Paramecium* that the G₁ timer is independent of all variable changes that affect the G₁ interval duration when changes are made prior to commitment to division. When cells are transferred to non-permissive conditions, the G₁ progression is arrested without the production of excess delay after the cells are returned to permissive conditions (Berger, 2001).

Our results showed that the greatest sensitivity to heat shock was manifested by *C. virens* cells in the D₀ phase of the cell cycle, during which the macronucleus condenses before division. One possible explanation is that heat shock inactivates a thermolabile protein necessary for the macronucleus division. The division cannot occur until more of this hypothetical protein has been accumulated. The existence of such heat-labile “division protein” was first postulated to explain heat shock-induced division synchrony in *Tetrahymena* (Zeuten, 1964). In *Tetrahymena*, the level of the accumulation of the nuclear proteins which should be closely involved in cell division increases markedly at the end of the G₂ phase, which roughly corresponds to the D₀ phase in our experiments. Extreme heat shock sensitivity

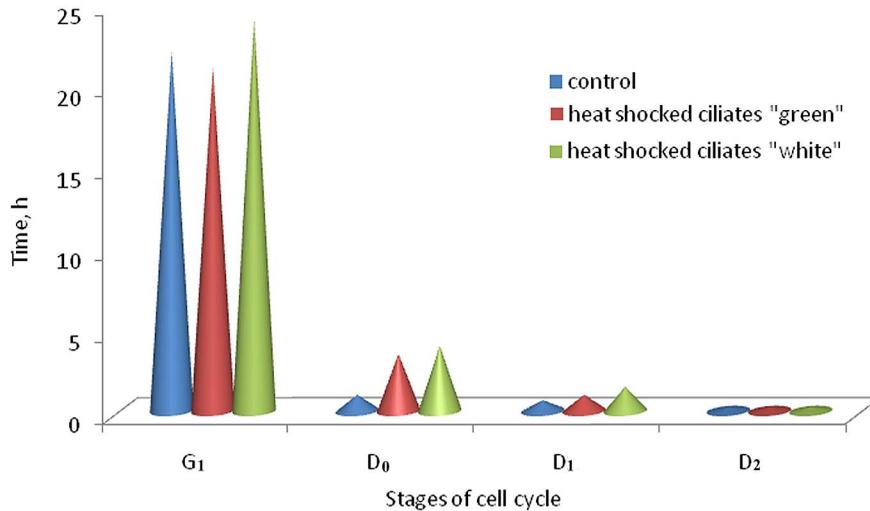


Fig. 2. The maximum time interval (h) between the beginning of heat shock at different cell cycle stages (G₁, D₀, D₁ and D₂) and separation of the daughter cells after the division of algae-containing (“green”) and algae-free (“white”) cells of ciliates *Climacostomum virens*.

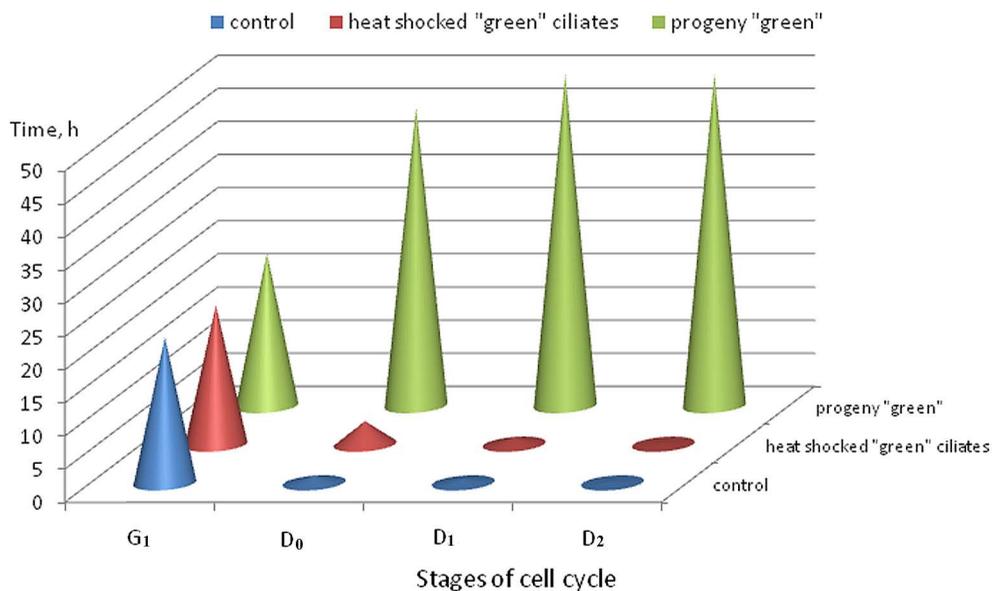


Fig. 3. The maximum time interval (h) between the beginning of heat shock at different cell cycle stages (G₁, D₀, D₁ and D₂) and separation of the daughter cells after the division of algae-containing (“green”) ciliates *Climacostomum virens*, and duration of the first cell cycle of their progeny cells.

of cells at the G₂ stage has been demonstrated in *Stylonychia mytilus* and in starved *Tetrahymena* (Frenkel et al., 1980; Hallberg et al., 1984; Kaul, 1991).

Scherbaum and Zeuthen (1954) established that in the *Tetrahymena* cell cycle there is a ‘stabilization point’ or physiological transition point, after which cells would proceed through division despite the heat shock. Analysis of the cell cycle regulation through

the use of mutant genes in *P. tetraurelia* has shown that near the end of the cell cycle, ciliates commit irreversibly to cell division. The point of commitment occurs at the time of oral polykinetid assembly and micronuclear anaphase. The commitment is a checkpoint which requisites a threshold cell mass/DNA ratio and stomatogenesis. Commitment to division in *P. tetraurelia* occurs at a point when cells reach a certain stage of oral morphogenesis.

Equivalent points of commitment to division operate at similar stages of oral morphogenesis in all ciliates that have been analyzed (Adl and Berger, 1996).

The existence of commitment point explains our experiments, which showed that when temperature shock was given at the late dividing stage (D_2) the ciliates *C. virens* in both investigated strains completed division almost simultaneously with the control cells. In addition, the different positions of the points D_0 , D_1 and D_2 of the *C. virens* cell cycle relative to the “commitment point” may explain the different extent of response of *C. virens* to the heat shock at these stages (Tables 1, 2).

However, in the next cell cycle of the heat shocked D_0 , D_1 and D_2 *C. virens* cells, progeny cells showed a 20–30 h delay of division. The cell cycle duration of the progeny heat shocked cells was 42–50 h vs. 21–26 h in the control cells. It can be assumed that heat shock at the division stages of the ciliate *C. virens* leads to damage of the cellular apparatus at the molecular level, the restoration of which takes a long time. However, the molecular mechanisms of this effect remain unclear and require further investigations.

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