
Short Communication

Timing of commitment to autogamy in *Paramecium bursaria*

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SUMMARY

To elucidate the timing of commitment to autogamy in *Paramecium bursaria*, cells were treated with an autogamy-inducing agent (1.25% methyl cellulose) for 0, 1, 2, 3, 5, and 24 h, and the duration of treatment required for the induction of autogamy was examined 24-25.5 h after the onset of treatment. Many cells treated for 0-2 h were at the vegetative stage, but many cells treated for 3-24 h were at stages from the third pre-zygotic division to the third post-zygotic division and normally underwent autogamy. Therefore, treatment with methyl cellulose for 3 h or more is considered necessary for the induction of autogamy. However, some of the cells treated for 3 h were at the stage of developing either a swollen and spindle-shaped micronucleus or an early round-shaped micronucleus, which is indicative of the pre-meiotic S phase. This suggests that if cells proceed beyond the stage of an early round-shaped micronucleus, they normally undergo autogamy, but if not, they terminate at the stage of either a swollen and spindle-shaped micronucleus or an early round-shaped micronucleus. Consequently, cells of *P. bursaria* are committed to autogamy just after the stage of an early round-shaped micronucleus (the pre-meiotic S phase).

Key words: Autogamy, Commitment, Meiosis, Methyl cellulose, *Paramecium bursaria*, Pre-meiotic S phase

INTRODUCTION

In ciliates, the major developmental pathways consist of the sexual (conjugation or autogamy) and asexual (cell division) pathways that can be easily switched each other, providing

us a useful experimental system to understand the mechanism of commitment to the sexual pathway. In studies of *Paramecium*, there are some reports concerning commitment to the sexual pathway in *P. caudatum* (Fujishima, 1983b, 1988), *P. multimicronucleatum* (Shimomura and Takagi, 1985) and *P. tetraurelia* (Berger, 1986). These three species of *Paramecium* belong to the “aurelia” group, though the species of *Paramecium* fall into two defined groups based on cell shape: the “aurelia” group and the “bursaria” group. Thus,

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in the “bursaria” group, there are no reports about the commitment to the sexual pathway.

In the present study, in order to determine the timing of commitment to autogamy in a species of the “bursaria” group, the period of methyl cellulose treatment required for the induction of autogamy in *P. bursaria* was examined. Autogamy is a sexual reproduction process occurring in single cells without the direct association of cells, and in *P. bursaria* autogamy can be artificially induced by the treatment with methyl cellulose (Yanagi, 2004); methyl cellulose-induced autogamous cells normally undergo nuclear processes.

MATERIALS AND METHODS

Strains and culture condition

Paramecium bursaria, syngen 1, stock Dd1 (mating type II) was used. The culture medium was 1.25% (w/v) fresh lettuce juice diluted with modified Dryl's solution (Dryl, 1959), pH 7.0, and inoculated with *Klebsiella pneumoniae* one day before use (Hiwatashi, 1968). In the modified Dryl's solution (K-DS), KH_2PO_4 was substituted for NaH_2PO_4 . The cells were incubated at 24–26°C.

Treatment with methyl cellulose

Methyl cellulose (100 centipoises, Wako Chemicals Co., Ltd., Japan) was dissolved in deionized water (Yanagi and Haga, 1996) as 2×stock solution containing 2.5% (w/v) methyl cellulose. The 2×stock solution (0.5 ml) was mixed with 0.5 ml of cell suspension (approximately 5,000 cells/ml) in depression slides (Toushinrikou Co., Ltd., Japan). Therefore, cells were treated with 1.25% methyl cellulose of the final concentration.

In order to examine the duration of methyl cellulose treatment required for the induction of autogamy in *P. bursaria*, cells were treated with 1.25% methyl cellulose for 0, 1, 2, 3, 5, and 24 h. The cells treated with methyl cellulose for 0 h

were isolated into half the original concentration of K-DS for 24 h in the wells of a 60-well micro test plate (Nalge Nunc International, U.S.A.). The reason why half the original concentration of K-DS was used for 0 h was due to the fact, for subsequent time periods (1, 2, 3, 5, and 24 h), when cells were treated with methyl cellulose, equal volumes of cell suspension in the original concentration of K-DS and 2.5% methyl cellulose were mixed; therefore, the concentration of K-DS in the mixed solution was half the original concentration.

The cells treated for 1, 2, 3, and 5 h were washed with K-DS following treatment with methyl cellulose and isolated into half the original concentration of K-DS in the wells of a micro plate. The cells to be treated for 24 h (incubated in 1.25% methyl cellulose solution) were isolated into the wells of a micro test plate 1–3 h after the onset of treatment. Finally, all cells treated for 0, 1, 2, 3, 5, and 24 h were stained with aceto-orcein 24–25.5 h after the onset of treatment. The induction of autogamy was detected by the observation of morphological changes of micronuclei in isolated single cells.

RESULTS AND DISCUSSION

Temporal sequence of micronuclear events in cells treated with methyl cellulose

To know the time course of micronuclear events in the methyl cellulose-induced autogamy of *P. bursaria*, the time course of nuclear processes in cells treated with methyl cellulose, which undergo either autogamy or conjugation, was examined. Because it is suggested that the time course of nuclear processes in the methyl cellulose-induced autogamy of *P. bursaria* is substantially similar to that in the methyl cellulose-induced conjugation (Yanagi and Haga, 1998; Yanagi, 2004).

At the vegetative stage, cells had a thin and spindle-shaped micronucleus (Fig. 1A). The micronucleus was swollen within 3 h of the treatment with methyl cellulose (Fig. 1B) and then began to

become round-shaped around 3 h after the onset of treatment (Fig. 1C). The round-shaped micronucleus gradually enlarged until 8 h after the onset of treatment (prophase of the first meiotic division) and elongated 10-12.5 h after the onset of treat-

ment (prophase of the first meiotic division). The micronucleus enlarged around 15 h after the onset of treatment (prophase and metaphase of the first meiotic division) and underwent the anaphase and telophase of the first meiotic division about 17.5 h

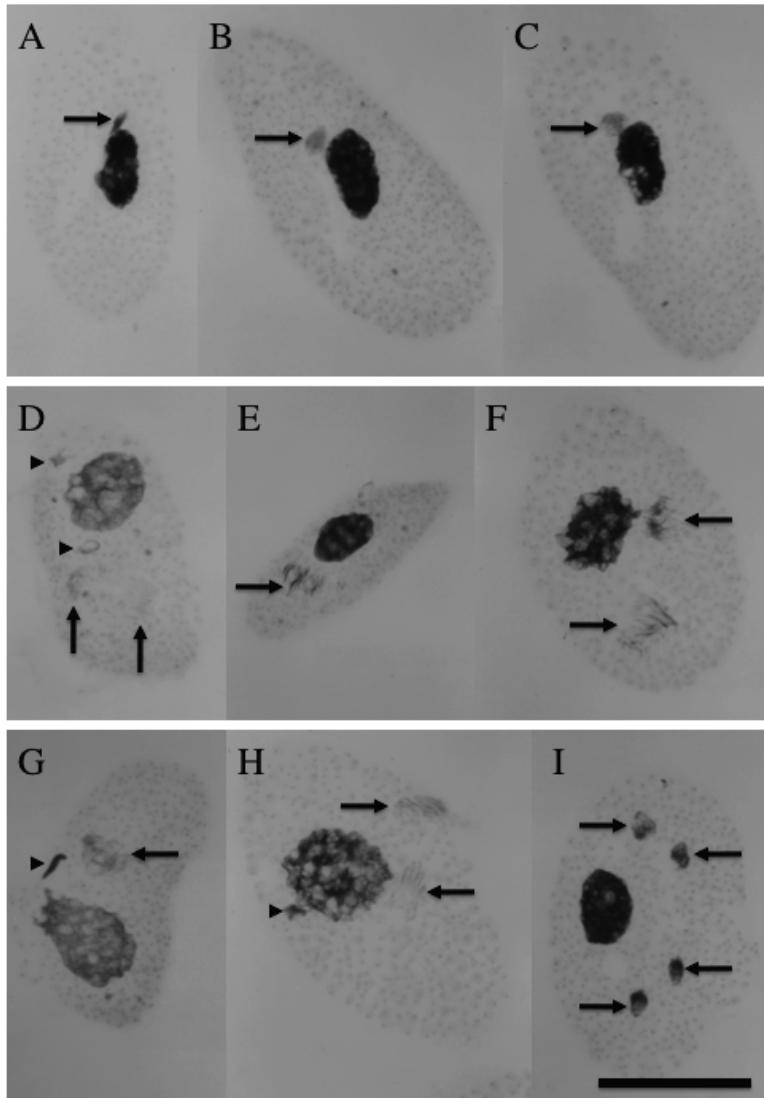


Fig. 1. Micronuclear changes induced by the treatment with methyl cellulose. Cells at micronuclear stages observed in Table 1 are shown. A, thin and spindle-shaped (vegetative stage); B, swollen and spindle-shaped; C, early round-shaped; D, 3rd pre-zygotic division; E, synkaryon; F, 1st post-zygotic division; G, pycnosis of one of two micronuclei produced by 1st post-zygotic division; H, 2nd post-zygotic division; I, 3rd post-zygotic division. Arrows and arrowheads indicate micronuclei and pycnotic micronuclei, respectively. Bar, 50 μ m.

after the onset of treatment. Thereafter the second meiotic division and the third pre-zygotic division (Fig 1D) occurred and a synkaryon was formed about 20 h after the onset of treatment (Fig. 1E). The synkaryon divided two times mitotically (the first and second post-zygotic division) 22-24 h after the onset of treatment (Fig. 1F, G and H) and the third post-zygotic division began to occur around 24 h after the onset of treatment (Fig. 1I).

Timing of commitment to autogamy

The duration of methyl cellulose treatment

required for the induction of autogamy in *P. bursaria* was examined (Table 1). All of the 0 h treated cells and most of the 1 h and 2 h treated cells had a thin and spindle-shaped micronucleus at the vegetative stage (Table 1 and Fig. 1A). Some of the cells treated for 3, 5 and 24 h were also at the vegetative stage. The percentage of the vegetative cells decreased with the length of the period of methyl cellulose treatment. The vegetative cells are not activated for autogamy.

Some of the 1 h and 2 h treated cells were at the stage of a swollen and spindle-shaped micronu-

Table 1. Duration of methyl cellulose treatment required for the induction of micronuclear events in autogamy

Stages of micronuclei#	Number of cells					
	0h*	1h*	2h*	3h*	5h*	24h*
Thin and spindle-shaped	15	19	18	10	5	1
Swollen and spindle-shaped	-	1	5	1	-	-
Early round-shaped	-	-	-	6	-	-
Meiosis	-	-	-	-	-	-
3 rd pre-zygotic division	-	-	-	1	-	2
Synkaryon	-	-	-	-	-	2
1 st and 2 nd post-zygotic division	-	-	-	3	2	14
3 rd post-zygotic division	-	-	-	4	8	3
Total number of cells examined	15	20	23	25	15	22

#Stages of micronuclei were classified on the basis of Figure 7.9 in R. Wichterman's *The Biology of Paramecium* (1986).

*Time indicates the duration of methyl cellulose treatment. Cells were treated with 1.25% methyl cellulose for 0, 1, 2, 3, 5, and 24 h, and stained with aceto-orcein 24-25.5 h after the onset of treatment.

cleus (Table 1 and Fig. 1B). The stage is normally observed 1-3 h after the onset of treatment with methyl cellulose. Some of the 3 h treated cells were at the stage of either a swollen and spindle-shaped micronucleus or an early round-shaped micronucleus (Table 1 and Fig. 1C). The stage of an early round-shaped micronucleus is normally observed around 3 h after the onset of treatment. The cells at the stages of a swollen and spindle-shaped micronucleus and an early round-shaped micronucleus are activated for the stages but could not proceed to the meiosis stage. Thus the cells are considered activated for autogamy, but not committed to autogamy.

On the other hand, one third of the 3 h treated cells and most of the 5 h and 24 h treated cells proceeded to later stages from the third pre-zygotic division to the third post-zygotic division (Table 1 and Fig. 1D-I), when they were stained with aceto-orcein 24-25.5 h after the onset of treatment with methyl cellulose. The stages are normally observed around 19-25 h after the onset of treatment with methyl cellulose and the time is similar to that of the staining with aceto-orcein (24-25.5 h after the onset of treatment). This shows that the cells at the stages from the third pre-zygotic division to the third post-zygotic division normally undergo nuclear events in autogamy. The cells are thought to complete the whole process of autogamy. Therefore, the cells are considered committed to autogamy. Moreover, none of the 3 h treated cells was during meiosis, though meiosis occurs between the stages of an early round-shaped micronucleus and the third pre-zygotic division and lasts more than 10 h. In the 5 h and 24 h treated cells, no cells were at the stages of a swollen and spindle-shaped micronucleus, an early round-shaped micronucleus and meiosis.

In brief, the present results show that, except for vegetative cells, all of the 1 h and 2 h treated cells and one half of the 3 h treated cells terminate at the stage of either a swollen and spindle-shaped micronucleus or an early round-shaped micronu-

cleus, but the remaining 3 h treated cells and all of the 5 h and 24 h treated cells undergo the normal process of autogamy. Therefore, the treatment with methyl cellulose for 3 h or more is considered necessary for the induction of autogamy. Furthermore, the results suggest that if cells proceed beyond the stage of an early round-shaped micronucleus, they normally undergo nuclear changes in autogamy, but if not, they terminate at the stage of either a swollen and spindle-shaped micronucleus or an early round-shaped micronucleus. This leads to the conclusion that cells are committed to autogamy just after the stage of an early round-shaped micronucleus.

The stages of a swollen and spindle-shaped micronucleus and an early round-shaped micronucleus are suggested to be in the pre-meiotic S phase undergoing pre-meiotic DNA synthesis, because the pre-meiotic S phase begins with the micronuclear swelling and ends immediately before meiotic prophase in conjugation of *P. caudatum* (Fujishima, 1983a). In *P. caudatum*, a round-shaped micronucleus, called a stage II micronucleus, is mainly in the pre-meiotic S phase (Fujishima, 1983a). Thus, it appears that cells of *P. bursaria* are committed to autogamy just after the pre-meiotic S phase, namely immediately prior to initiation of meiosis or at the beginning of meiosis.

In conclusion, it is thought that commitment to the sexual pathway (autogamy and conjugation) occurs immediately after the pre-meiotic S phase (immediately prior to initiation of meiosis or at the beginning of meiosis) not only in species of the "aurelia" group including *P. tetraurelia* (Berger, 1986), *P. multimicronucleatum* (Shimomura and Takagi, 1985) and *P. caudatum* (Fujishima, 1983b and 1988), but also in a species of the "bursaria" group, *P. bursaria* (the present study).

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