Short Communication

Changes in cell-cell adhesion ability of artificially induced autogamous cells in *Paramecium caudatum*

Satoshi OHTA and Akira YANAGI*

Department of Biotechnology, Senshu University of Ishinomaki, Ishinomaki 986-8580, Japan

SUMMARY

To analyze cell-cell adhesion in *Paramecium caudatum*, we examined methyl cellulose-induced autogamous cells for their ability to adhere. It was found that the autogamous cells have cell-cell adhesion ability. In addition, we examined the proportion of adhering (conjugating) pairs derived from autogamous cells at various stages (0.5, 1, 2, 4, 6, 9 and 12 h after the onset of induction of autogamy). The proportion of adhering cells reached a maximum about 2 h after the onset of induction of autogamy, and thereafter, rapidly decreased by 4 h after the onset, eventually reaching almost zero 6 h after the onset. These results suggest that there is a transient expression or activation of molecule(s) involved in cell-cell adhesion around 2 h after the onset of induction of autogamy.

Key words: Autogamy, Cell-cell adhesion, Cell adhesion molecule, Methyl cellulose, *Paramecium caudatum*

INTRODUCTION

In vertebrates, the mechanism of cell-cell adhesion is well documented and many kinds of cell adhesion molecules such as cadherins have been identified and characterized (Alberts et al., 2002). On the other hand, in the ciliate *Paramecium caudatum*, a unicellular eukaryote, no cell adhesion molecules have been identified and there have only been a few studies investigating the mechanism of adhesion between cell bodies without ventral cilia (Miyake, 1969), though there are many reports about mating reaction, in which cells adhere to each other on the surface of the ciliary membrane (Hiwatashi, 1981). In the present study, we refer to the adhesion between cell bodies without ventral cilia in *P. caudatum* as cell-cell adhesion. Cells of *P. caudatum* undergo cell-cell adhesion during their sexual reproduction process, also known as conjugation, which is one of examples of cell-cell adhesion in eukaryotic organisms. The ciliate *P. caudatum* occupies a key position in a major branch of eukaryotic evolution, the Alveolata. Therefore, an investigation of cell-cell adhesion in *P. caudatum* contributes to the understand-

*Corresponding author
Tel: ＋81-225-22-7716
Fax: ＋81-225-22-7746
E-mail: yanagi@isenshu-u.ac.jp
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ing of the evolution of cell-cell adhesion.

To examine cell-cell adhesion in *P. caudatum*, we used artificially induced autogamous cells. Autogamy is a sexual reproduction process occurring in single cells without the direct association of cells, and in *P. caudatum* autogamy can be artificially induced by treatment with chemical agents such as trypsin (Mikami and Koizumi, 1979), KCl and papain (Tsukii and Hiwatashi, 1979) and methyl cellulose (Yanagi and Haga, 1996). Under artificially induced autogamy, cells have the same characteristic as conjugating cells, in which the cilia on the ventral surface degenerate (Watanabe, 1978; Yanagi and Haga, 1996). But, in natural autogamy occurring in *P. tetraurelia*, ventral cilia do not degenerate (Watanabe, 1978). Thus, we presumed that methyl cellulose-induced autogamous cells in *P. caudatum* could adhere to each other on the ventral surface without cilia. In the present study, we examined this possibility and found that methyl cellulose-induced autogamous cells have the ability to conduct cell-cell adhesion. This is the first report showing changes in cell-cell adhesion ability during sexual reproduction process in *Paramecium*.

MATERIALS AND METHODS

Strains and culture conditions

Cells of a stock KNZ2 (mating type VI) in syngen 3 of *Paramecium caudatum* were mainly used. We also used stocks KoscA4 (V), 27aG3 (V) and KoscA3 (VI) in syngen 3 of *P. caudatum*. The culture medium was 1.25% (w/v) fresh lettuce juice diluted with modified Dryl's solution, pH 7.0, and inoculated with *Klebsiella pneumoniae* one day before use (Hiwatashi, 1968). The modified Dryl's solution (Dryl, 1959) (K-DS) substituted KH_2PO_4 for NaH_2PO_4. Cells were cultured at 24-25℃.

Induction of autogamy

Methyl cellulose (400 centipoises) (SIGMA) was dissolved in K-DS. Cells at a density of 1,500 cells/ml were treated with 1.25% methyl cellulose in a plastic Petri dish (6 cm in diameter, Corning) to induce autogamy according to the methods described by Yanagi and Haga (1996). Experiments were carried out at 25-26°C.

Examination of changes in cell-cell adhesion ability during autogamy

We examined whether methyl cellulose-induced autogamous cells can adhere to form pairs and whether cell-cell adhesion ability varies during autogamy by the following protocol.

1. Prepare cells (12,000 cells/8 ml) 0.5, 1, 2, 4, 6, 9 and 12 h after the treatment with methyl cellulose. Examine the percentage of conjugating pairs in the population of methyl cellulose-treated cells just after the treatment. This percentage is referred to as [control]. The population of methyl cellulose-treated cells contains not only autogamous cells but also conjugating pairs and vegetative cells, because methyl cellulose induces autogamy (Yanagi and Haga, 1996) and conjugation (Yanagi and Haga, 1998) in *P. caudatum*.
2. Wash methyl cellulose-treated cells three times with K-DS using a hand-operated centrifuge to remove methyl cellulose just after the treatment with methyl cellulose.
3. Suspend methyl cellulose-treated cells in 1 ml of K-DS to adjust the cell density to 12,000 cells/ml in order to increase the cell density and enhance the probability of collisions between the cells. The percentage of pairs formed after this was calculated and referred to as [experiment 1].
4. Incubate methyl cellulose-treated cells in K-DS for 1 h to allow them to form pairs. The percentage of pairs formed was calculated and referred to as [experiment 2].
5. Calculate the percentage of pairs derived from autogamous cells by subtracting [control] from [experiment 1] or [experiment 2].

RESULTS AND DISCUSSION

Changes in cell-cell adhesion ability during autogamy

To ascertain whether methyl cellulose-induced autogamous cells can adhere to form pairs and whether cell-cell adhesion ability varies during autogamy, the percentage of pairs was examined in autogamous cells derived at various stages (0.5, 1, 2, 4, 6, 9 and 12 h after the treatment with methyl cellulose) (Table 1). As a result, {[experiment 1] – [control]} values varied in a similar manner to those of {[experiment 2] – [control]} during autogamy (Table 1 and Fig. 1). This indicates that most adhesion-capable autogamous cells have already paired just after the adjustment of cell density to 12,000 cells/ml and continue to pair over the next 1 h.

The percentage of adhering pairs derived from autogamous cells varied during autogamy (Table 1 and Fig. 1). In the early stages of autogamy (0.5 and 1 h after treatment with methyl cellulose), the percentage of pairs formed was very low. This appears to be due to incomplete degeneration of the ventral cilia in autogamous cells, as the degeneration of cilia begins at the anterior tips of cells 0.5 h after methyl cellulose treatment and continues toward the posterior regions of the cells

Table 1. The percentage of pairs in methyl cellulose-treated cells

Time of methyl cellulose treatment (h)		0.5	1	2	4	6	9	12
1st experiment	[control]* (%)	0 (143#)	1.7 (115)	4.0 (100)	26.1 (46)	10.3 (78)	2.0 (99)	5.9 (102)
	[experiment 1]* (%)	0 (41)	4.4 (135)	22.7 (220)	12.8 (219)	8.5 (164)	3.1 (131)	1.8 (111)
	[experiment 2]* (%)	1.9 (212)	4.5 (219)	24.0 (225)	16.8 (226)	13.5 (223)	7.0 (228)	6.0 (232)
	[experiment 1]-[control]	0	2.7	18.7	-13.3	-1.8	1.1	-4.1
	[experiment 2]-[control]	1.9	2.8	20.0	-9.3	3.2	5.0	0.1
2nd experiment	[control] (%)	0 (167)	1.5 (133)	2.8 (143)	16.4 (159)	21.4 (168)	21.0 (124)	15.3 (183)
	[experiment 1] (%)	0 (73)	10.1 (179)	38.1 (126)	37.3 (177)	27.0 (111)	16.7 (192)	15.4 (169)
	[experiment 2] (%)	0 (149)	12.0 (217)	41.3 (213)	32.9 (225)	29.7 (229)	19.9 (261)	10.2 (235)
	[experiment 1]-[control]	0	8.6	35.3	20.9	5.6	-4.3	0.1
	[experiment 2]-[control]	0	10.5	38.5	16.5	8.3	-1.1	-5.1
3rd experiment	[control] (%)	0 (144)	0 (130)	0 (130)	20.0 (50)	12.2 (148)	14.3 (98)	0 (94)
	[experiment 1] (%)	0 (136)	0 (147)	28.4 (148)	24.7 (146)	14.3 (140)	7.0 (142)	5.1 (118)
	[experiment 2] (%)	0 (150)	2.6 (152)	34.8 (207)	35.6 (208)	6.1 (163)	9.6 (188)	6.8 (146)
	[experiment 1]-[control]	0	0	28.4	4.7	2.1	-7.3	5.1
	[experiment 2]-[control]	0	2.6	34.8	15.6	-6.1	-4.7	6.8

*See text for an explanation of [control], [experiment 1] and [experiment 2].
#Figures in parentheses show number of cells examined.

1 h after treatment (Watanabe, 1978). The percentage of paired cells reached a peak 2 h after methyl cellulose treatment. The percentage rapidly dropped by 4 h after the treatment and gradually decreased as autogamy progressed until 6 h after the treatment. Paired autogamous cells were hardly observed 6, 9 and 12 h after the treatment.

These results show that methyl cellulose-induced autogamous cells have the ability to conduct cell-cell adhesion, which reaches a maximum around 2 h after the induction of autogamy. This suggests that autogamous cells transiently express or activate molecule(s) involved in cell-cell adhesion around 2 h after the induction of autogamy. This also suggests that we must harvest autogamous cells 2 h after induction in order to identify the molecule(s) responsible for cell-cell adhesion. Furthermore, the cell adhesion molecule(s) may correspond to hypothetical holdfast substances appearing in the early stage of conjugation in *Paramecium* (Miyake, 1969).

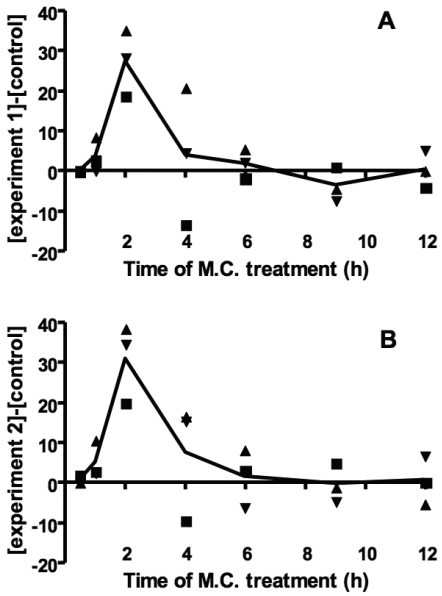

Fig. 1. Changes in the percentage of pairs derived from autogamous cells. The abscissa shows time of methyl cellulose treatment (h) and the ordinate shows values of {[experiment 1] – [control]} (A) or {[experiment 2] – [control]} (B). The mean values of {[experiment 1] – [control]} (A) or {[experiment 2] – [control]} (B) of three independent experiments (■, ▲ and ▼) in Table 1 are used to draw the lines. In A, the mean value of {[experiment 1] – [control]} at 2 h is significantly different at the $P < 0.01$ level from that at 9 h, and at the $P < 0.05$ level from that at 0.5, 1, 4, 6 and 12 h. In B, the mean value of {[experiment 2] – [control]} at 2 h is significantly different at the $P < 0.01$ level from that at 0.5, 6, 9 and 12 h, and at the $P < 0.05$ level from that at 1 and 4 h. All significance tests were performed using Tukey's multiple comparison test. See text for an explanation of [control], [experiment 1] and [experiment 2].

The present study demonstrates that the methyl cellulose-induced autogamous cells provide a useful model system to study the cell adhesion molecule(s) and mechanism(s) controlling cell -cell adhesion in *P. caudatum*.

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