Interspecific conjugation between distantly related species of *Paramecium*

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ABSTRACT

Interspecific conjugation was induced artificially between the distantly related species belonging to the two different morphological groups, 'aurelia' and 'bursaria' groups, of *Paramecium*. Cytological observations showed that the interspecific conjugants underwent normal nuclear changes, including meiosis. However, nuclear changes of co-conjugant cells did not synchronize each other. These results suggest that conjugation in *Paramecium* involves non-species-specific interactions and its interactions are sufficient for nuclear activation.

1. INTRODUCTION

Conjugation in ciliated protozoan *Paramecium* is a sexual process that is comparable to fertilization in metazoan. It involves a series of developmental events, such as meiosis, fertilization and nuclear differentiation (Metz 1954; Sonneborn 1957; Hiwatashi 1981, 1985). The first step of conjugation is sexual cell agglutination called mating reaction which is a specific cell interaction by the cilia of complementary mating types (Sonneborn 1937). In the second step, cells adhere at the anterior cell surfaces of cell bodies where cilia degenerate (Watanabe 1978). Following this cell union, a third connection including cytoplasmic exchange occurs from anterior to posterior end of the cell.

Paramecium species are classified into two groups, 'aurelia' and 'bursaria' groups based on their morphology (Wichterman 1986), the specificity of the immaturity substances which control the sexual immaturity after conjugation (Miwa 1979) and isozyme pattern of hemoglobin (Usuki and Irie 1982a, 1982b, personal communication). Hiwatashi (1951), Miyake (1968) and Tsukii and Hiwatashi (1983) demonstrated that the process following mating reaction does not involve mating-type specificity or species (including syngen and species complex) specificity, although the observations had been restricted to *Paramecium* belonging to 'aurelia' group. In contrast, it had been believed that there was the self-incompatibility that excludes the homotypic pair formation in *P. bursaria*. However, selfing pairs consisting of the same

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mating type do appear when conjugation is induced by mixing the detached cilia of complementary mating type (Endoh *et al.* 1987) or by the treatment of conjugation inducing-chemicals in chemically inducible mutants (Endoh, unpublished). This fact promotes me to test the possibility that the two distantly related groups of *Paramecium* have the potentials that exceed the prezygotic barrier of species. In order to test this possibility, experiments were designed to induce artificially interspecific conjugation between the two groups of *Paramecium*.

2. MATERIALS AND METHODS

Stock hr^d (high reacter, mating type VII) in *P. tetraurelia* and stock 27aG3 (mating type V) in *P. caudatum* from the 'aurelia' group and stock T151 (mating type IV) in *P. bursaria* from the 'bursaria' group were used in this study. hr^d is a mutant whose mating reaction lasts long and natural autogamy does not occur (Sonneborn 1974). 27aG3 (Tsukii and Hiwatashi 1979) is an exautogamous clone, derived from an early mature mutant (Myohara and Hiwatashi 1979). T151 is a mutant in which conjugation can be induced chemically (Endoh, unpublished). Cells were grown in Ca-poor fresh lettuce juice medium inoculated with *Klebsiella pneumoniae* one day before use (Hiwatashi 1968, Miyake 1968). Conjugation in *Paramecium* can be induced not only by the interaction of complementary mating types but also by certain chemical agents (Miyake 1958, 1968). In all three species, the chemical induction of conjugation was successful in the condition of 10 μ M Ca²⁺, 8 mM K⁺, 40 μ g/ml acriflavine, and 100 mM acetamide. Conjugating pairs began to appear 40 min in *P. tetraurelia*, 60 min in *P. caudatum* and 2 hrs in *P. bursaria*, respectively after treatment of chemical agents. In order to induce pairs simultaneously among three species, chemical inductions were started at different times. Cells of each two species were mixed just before intraspecific conjugants appeared.

3. RESULTS AND DISCUSSION

When conjugation was induced chemically between *P. bursaria* and *P. caudatum*, interspecific conjugants were observed besides each intraspecific ones (Figure 1A and C). At 5 hrs after the induction of conjugation, micronucleus in the co-conjugant cell of *P. caudatum* was in the crescent stage that is equivalent to pachytene-diplotene at meiotic prophase (Sugai 1974, 1976). In contrast, at 6 hrs after the induction, micronucleus in *P. bursaria* also entered the premeiotic S phase that is identified as a swollen micronucleus. Thus, nuclear changes proceeded depending upon the original time schedule of conjugation of each species and did not synchronize each other. Interspecific conjugants between *P. bursaria* and *P. tetraurelia* are shown in

Fig. 1. A: Photomicrographs of interspecific conjugation between *P. bursaria* (lower) and *P. caudatum* (upper). B: Interspecific conjugation between *P. bursaria* (left) and *P. tetraurelia* (right). C: Micronucleus entering the crescent shaped stage equivalent to pachytene-diplotene at meiotic prophase in *P. caudatum* (upper) and swollen stage (premeiotic S phase) in *P. bursaria* (lower). 5-6 hrs after induction of conjugation. D: Macronucleus in *P. tetraurelia* (lower) entering the stage of skein formation specific for the sexual process and micronucleus in *P. bursaria* (upper) entering the swollen stage. 4-6 hrs after induction of conjugation. Symbiotic algae, zoochlorella, are seen in cells of *P. bursaria*. Mi: Micronucleus Ma: Macronucleus

Figure 1B and D. In *P. tetraurelia*, skein formation specific for the sexual process began 4 hrs after the beginning of induction of conjugation. On the other hand, micronucleus of co-conjugant cells of *P. bursaria* entered the premeiotic S phase 6 hrs after the beginning. Nuclear changes also did not proceed synchronously in both partner cells of conjugants. The possible explanations to this are the followings: when conjugation is induced, there should be the mechanism that synchronizes the process of nuclear changes by the exchange of cytoplasm through the cell connection. In the interspecific conjugation, such mechanism may not be effective. A difference of the specificity of immaturity substances exists between 'aurelia' and 'bursaria' groups of *Paramecium* (Miwa 1979). The molecular incompatibility such as the species specificity of immaturity substances may be involved in the inter-

specific conjugation. Alternatively, cytoplasmic connection may not occur. The presence of meiosis-inducing factor (MIF) in *Paramecium* is suggested by micronuclear transplantation (Fujishima 1981) and the MIF is known to be effective in *Xenopus* oocyte (Fujishima, personal communication). Nuclear changes would not synchronize without cytoplasmic exchange even if conjugation-regulating molecules such as MIF are commonly effective beyond the barrier of species.

Autogamy occurs naturally in *P. tetraurelia* or can be induced artificially in *P. multimicronucleatum* and *P. caudatum* (Miyake 1968; Tsukii and Hiwatashi 1979; Mikami and Koizumi 1979). Thus, cell union is not indispensable for nuclear activation in the 'aurelia' group. However, no induction of autogamy has been reported in *P. bursaria*.

In order to attempt artificial induction of autogamy in *P. bursaria*, two kinds of experiments were performed. Single mating reactive cells were placed into depression slides separately with conjugation-inducing chemicals. Under this condition, each cell can not adhere. Ten hrs after transfer into chemical agents, cells were fixed and stained, and whether cells went through meiosis or not was examined. Although more than a thousand cells were observed, nuclear activation did not occur. It is known that when protease is added to the conjugation-inducing chemicals at various times from the start of the induction of conjugation, conjugants once united are gradually disjoined, and the separated cells undergo autogamy in *P. caudatum* (Tsukii and Hiwatashi 1979). Similar experiments were performed in *P. bursaria* as the second attempt of induction of autogamy. Although trypsin, pronase P and papain were used at various concentrations, no nuclear activation was detected. These results suggest that nuclear activation in *P. bursaria* is induced only when cell adhesion occurs even though its interaction is interspecific. In relation to this point, conjugation of *Tetrahymena* differs from that of *Paramecium*. Though homotypic pairs consisting of the same mating type are transiently formed in *Tetrahymena*, nuclear activation does not occur (Kitamura *et al.* 1986). Mating type specific interactions may be involved in the trigger mechanism of nuclear activation in *Tetrahymena* but not in that of *Paramecium*. Cell adhesion after mating reaction and nuclear activation of *Paramecium* occur in non-species-specific manner.

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REFERENCES

- ENDOH, H., WATANABE, T. and HIWATASHI, K. (1987) Artificial induction of selfing conjugation in *Paramecium bursaria*. *J. Exp. Zool.* (in press).
FUJISHIMA, M. and HIWATASHI, K. (1981) Transplantation of germ nuclei in *Paramecium cauda-*

- tum. II. Induction of meiosis in transplanted interphase nucleus. *Exp. Cell Res.* **131**, 63-71.
- HIWATASHI, K. (1951) Studies on the conjugation of *Paramecium caudatum* IV. Conjugation between individuals of two mating types marked by a vital staining method. *Sci. Rep. Tohoku Univ. (Biol.)* **19**, 95-99.
- HIWATASHI, K. (1968) Determination and inheritance of mating type in *Paramecium caudatum*. *Genetics* **58**, 373-386.
- HIWATASHI, K. (1981) Sexual interactions of the cell surface in *Paramecium*. In: *Sexual interactions in Eukaryotic Microbes*. (eds. O'Day, D. H. and Horgan, P. A.) pp. 351-378. Academic Press, New York.
- HIWATASHI, K. (1985) Fertilization of *Paramecium*. In: *Biology of fertilization*. (eds. Metz, C. B. and Monroy, A.) Vol. **1**, pp. 57-85. Academic Press, New York.
- KITAMURA, A., SUGAI, T. and KITAMURA, Y. (1986) Homotypic pair formation during conjugation in *Tetrahymena thermophila*. *J. Cell Sci.* **82**, 223-234.
- METZ, C. B. (1954) Mating substances and the physiology of fertilization in ciliates. In: *Sex in Microorganisms*. (eds. Whenrich, D. H.) pp. 284-334. *Am. Assoc. Adv. Sci.*, Washington, D. C.
- MIKAMI, K. and KOIZUMI, S. (1979) Induction of autogamy by treatment with trypsin in *Paramecium caudatum*. *J. Cell Sci.* **35**, 177-184.
- MIWA, I. (1979) Specificity of the immaturity substances in *Paramecium*. *J. Cell Sci.* **36**, 253-260.
- MIYAKE, A. (1958) Induction of conjugation by chemical agents in *Paramecium caudatum*. *J. Inst. Polytech. Osaka City Univ. (Biol.)* **9**, 251-296.
- MIYAKE, A. (1968) Induction of conjugation by chemical agents in *Paramecium*. *J. Exp. Zool.* **167**, 359-380.
- MYOHARA, K. and HIWATASHI, K. (1978) Mutants of sexual maturity in *Paramecium caudatum* selected by erythromycin resistance. *Genetics* **90**, 227-241.
- SONNEBORN, T. M. (1937) Sex, sex inheritance and sex determination in *Paramecium aurelia*. *Proc. Natl. Acad. Sci. U.S.* **23**, 378-385.
- SONNEBORN, T. M. (1957) Breeding systems, reproductive methods, and species problem in Protozoa. In: *The Species Problem*. (eds. Mayer, E.) pp. 155-324. *Am. Assoc. Advan. Sci.* Washington, D. C.
- SONNEBORN, T. M. (1974) *Paramecium aurelia*. In: *Handbook of Genetics*, vol. **2**. pp. 469-594. (eds. King, R. C.) Plenum Press, New York.
- SUGAI, T. and HIWATASHI, K. (1974) Cytologic and autoradiographic studies of the micronucleus at meiotic prophase in *Tetrahymena pyriformis*. *J. Protozool.* **21**, 542-548.
- SUGAI, T. (1976) Chromosomal association and arrangement at meiotic prophase of the micronucleus in *Paramecium*. *Jpn. J. Genetics* **51**, 385-392.
- TSUKII, Y. and HIWATASHI, K. (1979) Artificial induction of autogamy in *Paramecium caudatum*. *Genet. Res.* **34**, 163-172.
- TSUKII, Y. and HIWATASHI, K. (1983) Genes controlling mating-type specificity in *Paramecium caudatum*: Three loci revealed by intersyngenic crosses. *Genetics* **104**, 41-62.
- USUKI, I. and IRIE, T. (1983a) Inter- and intrasyngenic variation of *Paramecium* hemoglobin. I. *Paramecium aurelia* complex. *Comp. Biochem. Physiol.* **75B**, 415-420.
- USUKI, I. and IRIE, T. (1983b) Inter- and intrasyngenic variation of *Paramecium* hemoglobin. II. *Paramecium caudatum*, *Paramecium jenningsi* and *Paramecium multimicronucleatum*. *Comp. Biochem. Physiol.* **75B**, 421-424.
- WATANABE, T. (1978) A scanning electron-microscopic study of the local degeneration of cilia during sexual reproduction in *Paramecium*. *J. Cell Sci.* **32**, 55-66.
- WICHTERMAN, R. (1986) *Biology of Paramecium*. 2nd eds. Plenum Press, New York.