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Phenotypic conversion of mating type specificity induced by transplantation of macronucleoplasm in *Paramecium caudatum*

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Summary

According to the classical genetic analysis in *Paramecium caudatum* by Tsukii & Hiwatashi (1983), the E mating type of each syngen is expressed when the cell bears alleles specific for syngen at the *Mt* locus. The O mating type is expressed when cells are homozygous for the null allele, *mt*, at the *Mt* locus. In such *mt/mt* cells the O syngen specificity is determined by alleles at two other loci called *MA* and *MB*. In the study reported here, macronucleoplasmic transplantation technique was used to test the above hypothesis. When macronucleoplasm of type E^3 (mating type E of syngen 3) was injected into a macronucleus of type O^{12} (mating type O of syngen 12), some recipients changed to type E of the donor syngen but some others changed to type E of the recipient syngen. Thus, syngen specificity of donor macronucleoplasm controlling type E was converted into that of the recipients, even though the latter has no gene that controls type E. When this transformant expressing type E of the recipient syngen was re-injected back into E of the other syngen, the expression of the converted mating type in some way continued in the recipient. This suggests that syngen specificity of gene *Mt* of the donor was changed to that of the recipients by intersyngenic transplantation of macronucleoplasm. We also obtained results suggesting that the gene dosage ratio of *Mt* to *mt* or *Mt* to *MA* and *MB* may be important for syngen specific expression of type E.

1. Introduction

Morphological species *Paramecium caudatum* consists of several biological species known as syngens which are sexually isolated from each other. Each syngen is composed of two complementary mating types, E (even-numbered) type and O (odd-numbered) type. In this species, however, sexual isolation between syngens is not complete as in the *Paramecium aurelia* complex (Sonneborn, 1975) and intersyngenic hybrids have fertility to some extent (Tsukii & Hiwatashi, 1983). Using these fertile hybrids, Tsukii & Hiwatashi (1983) revealed that the syngen specificity of E mating types is controlled by multiple codominant alleles at the *Mt* locus (e.g. type E^3 is controlled by Mt^3) and that of O mating types by interactions of syngen specific codominant multiple alleles at the two loci, *MA* and *MB*, *Mt* being epistatic to *MA* and *MB*. Thus O mating type is expressed only when cells lack the dominant mating type allele *Mt* and instead are homozygous for the recessive allele *mt* at the *Mt* locus (see Table 1 for examples). This genetic determination system of mating types in *P. caudatum*, called the three-gene hypothesis, was established exclusively by crossbreeding analyses. The main objective of the study reported here is to test whether the three-gene hypothesis will hold true even when different alleles controlling mating types are put together by transplantation during vegetative reproduction, and not by crossbreeding through sexual reproduction. In this study, a phenomenon which cannot be interpreted by the genetic determination system of mating types reported by Tsukii & Hiwatashi (1983) was observed. When macronucleoplasm of type E was injected into cells of type O belonging to a different syngen, recipient cell lines expressed type E, the controlling gene of which was neither in the donor nor in the recipient. The possible mechanisms of this conversion of syngen specificity in the intersyngenic macronucleoplasmic transplantation are discussed. In addition, as shown in this study, the technique of macronucleoplasmic transplantation may lead to new evidence on gene interactions which germ-line genetics

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Table 1. *Stocks of* P. caudatum *used in this study*

Stocks	Phenotypes: Mating type	Behaviour	Trichocyst exocytosis	Mating type genotypes§
Syngen 3				
C103	E^{3}*	Wild type	TND‡	Mt^{3}/Mt^{3}; MA^{3}/MA^{3}; MB^{3}/MB^{3}
Kyk402	E^{3}	Wild type	Wild type	Mt^{3}/mt; MA^{3}/MA^{3}; MB^{3}/MB^{3}
16B909	E^{3}	CNR†	TND	Mt^{3}/mt; MA^{3}/MA^{3}; MB^{3}/MB^{3}
16B1002	O^{3}	CNR	TND	mt/mt; MA^{3}/MA^{3}; MB^{3}/MB^{3}
Syngen 12				
Yo3-1	E^{12}	Wild type	Wild type	Mt^{12}/mt; MA^{12}/MA^{12}; MB^{12}/MB^{12}
Yo16	O^{12}	Wild type	Wild type	mt/mt; MA^{12}/MA^{12}; MB^{12}/MB^{12}
GT105	O^{12}	Wild type	Wild type	mt/mt; MA^{12}/MA^{12}; MB^{12}/MB^{12}

* The mating type of each syngen is designated as E or O with the number of syngen as the superscript.
† CNR is a membrane-inexcitable mutant and its genotype is *cnrB/cnrB*.
‡ TND is a mutant that cannot discharge trichocysts and its genotype is *tnd2/tnd2*.
§ This column shows the genotypes according to Tsukii & Hiwatashi (1983).

cannot disclose. Because the macronucleus of *P. caudatum* is highly polyploid (Soldo *et al.* 1981) as in other ciliates, dosage effects among genes may appear.

P. caudatum is a convenient material for transformation experiments by injection of macronucleoplasm, because it has a large macronucleus and there have been no reports of phenotypic assortment and autogamy. Expression of mixed chromatin must be stable.

Transformation experiments by macronucleoplasmic injection in *Paramecium* have so far been reported by a few authors. Harumoto & Hiwatashi (1992) rescued behavioural mutants *cnrA* and *cnrB* by injection of wild type nucleoplasm. On the other hand in *P. tetraurelia*, Koizumi & Kobayashi (1981) transformed cells of mating type O to mating type E and Harumoto (1986) induced a change in a non-Mendelian determinant by transplantation of macronucleoplasm.

2. Materials and methods

(i) *Stocks and culture method*

The stocks of *Paramecium caudatum* belonging to syngen 3 and 12 were used (listed in Table 1). CNR (P. caudatum non reversal) is a membrane inexcitable mutant (Takahashi & Naitoh 1978; Takahashi, 1979) and TND (trichocyst non discharge) is an exocytosis mutant that cannot discharge trichocysts (Takei *et al.* 1986). The culture medium was 2·5% (w/v) fresh lettuce medium inoculated with *Klebsiella pneumoniae* one day before use (Hiwatashi, 1968). All cultures were kept at 25 °C.

(ii) *Microinjection techniques*

Microinjection was performed by the modified method of Ohba *et al.* (1992), using two needles; one for injection of macronucleoplasm and the other for supply of the medium. Cells to be injected were deciliated with 5% ethanol (Ogura, 1981) and embedded in mineral oil (Squibb & Sons, Inc.). Deciliated cells can be injected with a larger volume of macronucleoplasm compared with normal ciliated cells which are flattened and immobilized for injection. About 2–20 pl of the macronucleoplasm of the donor was injected into a macronucleus of the recipient. After injection, recipient cells became reciliated within 1 h when they were incubated in modified Dryl's solution [substitute NaH_2PO_4 in original Dryl's solution (Dryl, 1959) for KH_2PO_4] containing 0·01% BSA and 0·02% methyl cellulose. After about 16 h of incubation, cells were transferred to the culture medium. Then, each recipient clone was transferred to a test tube. The tube cultures were fed with 4, 8 and 8 ml of fresh culture medium on successive days, and on the day after the last feeding, cells were transferred to new tubes containing 2 ml culture medium, after which the same feeding schedule was continued.

(iii) *Observations on transformants*

Three kinds of phenotype – mating type, behaviour and exocytosis of trichocysts – were examined each time when transfers to new tube cultures were made. If recipient cell lines expressed the donor character more than twice among a total of 10 examinations, they were judged to be transformants.

The mating types of the recipient cell lines were determined by reactions with testers of standard mating types expressing high mating reactivity. To determine the mating type of a single cell, CNR mutants were used as testers. A recipient cell expressing wild-type phenotype was mixed with a tester, and after determining the mating type the recipient cell was recovered by transferring to a K^+ test solution and was then mixed with the next tester.

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Table 2. *Transformation of mating types in intrasyngenic transplantation of macronucleoplasm**

Donor	Recipient	No. of cells injected†	Transformation of behaviour‡	Transformation of mating type§
E^3	O^3			
(C103, Mt^3/Mt^3)	(16B1002, *mt/mt*)	24	21	3
(Kyk402, Mt^3/mt)	(16B1002, *mt/mt*)	23	22	2
E^{12}	O^{12}			
(Yo3-1, Mt^{12}/mt)	(Yo16, *mt/mt*)	22	Not applicable	0
(Yo3-1, Mt^{12}/mt)	(GT105, *mt/mt*)	15	Not applicable	0

* Volume of macronucleoplasm injected was from 5 to 20 pl.
† This column indicates the number of cells that survived 16 h after injection.
‡ No. of recipient cell lines showing backward swimming when cells in culture medium were transferred to modified Dryl's solution containing 20 mM KCl. Not applicable when recipient was wild type.
§ No. of recipient cell lines showing mating type of the donor. Mating types were judged by mating reaction with the tester cells.

The behaviour was tested in a modified Dryl's solution containing 20 mM-KCl. *Paramecium* is known to swim backwards when transferred to stimulating solutions, such as a high K^+-solution (Naitoh, 1968). CNR mutants cannot swim backwards because they have a defect in membrane excitability. We judged cells to be transformants when backward swimming of cells was very fast for a long period and indistinguishable from wild-type cells. When cells were whirling or showed slow backward swimming for a very short period, they were judged to be CNR.

Exocytosis of trichocysts was stimulated with picric acid. The mutant TND cannot discharge trichocysts after this treatment (Takei *et al.* 1986). An ordinary stereomicroscope was used for this observation.

3. Results

(i) *Intrasyngenic transplantation*

We examined the mating type of recipient cell lines when macronucleoplasm from mating type E was transplanted to type O of the same syngen (Table 2). When cells homozygous for Mt^3 (C103) were used as the donor, the behavioural mutant character was rescued in a ratio of 21 out of 24 recipients but change of mating type from O^3 to E^3 was observed only in three cases. When macronucleoplasm of the E^3 heterozygous for Mt^3 (Kyk402), i.e. Mt^3/mt, was injected into O^3 cells, two out of 23 recipients expressed type E^3. Thus, the ratio of the transformation of mating type of the recipients was not different after injection of macronucleoplasm from homozygous or heterozygous donors, in regard to the *Mt* alleles. On the other hand, when macronucleoplasm of type E^{12} was injected into cells of two different stocks of type O^{12}, neither recipient changed to E^{12}.

In transplantation within syngen 3, recipients changed their mating type from O^3 to E^3 by the injection of about 10–15 pl of macronucleoplasm (about $\frac{1}{3}$–$\frac{1}{2}$ of the volume of a macronucleus), while in syngen 12, change in recipient O^{12} to mating type E^{12} was not observed with the injection of even more than 20 pl of macronucleoplasm of E^{12}. Two different stocks of type O^{12} were used as recipients in these experiments.

(ii) *Intersyngenic transplantations of type E macronucleoplasm to type E cells and type O macronucleoplasm to type O cells*

When cells of E^{12} were injected with macronucleoplasm of type E^3, four out of 17 recipients expressed the dual type E^3E^{12}, and when cells of E^3 were injected with E^{12} macronucleoplasm, 10/15 expressed dual type E^3E^{12} (Table 3). The dual mating type E^3E^{12} was not a mixture of cells of different mating types, because whenever samples from several recipient clones were tested, a single cell expressed both type E^3 and E^{12} simultaneously (data not shown). In *P. caudatum*, cells of type E change to type O when they age and are in late stationary phase (Myohara & Hiwatashi, 1975). Both the donor and the recipient were aged clones. When clones of cells showing type E^3E^{12} in these experiments reached the late stationary phase, they were converted to O^3O^{12}, which also means that a single cell expresses both O^3 and O^{12} simultaneously. Thus it is concluded that not only Mt^3 but also MA^3 and MB^3 are transferred by the injection.

When intersyngenic transplantation of macronucleoplasm was carried out between O^3 and O^{12} reciprocally, clones expressing dual O types, O^3O^{12} were produced (Table 3). These results are consistent with the genetics of mating type reported by Tsukii & Hiwatashi (1983).

As seen in Table 2, when macronucleoplasm of type E^{12} was injected into cells of type O^{12} the latter did not express E^{12}. Here, however, when the same macronucleoplasm of type E^{12} was injected into cells of E^3 which belong to a different syngen, the latter expressed E^{12} together with E^3.

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Table 3. *Intersyngenic transplantation between cells of different E types and different O types**

Donor	Recipient	No. of cells injected	Transformation of behaviour	Transformation of mating type	
				Hybrid†	Complete‡
E^3 (16B909, Mt^3/mt)	E^{12} (Yo3-1, Mt^{12}/mt)	17	Not applicable	4	0
E^{12} (Yo3-1, Mt^{12}/mt)	E^3 (16B909, Mt^3/mt)	15	15	10	3
O^3 (16B1002, mt/mt)	O^{12} (Yo16, mt/mt)	18	Not applicable	12	0
O^{12} (Yo16, mt/mt)	O^3 (16B1002, mt/mt)	22	17	11	0

* Volume of macronucleoplasm injected was from 5 to 10 pl.
† Recipients showed dual mating types.
‡ Recipients showed mating type of the donor. Other conditions are the same as those in Table 2.

Table 4. *Phenotype transformation in intersyngenic transplantation*

Donor	Recipient	No. of cells injected	Transformation of behaviour	Transformation of mating type		
				O hybrid	Complete	Others
E^{12} (Yo3-1, Mt^{12}/mt)	O^3 (16B1002, mt/mt)	62	52	34	4	11*
E^3 (16B909, Mt^3/mt)	O^{12} (Yo16, mt/mt)	23	Not applicable	1	0	1†
O^{12} (Yo16, mt/mt)	E^3 (16B909, Mt^3/mt)	23	20	0	3	0
O^3 (16B1002, mt/mt)	E^{12} (Yo3-1, mt^{12}/mt)	17	Not applicable	1	0	0

* Unusual E, that is E^3.
† Unusual E, that is E^{12}. Three of these unusual E lines are described in Table 5, and then used in the Table 6 experiments. Other conditions are the same as those in Table 2.

Table 5. *Examples of the expression of mating types in recipient clones of intersyngenic injection through successive tube-transfer cultures*

Injection (clone No.)	Mating types of recipients after successive numbers of tube transfer cultures									
	1	2	3	4	5	6	7	8	9	10
$E^{12} \rightarrow O^3$										
(108)	E^3	E^3	E^3	E^3	ND*	E^3	ND	E^3	E^3	E^3
(350)	E^{12}	E^{12}	E^{12}	E^{12}	E^{12}	E^{12}	E^{12}	E^{12}	E^{12}	E^{12}
$E^3 \rightarrow O^{12}$										
(133)	O^{12}	O^{12}	O^{12}	O^{12}	ND*	E^{12}	E^{12}	E^{12}	O^{12}	O^3

* Mating type was not determined.

(iii) *Intersyngenic transplantation of E macronucleoplasm to type O cells*

When macronucleoplasm from type E cells is injected into cells of type O of a different syngen, it might be expected that the recipient would express the single O type of the recipient, the dual O type of the donor and the recipient or the single E type of the donor, according to the theory of mating-type determination system by Tsukii & Hiwatashi (1983). The results are summarized in Table 4. When cells of type O^3 were injected with macronucleoplasm of E^{12}, 13 cell lines from the 62 recipients showed the mating type of the recipients, 34 cell lines expressed dual type O^3O^{12} and four changed to type E^{12}. An unexpected result was the occurrence of 11 injected clones which expressed stable type E^3. Type E^3 was thought to contain the gene Mt^3 but neither donor nor recipient in these experiments has gene Mt^3. Similarly, one of the 23 type O^{12} cells which were injected with E^3 macronucleoplasm expressed type E^{12} controlled by Mt^{12} which is in neither the donor nor the recipient. Three

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Table 6. *Re-transplantation from the converted type E to other type E cells*

Donor	Recipient	No. of cells injected*	Transformation of behaviour*	Transformation of mating types*		
				E^3	E^{12}	Hybrid E^3E^{12}
tE^3-108	E^3 (16B909, Mt^3/mt)	12	12	12	0	0
	E^{12} (Yo3-1, Mt^{12}/mt)	14	Not applicable	0	0	14
tE^{12}-350	E^3 (16B909, Mt^3/mt)	16	16	0	0	16
	E^{12} (Yo3-1, Mt^{12}/mt)	10	Not applicable	0	6	4

* For details, see footnotes in Table 2.

Table 7. *The relation of the volume of injected macronucleoplasm to the expression of phenotypes**

Volume of injected macronucleoplasm (pl)	No. of cells injected	Transformation of behaviour	Transformation of exocytosis†	Transformation of mating types	
				Hybrid O^3O^{12}	Complete
0·5–2	12	6	6	1	0
2–5	13	13	13	9	0
5–10	13	13	13	11	0
15–20	13	13	13	9	4

* The donor was E^{12} (Yo3-1, Mt^{12}/mt) and the recipient was O^3 (16B1002, mt/mt).
† Number of recipient cell lines that discharged trichocysts by picric acid. Others are the same as in Table 2.

of these exceptional recipients are described in Table 5. These unexpected results show that when E type macronucleoplasm is injected into O type cells of a different syngen, expression of the syngen specificity of the donor E type in macronucleoplasm of the recipient is sometimes converted by the syngen specificity of the recipient and becomes that of the recipient.

(iv) *Re-transplantation of the converted E type macronucleoplasm with altered syngen specificity to original E type cells*

Re-transplantation was done to see if this conversion was stable. Clone tE^3-108 (clone 108 in Table 5) which expressed complete E^3 by the transplantation of E^{12} to O^3 was re-injected into E^3 and E^{12} cells. As shown in Table 6, all recipients expressed stable dual type E^3E^{12} when tE^3-108 was re-transplanted to E^{12} cells. In the control transplantation of tE^3-108 to E^3 cells, all recipients expressed only type E^3. This suggests that tE^3-108 converted from type O^{12} has stable gene Mt^3. In addition, clone tE^{12}-350 (clone 350 in Table 5) which expressed E^{12} without changing syngen specificity by the injection of E^{12} into O^3 was also re-injected into E^3 and E^{12} cells. When tE^{12}-350 was injected into E^3 cells, all recipients expressed E^3E^{12} as in the re-injection of tE^3-108. However, when tE^{12}-350 was injected into E^{12}, six out of 10 expressed E^{12} as expected but the remaining four expressed E^3E^{12}. This result was quite unexpected because the transformant tE^{12}-350 expressed only type E^{12} for 10 successive tube transfers (about 70 fissions) (Table 5). This suggests that in tE^{12}-350 the conversion of mating type from recipient O^3 to donor E^{12} occurred so extensively that it could not express type E^3 as tE^3-108, but still, in some conditions as re-injection, it expresses E^3 by the effect of the first recipient O^3.

(v) *Relation of the volume of injected macronucleoplasm to the transformation of phenotypes*

When the recipient used for the transplantation of macronucleoplasm was a recessive behavioural mutant *cnrB*, the behavioural marker of the recipients was mostly changed (Table 2, 3, 5 and 6). This suggests that, compared to transformation of mating type, CNR can be 'cured' with a smaller volume of injected macronucleoplasm. We examined the relation between the volume of the injected macronucleoplasm and transformation of phenotypes. In the experiments, cells of type O^3 were injected with 0·5–2, 2–5, 5–10 and 15–20 pl of E^{12} macronucleoplasm and the behaviour, exocytosis and mating type phenotypes of the recipients were examined. As shown in Table 7, recessive characters of *cnrB* and *tnd2* began to be cured by the transplantation of as small as 0·5–2 pl of the macro-

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nucleoplasm. This result is consistent with the result of Harumoto & Hiwatashi (1992) who showed that *cnrB* was rescued by transplantation of 0·5 pl of macronucleoplasm. As to the mating type transformation, more than 15 pl of macronucleoplasm (about $\frac{1}{2}$ of the volume of the macronucleus) was necessary for the change from O^3 to E^{12}, though the dual O type, O^3O^{12}, appeared in the injection of 2–5 pl (Table 7). When macronucleoplasm was injected into the cytoplasm and not into the macronucleus, recipients did not change any of the above three characters (data not shown).

4. Discussion

The most unexpected phenomenon in this report is the conversion of syngen specificity when E type macronucleoplasm was injected into O type cells of a different syngen. As shown in Table 4, when E^{12} macronucleoplasm was injected into O^3 cells, 11 out of 62 recipients changed to E^3 within 10 fissions after injection and when E^3 was injected into O^{12}, one out of 23 changed to E^{12}. According to the three-gene hypothesis of mating type determination in *P. caudatum* (Tsukii & Hiwatashi, 1983), *Mt* determines not only the mating type E but also its syngen specificity. The change of syngen specificity upon injection of macronucleoplasm mentioned above cannot be explained by the three-gene hypothesis. The following two interpretations may be possible explanations of this peculiar phenomenon; (1) not only *Mt* but also *mt* has a site controlling the syngen specificity and the change of syngen specificity upon injection of macronucleoplasm occurred by the gene dosage effect between *Mt* and *mt* or (2) syngen specificity of the *MA* and *MB* loci in some way affects expression of the injected *Mt* and changes the syngen specificity of the latter.

In both cases of hypotheses (1) and (2) gene dosage effect may be important. The amount of macronucleoplasm to be injected had to be more than $\frac{1}{3}$ of the volume of the macronucleus for the syngen-specific change of mating type E. Tsukii & Hiwatashi (1985) and Tsukii (1988) reported that polysomic (aneuploid) cell lines for mating type loci can be made using meiotic non-disjunction. If we can obtain tetrasomic cell lines as $Mt^{12}/mt/mt/mt$ where all *mt*s are from syngen 3 and those as Mt^{12}/mt; $MA^{12}/MA^3/MA^3/MA^3$; $MB^{12}/MB^3/MB^3/MB^3$, and test the change of syngen specificity of mating type, we shall be able to test the validity of hypotheses (1) and (2).

A completely different interpretation of the above phenomenon is that the *mt* allele in syngen 3 is actually a Mt^3 allele in an inactive state and change to the active state was induced by the macronucleoplasm of E^{12} cells which have active Mt^{12} alleles. This interpretation is much more like the nature of the *Mt* gene in *P. tetraurelia*, where expression of *Mt*, not its presence or absence, controls the difference of E and O types (Sonneborn, 1947, 1974). In *P. caudatum*, however, no effect of cytoplasm or environment upon the expression of the *Mt* allele was known.

For the transformation by macronucleoplasmic injection, the amount of injection must be important. In the transplantation from cells of E^{12} to those of O^3 reported in this paper, 2 pl of the macronucleoplasm rescued *cnrB* and *tnd2* mutants. As to the transformation of mating type, however, injection of 5 pl E^3 macronucleoplasm changed cells of E^{12} to those of E^3E^{12} and the same amount of O^3 macronucleoplasm changed cells of O^{12} to O^3O^{12} (Table 3). Moreover, when E^{12} was injected into O^3, the expression of type E^{12} occurred only when the volume of injected E^{12} macronucleoplasm was more than 15 pl. Why is a larger amount of injection necessary for the transformation of mating type, especially from O to E than for transformation of behavioural and exocytotic genes?

One possible interpretation is that *cnrB* and *tnd2* are null alleles. Then no competition would occur between the mutants and their wild-type alleles for gene products, whereas mating type alleles of different syngens equally have their gene products and their competition for larger gene dosage must be necessary. By the three-gene hypothesis of mating type determination (Tsukii & Hiwatashi, 1983), the gene *Mt* controlling type E is epistatic to *MA* and *MB* which control type O. This suggests that there are some interlocus interactions working between *Mt* and *MA* and *MB* for the expression of mating types. If we assume that the gene dosage ratio of *Mt* to *MA* and *MB* is important for the epistatic expression of *Mt*, the result of the expression of injected Mt^{12} in cells of O^3 only when more than 15 pl of macronucleoplasm were injected would mean that for the epistatic expression Mt^{12} in O^3 cells, the ratio of Mt^{12} to *MA* and *MB* (MA^3 and MB^3 of recipient, and MA^{12} and MB^{12} from donor) should be more than 1:3. In the intrasyngenic injection of E^{12} macronucleoplasm into O^{12} cells, however, injection of even more than the above ratio was unable to change the recipient from O^{12} to E^{12} (Table 2). When injections of macronucleoplasm from *Mt* homozygote and heterozygote were compared, no difference was found in their effects, not only on the change of mating type from O to E but also on the rescue of *cnrB* (data not shown). Whether this is due to gene dosage compensation is unknown.

Important evidence relevant to the change of syngen specificity in intersyngenic transplantation of macronucleoplasm reported here has been reported by Sonneborn (1974) on the mating type of interspecies (intersyngenic) hybrids in the *P. aurelia* complex. In this species complex, interspecies matings occur in some combinations of species (syngens) (Sonneborn, 1947, 1974). In the interspecies cross between an E-type stock of one species and an O-type stock of another species, the E type hybrid progeny expressed not only dual E types but also the E type specificity

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which is genetically missing in either of the parental stocks (Sonneborn, 1974). This interspecies combination of stocks in the *P. aurelia* complex, if still available, should be very interesting material for analysis by the macronucleoplasmic transplantation technique.

Harumoto & Hiwatashi (1992) reported that clones transformed by the injection of macronucleoplasm can be classified into two groups: stable and unstable transformants. The stable transformant maintains chromatin of both donor and recipient and expresses the dominant phenotype. The unstable transformant assorts the injected macronucleoplasm and produces the appropriate phenotype. Thus an injected cell line segregates into sublines of donor and recipient phenotype. In our experiments, the transformation of unstable type as reported by Harumoto & Hiwatashi rarely appeared (less than 1 %, personal observation). They reported that the unstable type was seen when the donor and recipients were in the same stage of the cell cycle, i.e. G1. But we did not obtain the unstable type even when the cells of G1 phase were used to inject into recipients of G1 (data not shown). Furthermore, when injected cells were transferred into the culture medium at various times such as 4, 8, 16, 24 and 36 h after injection, all cells were of stable type (data not shown). The reason for this discrepancy is unknown.

Whatever the explanation is, analysis of the mechanisms of the mating type transformation induced by the nucleoplasmic transplantation will lead not only to better understanding of mating type genetics in ciliated protozoa but also the elucidation of important mechanisms of gene interaction. Furthermore, change of genotype by nucleoplasmic transplantation in ciliates has some general advantage over conventional classical experimental genetics using crossbreeding. Ciliates often have a long sexually immature period after conjugation and a high post-conjugational mortality in the senescent period, both of which hinder efficient crossbreeding analysis. Analysis by nucleoplasmic transplantation can eliminate the immature period and avoid post-conjugational death. On the other hand, more than two genes from different sexually isolated groups (syngens or species) can easily be put together and thus their expression and interactions can be analysed.

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