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A modifier gene involved in the expression of the dominant mating type allele in *Paramecium caudatum*

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SUMMARY

Mating type in *Paramecium caudatum*, syngen 3 is determined by a pair of alleles with simple dominance; the recessive allele restricts homozygotes to mating type V and the dominant allele permits expression of mating type VI. Clones of mating type V never show natural selfing, but most clones of mating type VI self naturally. A mutant clone of mating type VI which never selfed over a period of more than 3 years was obtained by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. When this mutant clone was crossed to a wild-type stock of mating type V, all F_1 clones of mating type VI gave rise to selfers. From selfing of these F_1 of mating type VI, clones of F_2 were obtained. Nearly 3:1 segregation of selfer to non-selfer clones was observed among the F_2 clones of mating type VI. The results were consistent with the interpretation that a dominant modifier gene, $Su(+^{mtV})$, controls the instability in the expression of mating type VI.

1. INTRODUCTION

Mating type in *Paramecium caudatum* is determined by a pair of alleles with simple dominance; the recessive allele restricts homozygotes to the odd mating type, and the dominant allele permits expression of the even mating type (Hiwatashi, 1968). Clones of recessive homozygotes express the odd mating type faithfully in the course of the clonal life history and never change to the even mating type. Clones with the dominant mating-type allele, both homozygotes and heterozygotes, express the even mating type during about the first month of the clonal period of maturity but become unstable in the later period of the clonal life-cycle. The latter period is associated with senility in this species. In the period of senility, clones with the dominant mating type allele express the even mating type in the early stationary phase but are odd mating type in the late stationary phase. Thus, a change of mating type without cell division is observed during the stationary phase (Hiwatashi, 1969).

In *Paramecium aurelia*, Butzel (1955) proposed the hypothesis that the odd mating-type substance is a precursor of the even mating-type substance and that a dominant allele at the mating-type locus controls the conversion reaction. It has been suggested (Hiwatashi, 1968) that this hypothesis can be applied to the mating-type system in *P. caudatum*. According to this hypothesis, the change of mating type from even to odd in *P. caudatum* would mean that there might occur

inactivation or suppression of the dominant mating type allele during the stationary phase when clones of the even mating type enter into the senility period. Based on experiments with actinomycin D, Hiwatashi (1969) suggested that there might be another gene which controls the expression of the dominant mating type allele. This article reports the discovery of a modifier gene which controls the expression of the dominant mating type allele.

2. MATERIALS AND METHODS

(i) *Stocks and culture methods*

All stocks used belonged to syngen 3 of *P. caudatum*. Stock d-K20a, mating type VI, was used for mutagenesis because this stock showed comparatively high survival (about 60%) after selfing conjugation. Stock d-K20a was one of the progeny of a cross between stock d^m-1 and a selfing progeny of stock Ky. Stocks Kok 1, Ksy 1 and d^m-1, all belonging to mating type V, were used for crosses and for tests of mating type (mating type V testers). Stock d-N14a, mating type VI, was used as the mating type VI tester.

Culture medium was 1·25% (w/v) lettuce juice in Dryl's solution (Dryl, 1959) inoculated with *Klebsiella aerogenes* one day before use (Hiwatashi, 1968). Clones were cultured in test-tubes except during the period of a few fissions immediately after conjugation, during which cultures were maintained in depression slides. In the test-tube cultures 60–100 cells were inoculated in a test-tube containing 2 ml fresh culture medium and fed the following volumes on 3 successive days: 4, 10 and 10 ml. At every 4th day, 60–100 cells were transferred into new test-tubes and grown with the same feeding schedule. Under these cultural conditions, cells were grown at the rate of about 2 fissions per day. The cumulative number of fissions that a given clone underwent after conjugation was estimated from the number of transfers to new test-tube culture. The temperature for growth was 25 °C.

(ii) *Mutagenesis*

Mutagenic treatment was essentially the same as that reported by Sonneborn (1970). Cells in the logarithmic phase of growth were washed with Dryl's solution and treated for 60 min at room temperature with a freshly prepared solution of 75 μg/ml *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Wako Chemical Industries) in Dryl's solution. After treatment, the cells were washed three times with Dryl's solution and then transferred into a large volume of fresh culture medium. After an hour or two, single cells were isolated into depression slides with fresh culture medium and grown for 3 days. From those slide depressions in which cells showed good growth, cells were transferred into test-tubes containing 6 ml of culture medium. In 4–5 days the culture entered into the late stationary phase and produced selfing pairs. Twenty selfing pairs were isolated from each mutagenized clone, and one caryonide from each mate of a pair was grown to establish progeny clones. These were used for screening mutants.

(iii) *Crosses*

Cross-breeding analysis was made difficult by poor survival after conjugation, especially in the F_2 generations. Very few F_1 clones yielded sufficient F_2. In all crosses and selfings, pairs and exconjugants were isolated into exhausted culture medium (Takahashi & Hiwatashi, 1970) and then transferred into fresh culture medium about 24 h later. This process is necessary to avoid the occurrence of macronuclear regeneration (Mikami & Hiwatashi, 1975).

3. RESULTS

(i) *Features of the expression of mating type VI*

As mentioned in the Introduction, clones with the dominant mating-type allele, both homozygotes and heterozygotes, express mating type VI faithfully during 30–50 fissions after reaching the maturity period, and then begin to undergo

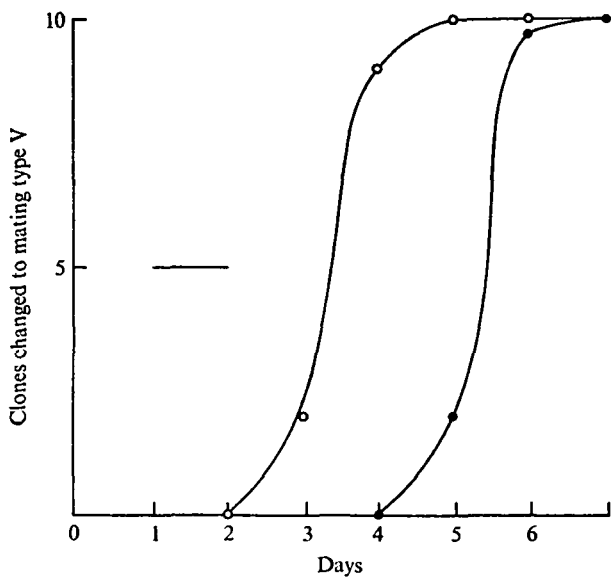

Fig. 1. Retardation of the change of mating type by treatment with cycloheximide. ●, Clones treated with 6 mM cycloheximide for 24 h; ○, control. Bar indicates the period of the treatment.

selfing conjugation. This selfing is due to the presence of cells of mating type V in the clone of mating type VI (Hiwatashi, 1960). When cells grow rapidly, the whole population enters into the stationary phase rather synchronously. Under such conditions, selfing does not occur in the first 2 days of the stationary phase. Selfing conjugation appears on the 3rd and 4th days, and from the 5th day the whole population expresses mating type V (Fig. 1). This change of mating type is not due to one subpopulation (mating type VI) becoming mating-reactive early and the other (mating type V) late. When single cells were isolated and tested for

mating reactivity, more than 80 % of them showed strong mating reactivity from the 1st to the 6th day of the stationary phase. Apparently the change of mating type is due to a change in single cells without cell division as in the case reported in *P. aurelia* by Taub (1966*b*).

Previous study (Hiwatashi, 1969) showed that the change of mating type is delayed by treatment with actinomycin D and suggested that protein synthesis is necessary for the change. A similar experiment using cycloheximide strengthens this idea. When cells in stationary phase were treated with 6 mM cycloheximide for 24 h preceding the occurrence of the change of mating type, about 48 h retardation of the change was observed (Fig. 1). Though mating reactivity was also lost by the treatment, it recovered completely 24 h after the treatment. This result again suggests that protein synthesis is necessary for the change of mating type. The action of the protein synthesis inhibitors is such that they neither induce nor accelerate the mating type change but on the contrary inhibit or retard the change. This means that they are not blocking the action of the dominant mating-type gene but may be blocking another gene controlling the expression of the dominant mating-type gene. Since such a gene would suppress the dominant mating-type allele, recessive mutants might be found in which the dominant mating-type allele continues to be expressed throughout the period of the stationary phase.

(ii) *Mutagenesis and cross-breeding analysis*

Among 800 selfing pairs isolated from 40 mutagenized clones, only 172 clones survived as true progeny of the selfing conjugation. Among them, 121 clones were identified as mating type VI when matured after a month of growth. These mating type VI clones were grown under the conditions of about 2 fissions per day over a period of 6 more months. After every transfer to new test-tube cultures the remaining cells in the old cultures (discard cultures) were examined for the appearance of selfing conjugation throughout the 6 days of the stationary phase when the cultures are mating-reactive. Among the 121 clones of mating type VI, 120 underwent selfing conjugation during the 1st month of the maturity period (earlier than 120 fissions after conjugation). Only one clone, NG-601, never selfed during the above period. This clone was grown for 4 more months and examined for the occurrence of selfing conjugation during that time. Selfing was never observed, and the culture was tested as pure mating type VI throughout its mating-reactive phase.

NG-601 was crossed to three different stocks of mating type V: Ksy 1, Kok 1 and d^m-1. In all three crosses, survival after conjugation was less than 10 %, and two crosses yielded no mating type VI clones. Only NG-601 × Kok 1, yielded 4 mating type VI clones of true F_1. All 4 of these clones were tested as selfers after about 2 months of growth (120 fissions). As soon as the F_1 mating type VI clones reached maturity, clones of F_2 were obtained from each by selfing conjugation induced by formalin-killed cells of mating type V. This method of selfing induction (Hiwatashi, 1949) was necessary because natural selfing yielded no viable F_2 clones, probably due to ageing. Non-selfing clones were recovered in the F_2 from only one among the

four F_1 clones. Since survival after selfing conjugation of the F_1 was only 5%, one of the F_2 non-selfing segregants, d-8b, was further crossed to stock Kok 1. This time, F_1 survival was 40·5%, and all F_1 clones of mating type VI were again selfers. Among 35 F_1 synclones of mating type VI selfer, 2 clones were selected to produce an F_2, using formalin-killed cells of mating type V. Segregation of selfer and non-selfer in the F_2 synclones was approximately 3:1, where segregation of mating type alleles was fairly normal (Table 1).

Table 1. *Genetic data from the cross between the non-selfer clone, d-8b, mating type VI and the wild-type stock, Kok 1, mating type V**

Generation		Mating type		Mating-type instability		Survival (%)
				Unstable	Stable	
		VI	V	VI	VI	
F_1	Observed	35	31	35	0	40·5
	Expected	33	33 (1:1)	—	—	—
	Chi square†	0·03		—	—	—
	P value	0·90 > *P* > 0·80		—	—	—
F_2	Observed	31	14	24	7	32·5
	Expected	33·9	11·3 (3:1)	23·1	7·7 (3:1)	—
	Chi square†	0·16		0·005		—
	P value	0·70 > *P* > 0·50		0·95 > *P* > 0·90		—

* Data are listed as number of synclones, including those in which only one clone of a synclone survived.

† For the calculation of chi-square, Yates's correction was applied.

The results suggest that stable expression of mating type VI, i.e. the non-selfer condition, is controlled by a recessive gene, which is designated $su(+^{mtV})$. In the wild type, the dominant allele $Su(+^{mtV})$ suppresses expression of the dominant mating-type allele, $+^{mtV}$, in the late stationary phase. The results of the breeding analysis also suggest that stock Kok 1, mating type V, is a dominant homozygote at this locus. Therefore, this locus does not affect the expression of mating type V.

4. DISCUSSION

Intraclonal conjugation (selfing) due to change of mating type without change in genotype has been reported for many ciliates (Taub, 1966*a*, *b*; Bleyman, 1967; Heckmann, 1967; and others). Intraclonal conjugation is prevalent in stocks of even mating types in *P. caudatum* (Hiwatashi, 1960, 1968). In this species, selfing conjugation due to recombination of mating type alleles by autogamy is excluded because (*a*) no autogamy has ever been found and (*b*) the change of mating type is known to be reversible (Hiwatashi, 1960). This information suggests that the selfing in *P. caudatum* is due to a change in the expression of the mating-type locus. The present study demonstrates that the change in the expression of the dominant mating-type allele is controlled by a modifier gene at another locus. As shown in the Results, this modifier gene interacts only with the dominant allele

at the mating type locus though it is contained in clones homozygous for the recessive mating-type allele. These features can best be explained by applying Butzel's hypothesis of mating-type determination (Butzel, 1955), i.e. the odd mating-type substance is a precursor of the even mating-type substance. As shown in Fig. 2, the modifier gene, $Su(+^{mtV})$, acts to suppress the terminal reaction in the synthesis of even mating-type substance, which is controlled by the dominant mating-type allele. In the recessive homozygote, $su(+^{mtV})/su(+^{mtV})$, the terminal reaction is not suppressed and stable expression of the dominant mating-type allele continues.

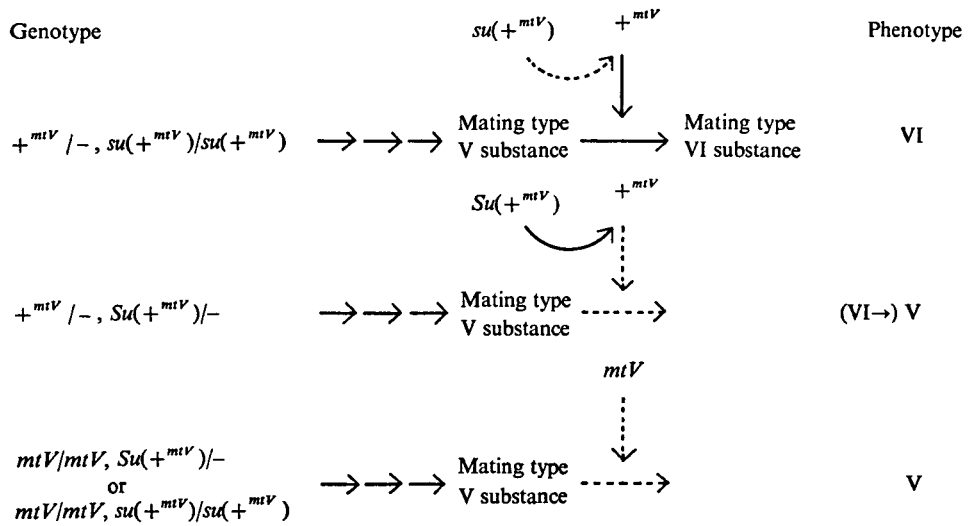

Fig. 2. Scheme for the expression of mating type in *Paramecium caudatum*, syngen 3. See text for various gene symbols.

The modifier gene is expressed only in aged clones. In this respect, the phenomenon resembles age-dependent intraclonal conjugation in *Euplotes crassus*, which is controlled genetically (Heckmann, 1967). The only difference between the phenomena in the two species is the dominant–recessive relationship. In *E. crassus*, mating-type instability is controlled by a recessive gene, but in *P. caudatum* it is controlled by a dominant gene.

Because of poor survival after conjugation, analysis of segregation ratios was so limited that linkage relationships between the modifier and the mating type-locus could not be elucidated. What induces activation of the modifier gene in the late stationary phase is an interesting problem remaining for future study.

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