Paramecium caudatumの接合能力の発現に対するマイトマイシンCの影響

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EFFECT OF MITOMYCIN C ON THE EXPRESSION OF MATING ABILITY IN *PARAMECIUM CAUDATUM*

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It has been known that an exconjugant clone of *Paramecium*, as many other ciliates, shows the sequence of sexual immaturity, maturity and senility (Jennings 1944; Sonneborn 1954, 1957; Siegel 1957; Hiwatashi 1958, 1960). *Paramecium* has no mating reactivity in immaturity, strong reactivity in maturity and weak or no reactivity in sensility. As other species of *Paramecium*, *Paramecium caudatum* has no ability to mate in the immature period of life cycle even under appropriate conditions. In maturity they show ability to mate if they are neither supplied with enough food nor in extremely starved condition and temperature is appropriate. Since the mating ability depends primarily upon the existance of complementary substances, so-called mating-type substances, on the cilia (Metz 1948, 1954; Cohen and Siegel 1963; Miyake 1964; Hiwatashi 1969), expression of mating reactivity in maturity might reflect any of the following phenomena; biosynthesis, activation and inactivation, or transportation of mating-type substances inside of the cells to the cilia. With *Paramvcium bursaria* which shows diurnal periodicity of mating reactivity, Cohen (1965) demonstrated that the change of reactivity actually reflects the biosynthetic process, and concluded that the cellular manifestation of mating reactivity requires protein synthesis. In the case of *Paramecium caudatum* also, expression of mating reactivity seems to require protein synthesis because at any time during the period of mating competence mating ability was lost by the treatment with actinomycin D (Hiwatashi, unpublished). These facts strongly suggest that expression of mating ability in maturity might reflect the expression of a particular gene concerning the appearance of active mating-type substances on the cilia. Thus the mechanism of the change from immaturity to maturity may include problems of regulation of gene expression.

Studies on the length of immaturity period and cell fission in *Paramecium* have been reported by several investigators (Sonneborn 1957; Kroll and Barnett 1968; Takagi 1970). In those studies the immature period was measured in days and fissions, and it was reported that the immature period usually depends upon cell divisions rather than days where temperature or quantity of nutrients limits the fission rate. The writers confirmed the same evidence and further tried to change the length of immaturity by artificial means. Since the length of immaturity depends upon the fission and mitomycin C is known to interfere with DNA replication (Shiba *et al.* 1959), the writers tested the effect of mitomycin C on the length of immaturity measured in fissions and succeeded to shorten it.

The present paper also provides some other informations relevant to the problems of immaturity.

MATERIALS AND METHODS

Materials used for the clonal expansion after conjugation were progenies from a cross between stocks Kok 1 and Koj which belong to mating types V and VI respectively in syngen 3 of *Paramecium caudatum* and stocks used for the tester were Ksy 1 and d-N14a which belong to types V and VI respectively. The exconjugant clones from the cross between Kok 1 and Koj showed a high percentage of survival (94%) and little or no macronuclear regeneration. Stocks of Kok 1, Koj and Ksy1 were collected in Kyoto by Mr. Y. Ito. Stock d-N14a is a progeny from a cross between stocks Nd3 and K4s15b.

Culture fluid used for the present experiment was the same as reported by Hiwatashi (1968). Each exconjugant was grown at 25°C with daily isolation and expanded to sub-caryonide or the next fission products and then single cells were randomly selected from each exconjugant clone and further expanded for two or three more fissions. In each exconjugant line, three cells from the final fission products were distributed into control culture at 25°C, low temperature culture at 15°C and the culture containing 0.1 mM mitomycin C at 25°C. Every culture was maintained in daily isolation line. Mean fission rates were 3.1 per day in control culture, 1.4 in low temperature culture and 1.3 in mitomycin C containing culture. Mitomycin C was added to the culture medium immediately before use to avoid the effect on the bacterial growth in the medium, since amount of the bacteria affects the fission of paramecium.

After daily isolation in each line the remaining cells in the depression were allowed to starve for two days where the cells divided approximately six more times. The starved cell were used to test mating reactivity by mixing with tester cells. From the 15th day after the conjugation to the time when every member of the exconjugant clone shows mating reactivity, the test was made every day. The fission count of the first positive test was used in calculating the length of immaturity.

RESULTS

In the first experiment, 21 pairs, 42 exconjugant clones from a cross between stocks Kok1 and Koj, were isolated and expanded till sub-caryonide or the next fission product in slide depression. As mentioned in the Materials and Methods, single cells were reisolated from sub-caryonide or the next fission product in each exconjugant clone and were allowed to grow for two or three more fissions. Single cells from the last fission products were distributed to control culture at 25°C, low temperature culture at 15°C and the culture containing 0.1 mM mitomycin C at 25°C. The three kinds of culture from each clone were continued under the condition of daily isolation. At about 15 fissions after the conjugation, samples were tested for mating reactivity to know whether they are descendants from true conjugation or those from cells undergone

macronuclear reorganization. Descendants from true conjugation have a period of immaturity but those undergone macronuclear regeneration show mating reactivity immediately after conjugation. The test was made with cells left after daily isolation and also those transferred into test tube culture. All clones showed no mating reactivity and were confirmed to be descendants from true conjugation.

Features of growth in three kinds of culture were shown in Fig. 1. As seen in the figure, control culture showed mean fission rate of 3.1/day, while both low temperature culture and the culture with mitomycin C showed mean fission rate of about 1.3/day until about the 15th day after the conjugation. In the culture with mitomycin C, however, the fission rate decreased gradually and finally ceased to divide and went to death about at the 20th day after the conjugation. To avoid the perish of the line, cells in the mitomycin C containing culturs were transferred into normal culture medium at the 18th day after the conjugation. After the transfer, the line showed low fission rate for about two days probably due to the remaining effect of mitomycin C but then the fission rate recovered almost to the same rate as control. Every day after 13th day from

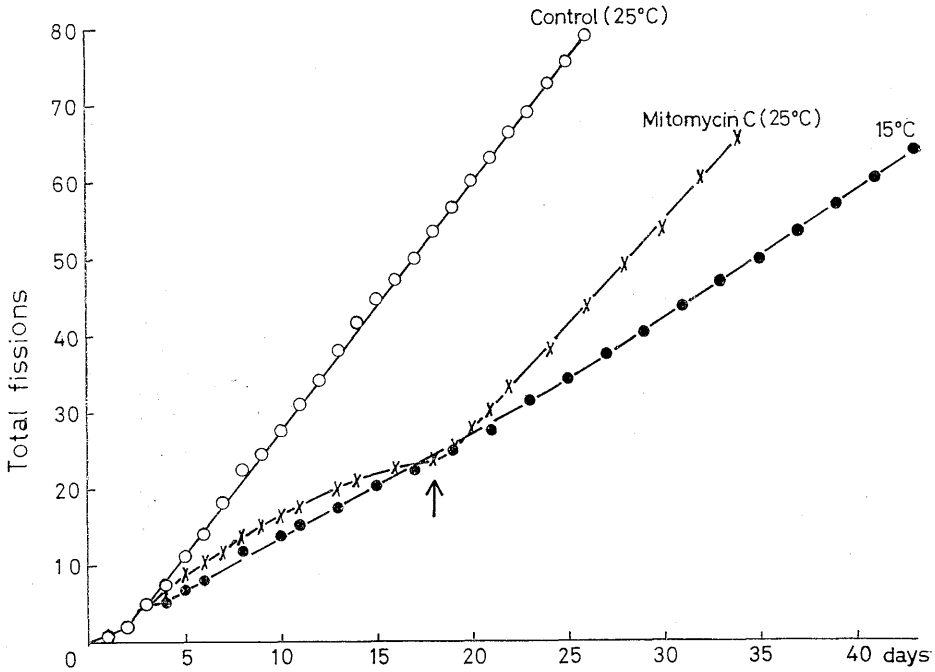

Fig. 1. Features of growth in three kinds of isolation line culture. Arrow indicates the time when the culture with mitomycin C was transferred to normal medium.

the conjugation, cells remained in the depression after the isolation of single cell to maintain the line were allowed to starve for two days and used for the test of maturity. The days and the fission counts of the first positive test were used to show the onset of maturity. Lengths of immaturity measured in days were 20-25 days in control at 25°C, 39-45 days in the cultures at 15°C and 28-34 days in the cultures with mitomycin

C. However, lengths of immaturity measured in fissions were 54-68 in both control and the cultures at 15°C, while they were 45-55 fissions in the cultures with mitomycin C. Mean fissions and days required were shown in Table 1. The results show that the length of immaturity depends upon times of fissions rather than days where temperature limits the fission rate. In the cultures with mitomycin C, however, the length of immaturity measured in fissions was about 10 fissions shorter than in the control, where temperature was the same but mitomycin C limits the fission rate for 15 days.

Table 1. The length of immature periods measured in days and fissions

		Type V	Type VI	Mean
Control (25°C)	Fissions	53.0 ± 2.0	62.9 ± 1.0	61.5 ± 1.4
	Days	22.7 ± 1.4	22.8 ± 0.4	22.8 ± 0.4
15°C	Fissions	53.5 ± 1.4	64.4 ± 1.0	62.9 ± 1.5
	Days	39.0 ± 1.0	43.1 ± 0.7	42.5 ± 0.8
Mitomycin C (25°C) 0.1 mM 15 days	Fissions	42.7 ± 2.8	51.7 ± 1.1	50.2 ± 1.3
	Days	29.0 ± 1.0	31.2 ± 0.8	30.9 ± 0.7

Another interesting feature of the length of immaturity was the difference between clones of different mating type. Mating type of *Paramecium caudatum,* syngen 3, is known to be determined synclonally and controlled by a pair of alleles (Hiwatashi 1968). In this experiment, segregation of mating type was synclonal but the ratio of segregation was abnormal, synclones determined to mating types V and VI being 3 and 18 respectively. Both the ratio and actual number of two mating types were exactly the same in three cases of different culturs. Thus the low temperature and mitomycin C in the period of immaturity did not affect the segregation ratio of mating type. However, mean length of immaturity in clones determined to mating type V was about 10 fissions shorter than those determind to VI in all three cases of different cultures (Table 1). In one exceptional clone in control culture determined to VI, precocious expression of mating type V was observed at about 3 days (10 fissions) earlier than the time when VI was first expressed. This expression of V was temporary and disappeared in the following two days and then VI was expressed. In this case also, fissions or days required for the expression of VI was the same as other clones determined to VI.

Second experiment was carried out to know the effect of higher temperature and also that of mitomycin C with different concentration and periods of treatment. Fifteen pairs of a cross between Kok1 and Koj were isolated and exconjugant clones were expanded in the same way as previous experiment. Single cells from each exconjugant clone were distributed to control culture at 25°C, high temperature culture at 33°C, and cultures with 0.1 mM and 0.2 mM mitomycin C. The culture with 0.1 mM mitomycin C was divided into two lines, one of which was transferred to normal culture medium at the 7th day after the onset of the treatment and the other at the 15th day as previous experiment. The culture with 0.2 mM mitomycin C was transferred into normal culture at the 3rd day after the onset of the treatment.

Unexpectedly, mean fission rate of the cultures at 33°C was not significantly different

from that of control (3.0/day) and days and fissions required for the onset of maturity were not very different from control, though fissions decreased slightly (Table 2). In the cultures treated with 0.1 mM mitomycin C, mean fission rate was 1.4 during the treatment. In those with 0.2 mM mitomycin C, it was 1.4 in the first day, 0.9 in the second day, 0.9 in the first day after the transfer to normal medium and 1.4 in the second day after the transfer, and then gradually recovered to normal (3.0/day) until 4th day in the normal medium. The effect of mitomycin C to diminish the length of immaturity period measured in fissions was also confirmed in this experiment. However, cultures treated with 0.1 mM for 7 days and 0.2 mM for 2 days showed immaturity periods of 3 to 5 fissions longer than those treated with 0.1 mM for 15 days (Table 2).

Table 2. The length of immature periods measured in days and fissions

		Type V	Type VI	Mean
Control (25°C)	Fissions	54.1 ± 1.4	64.1 ± 1.6	58.7 ± 2.1
	Days	21.6 ± 0.6	23.7 ± 1.2	22.6 ± 0.7
33°C	Fissions	53.1 ± 1.2	60.7 ± 2.7	56.5 ± 2.0
	Days	21.6 ± 0.9	23.4 ± 1.4	22.4 ± 0.8
Mitomycin C (25°C) 0.1 mM 15 days	Fissions	43.9 ± 0.8	52.2 ± 1.5	49.3 ± 1.8
	Days	24.9 ± 0.7	27.4 ± 0.9	26.1 ± 0.7
0.1 mM 7 days	Fissions	48.7 ± 0.8	57.4 ± 1.9	52.7 ± 1.9
	Days	23.4 ± 0.6	25.7 ± 1.0	24.5 ± 0.7
0.2 mM 2 days	Fissions	49.3 ± 1.4	55.0 ± 2.0	52.0 ± 1.6
	Days	23.3 ± 0.9	24.4 ± 1.2	23.8 ± 0.8

In this experiment, segregation of mating type between V and VI was 8 to 7 showing nearly 1:1 segregation as would be expected if VI was heterozygous for the mating-type alleles. Here again difference in the length of immaturity periods between different mating types was observed. As seen in Table 2, clones determined to V showed about 10 fissions shorter immaturity period than those determined to VI, though the difference was much less in the clones treated with 0.2 mM mitomycin C for 2 days. In one exconjugant clone in the control, precocious expression of mating type V as described in the first experiment was again observed. This clone expressed mating type V 4 days before the first expression of mating type VI and then returned to immaturity until it expressed VI.

DISCUSSION

In *Paramecium caudatum,* as mentioned in the results, the length of immaturity depends upon times of fissions rather than days where temperature limits the fission rate. In *P. multimicronucleatum,* Kroll and Barnett (1968) have reported that the immature period is more appropriately measured in cell divisions rather than days where temperature limits the fission rate. In *P. multimicronucleatum* and *P. caudatum,* however,

Takagi (1970) has reported that in many clones of both species the length of immature stage was closely related to the number of fissions rather than physical time, but in some other clones of both species it was related to both the number of fissions and physical time. If we assume that a particular gene concerning the appearance of active mating-type substances on the cilia is repressed in immature period and that the expression of the gene depends upon times of fissions, one of the simple explanations for the expression of mating ability after a certain number of fissions might be that a certain amount of substances which repress the genic activity exist in the exconjugant cell and are diluted with multiplication of the cells. The gene would be expressed when the amount of the repressing substances are diluted below a certain level with repeated fissions. In such a case, one exconjugant cell has to contain the substances at least over 10^{18} molecules. The 10^{18} molecules are about 6.7×10^{3} mol. Such abundant molecules in one exconjugant cell are not considered. Next possibility is to account for the phenomena by the same way as the appearance of sensitive cells from killer paramecia (Preer 1946) assuming some self-replicating factor which represses the genic activity. In this assumption, the factor must be considered to have a slower multiplication rate than the fission rate of the cell. The action of mitomycin C could be accounted for by assuming that the reagent destroys the factor or retards the multiplication of it. By this assumption, however, it is difficult to explain the fact that the length of immature period depends upon times of fissions when temperature limits the fission rate. Another possible explanation is to consider that for the appearance of mating ability maintenance of a fixed amount of some metabolic reactions for a certain period of time is necessary. In low temperature culture, the fission rate decreases but the amount of the metabolic reactions may also decreases proportionally. In the culture treated with mitomycin C, however, the reagent retards the fission rate but would affect the metabolic reactions less extensively. In any way, further study is necessary to answer the question what determines the length of immaturity. In *Tetrahymena pyriformis,* Bleyman and Simon (1967) reported that immature period can be drastically curtailed in the presence of mendelizing factors called Early mature (EM). Analysis of the action of such gene will be very helpful for the elucidation of the problem.

In one or two exceptional clones in control culture determined to VI, precocious expression of mating type V was observed at about 3 days (10 fissions) earlier than the time when VI was first expressed. And also mean length of immaturity in clones determined to mating type V was about 10 fissions shorter than those determined to VI in all three cases of different cultures. Whether these two phenomena have a common mechanism is unknown. As reported by Hiwatashi (1968), mating type of *Paramecium caudatum,* syngen 3 is controlled by a pair of alleles. Why the expression of the recessive allele comes earlier than the dominant allele is an interesting problem for future study.

SUMMARY

The length of immaturity in *Paramecium caudatum* was investigated counting the fissions exactly in daily isolation culture in depression slides and the following results were confirmed.

(1) The length of immaturity depends upon the times of fissions rather than days when temperature limits the fission rate.

(2) Mitomycin C shortens the length of immaturity about 10 fissions.

(3) The maturity in the clones determined to mating type V appears about 10 fissions earlier than those determined to mating type VI.

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