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Protoplasmic Streaming and Nuclear Differentiation during Conjugation of *Paramecium caudatum*

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ABSTRACT—It is known in *Paramecium* that the fate of the micronucleus is determined by the intracellular localization of the nucleus and that protoplasmic streaming named cyclosis is very active during vegetative phase. The active streaming, if it occurs during conjugation, may interfere with the correct positioning of micronuclei and/or cytoplasmic determinants in a cell. In the present work, the velocity of protoplasmic streaming was measured during conjugation of *P. caudatum*. The results showed that the velocity changed remarkably at 3 stages. (1) It increased with the expansion of its active area 3 to 5 minutes after the mating reaction and then decreased again to the ordinary speed within 3 hours. The results of micro- or macronuclear removal showed that the increase in the velocity was not mediated either by the micronucleus or by the macronucleus. Moreover, injection of the anti-α-tubulin antibody at the mating reaction did not suppress the protoplasmic streaming effectively, although a role of microtubules on the protoplasmic streaming is not ruled out completely. (2) At the stages of disintegration of meiotic products and the exchange of gametic nuclei, the cytoplasm ceased streaming and then resumed it soon after synkaryon formation. (3) The streaming ceased again at the critical stage of macro- and micronuclear differentiation after the separation of mating pair, although the streaming was very active before and after this stage. Both cessations of (2) and (3) were not influenced by removal of the micronucleus during conjugation. The velocity of protoplasmic streaming during conjugation does not directly correlated with the micronuclear behavior. The cessation seems to be very convenient for intracellular positioning of the cytoplasmic determinants and/or of the micronuclei for the nuclear differentiation.

INTRODUCTION

Protoplasmic streaming is very active and is known as cyclosis in the species of a unicellular animal *Paramecium*. In the cells, the cytoplasm flows constantly in the same direction, forming a closed circuit channel (Sikora *et al.*, 1979; Sikora, 1981). The protoplasmic steaming depends on the proteins of actin and myosin in many organisms, especially in plant cells. In *Paramecium*, however, we have no evidence to conclude whether these proteins or microtubules take part in it or not.

By the way, each cell of *P. caudatum* has a germinal micronucleus and a somatic macronucleus. The macronucleus differentiates from the micronucleus during the sexual process, i. e., conjugation and autogamy. The process of nuclear differentiation is controlled at two stages by intracellular positional information (reviewed by Hiwatashi and Mikami, 1989). At these stages, nuclear differentiation depends on intracellular localization. If active streaming occurs, it may influence on the complex behavior of the micronuclei during conjugation.

One of the stages of nuclear differentiation is survival versus disintegration of meiotic products of the micronucleus. After the induction of conjugation, the micronucleus undergoes meiosis and gives rise to 4 haploid nuclei in each cell of the mating pair. Of the 4 resulting haploid nuclei, all but one disintegrate and disappear. The surviving one lies in a bulge of the cell body, formed after meiosis, and is called a paroral cone because it lies near the region of the oral apparatus. The three nuclei that degenerate are localized out of the paroral cone. Thus, it is known that the intracellular position of the nucleus plays an important role, but it is not clear whether streaming disturbs the positioning of nuclei and/or cytoplasmic determinants. In *P. tetraurelia*, however, protoplasmic streaming ceases at the time of the presumable exchange of gametic pronuclei in conjugation (Sikora and Kuznicki, 1976).

The other stage of this phenomenon is the differentiation of macro- and micronuclei that occurs at the later stage of conjugation. A fertilization nucleus that is formed after the reciprocal exchange of gametic nuclei between mating cells divides three times and gives rise to 8 nuclei. These division products localize for a moment anteroposteriorly; 4 nuclei positioned at the anterior part of the cell and the other 4 localized at the posterior part of the cell. In the fission yeast

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Schizosaccaromyces pombe, nuclear positioning is achieved by regulation of spindle pole body-mediated nuclear migration (Hagan and Yamada, 1997). In *Paramecium* as well as in *S. pombe*, the nuclear envelope does not break down during nuclear division and the mitotic spindle forms inside the microtubules so that elongation of the spindles resulted in localizing the daughter nuclei anteroposteriorly. The nuclei positioned at the anterior part of the cell differentiate micronuclei and those localized at the posterior part differentiate into new somatic nuclei (reviewed by Hiwatashi and Mikami, 1989). The macro- and micronuclear differentiation seems to be determined by the cytoplasmic determinant localized anteroposteriorly. How is the protoplasmic streaming at the stages? Careful observation, so far, suggests that the velocity of the protoplasmic streaming is dependent to some extent on the stages of conjugation (Wichterman, 1940; Sikora and Kuznicki, 1973, 1976). However, the velocity has never been measured over the conjugational process. The present paper examines the velocity of cytoplasmic streaming in correlation with nuclear events during conjugation in *P. caudatum*.

MATERIALS AND METHODS

Stocks and culture

The stocks used were G3-3508, G3-9308 and amicronucleate clone 27aG3am (mating type V), and G3-9022 and G3-9306 (mating type VI) belonging to syngen 3 of *P. caudatum*. G3-9308 were kindly supplied by M. Takahashi (University of Tsukuba, Tsukuba, Japan). The culture and handling techniques for paramecia followed those of Hiwatashi (1968). Culture medium was fresh lettuce juice diluted with Dryl's salt solution (Dryl, 1959) modified by the substitution of KH_2PO_4 for NaH_2PO_4 (pH 7.0) (abbreviated here as K-DS for the modified Dryl's salt solution) and inoculated with a nonpathogenic strain of *Klebsiella pneumoniae* as food organisms the day before use. Cultivation and experiments were carried out at 25±1°C. Conjugation was induced by mixing mating-reactive cells of complementary mating types. Two or 3 hr after mixing the complementary types, mating pairs were isolated into K-DS as mentioned above.

Removal of the macronucleus from a vegetative cell

The macronuclei were removed from cells at vegetative G1. Prior to surgery, cells were placed in K-DS containing bovine serum albumin (2 mg/ml, Wako) and their macronuclei were withdrawn with a glass microinjection needle (inside diameter 7 to 10 μm) (Koizumi, 1974; Mikami, 1979). After the operation, cells were placed in K-DS supplemented with BSA (1 mg/ml). About one hour after, cells were then moved to K-DS and their cytoplasmic velocity was measured 1.5 to 3 hr after macronuclear removal.

Removal of the micronucleus during conjugation

Cells cultured in a nutritional food for human New Calorie Mate liquid type (Ohtsuka, abbreviated here CM) were opaque milky-white under the microscope, while they grown in an ordinary lettuce juice medium were semitransparent. To distinguish one type cell from the other type cell of a mating pair, cells of one mating type were cultured in a diluted CM with K-DS (2.5 μl/ml) and the other types were cultured in the ordinary lettuce juice. Conjugation was induced by mixing the cells grown in the CM and those grown in the lettuce medium. Three to 4 hr after the mixing, pairs of a semitransparent cell and an opaque cell were isolated. The micronucleus was removed from the semitransparent cell of each pair at the crescent stage about 4.5 hr after mixing or at the synkaryon formation 12 to 13 hr after the mixing. The method of micronuclear removal was the same as those of the macronuclear removal as mentioned above.

Measuring the velocity of protoplasmic streaming

Cells were kept in the culture medium diluted by adding the same amount of Ca^{2+}/EGTA buffer (2.0×10^{-6}M Ca^{2+}/EGTA) before measurement. The buffer was prepared by mixing 10 mM EGTA (final 2 mM), 10 mM $CaCO_3$ (final 1.7 mM) and 100 mM HEPES (final 10 mM), titrated with 1 N KOH to pH 7.0 (Xianyu and Haga, 1993). Prior to measurement, two capillaries (0.5～1 mm in outside diameter and about 2 cm in length) were laid in parallel about 1 cm apart on a slide glass. A drop of the medium containing some cells was put in the center of a cover glass and then a small drop of BSA solution (10% in the buffer) was added to it. The final concentration of BSA was estimated to be about 2%. The cover glass was turned upside-down and placed on the capillaries that had been laid on the slide glass. Flattening the normal shape of the cell improves observation of the specimen under a phase contrast microscope but also affects the cell in such a way that protoplasmic streaming is stopped (Sikora unpublished, cited by Sikora and Kuznicki, 1976). Therefore, the distance between the slide glass and the cover glass was set at 0.5～1 mm so as not to flattening the cells , which are about 50 μm in width and about 180 μm in length.

For measurement of the velocity, swimming cells were followed under the phase contrast microscope and recorded on videotape when the cells slowed down and stopped spontaneously. The velocity was determined on the monitor screen by measuring migration distance of a granule (0.3 to 0.6 μm in diameter) for 2 sec. Five granules were randomly selected and their migratory distances were measured. Thus, each value of velocity in the results represents the mean value of 5 granules in different parts of the cell. The velocity of protoplasmic streaming was examined just under the trichocyst layer and around the macronucleus (Fig. 1).

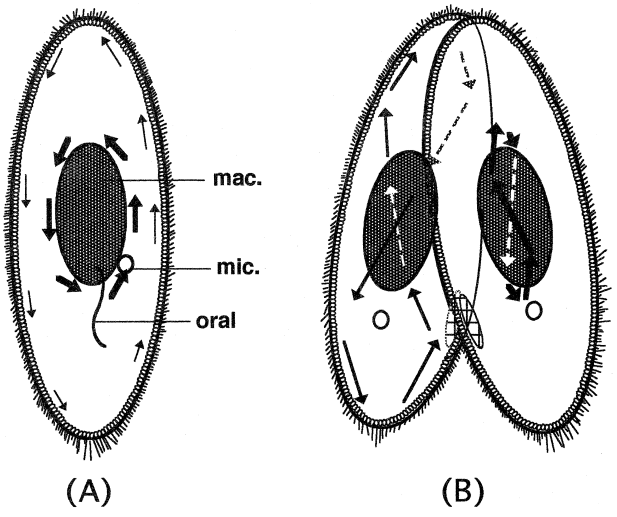

Fig. 1. Protoplasmic streaming. (A) The streaming just under the trichocysts layer and around the macronucleus viewed from the oral side of a vegetative cell. (B) The streaming in a mating pair. The cell on the left shows the streaming just under the trichocysts layer and that on the right shows around the macronucleus.

Injection of monoclonal anti-α-tubulin antibody

An anti-α-tubulin antibody (monoclonal antibody N356, Amersham) purchased is supplied as a solution (0.5 ml, 150 mg protein) in PBS (0.15 M NaCl, 0.05 M phosphate buffer pH 7.4) containing 0.1% (w/v) sodium azaid and 1.0% (w/v) BSA. Prior to microinjection, sodium azaid was removed by filtration with Centricon30

(Amicon). The monoclonal anti-α-tubulin antibody solution (20 to 30 pl) were injected into cells (Koizumi, 1974). After the injection, the cells were placed in K-DS supplemented with BSA (1 mg/ml) to heal the injury brought by the operation and transferred into K-DS after about 20 min.

RESULTS

Protoplasmic streaming in vegetative cells

The macronucleus is located at the center of the cell and the micronucleus is attached to the macronucleus. Small granules, suspended in the cytoplasm, flow constantly around the macronucleus in the same direction (Fig. 1-A). Mean values of the velocity at the vegetative G1 phase were 4.42±0.23 (±SD, n=4 cells) μm/sec in G3-9022, 4.37±0.21 (±SD, n=7 cells) μm/sec in G3-3508 and 4.46±0.53 (±SD, n=7 cells) μm/sec in G3-9308. There was no statistical significance between them, which suggests that the velocity is not so different among strains.

In the present study, the velocity of protoplasmic streaming was examined also just under the trichocysts layer where trichocysts were packed closely side by side about 3 μm from the cell surface, because the nuclei touched to the trichocysts layer at opposite ends of the cell when the direction of macro- and micronuclear differentiation was determined as mentioned above (Yashima and Mikami, unpublished). During vegetative phase, the cytoplasm was calm in an outer layer just under the trichocysts layer. In this outer layer, cytoplasmic granules streamed very slowly, and the mean value was 1.97±0.32 (n=10 cells) μm/sec in G3-3508.

Time course of nuclear events during conjugation of stocks used

When complementary mating types were mixed, mating agglutination occurred and continued for about an hour and then the holdfast unions, pairs united at the anterior ends, were released from the agglutinates and went into conjugation. In two hours after mixing, cells unite much more tightly at their paroral region. The micronucleus commenced meiosis about 3 hours after mixing of complementary mating types. In prophase of the first meiotic division, the micronucleus formed a structure called the crescent, in which the formerly spherical micronucleus elongated and formed a large crescent-shaped structure 6 to 7 hr after mixing (Fig. 2). After the two divisions of meiosis, one of the four haploid nuclei entered into the paroral cone and survived about 12 hr after mixing, but the other three products degenerated and were soon resorbed. The surviving nucleus underwent a mitotic division, producing migratory male and stationary female pronuclei.

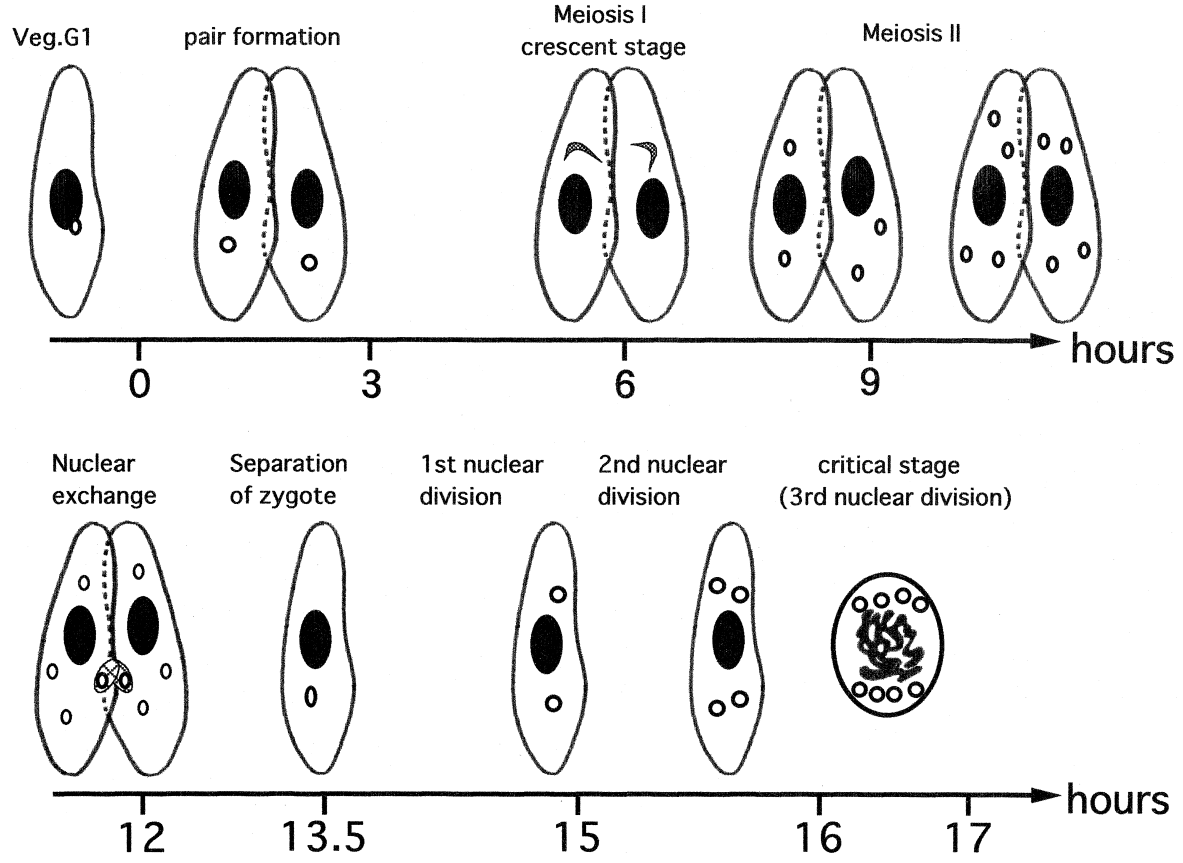

Fig. 2. Time course of nuclear events during conjugation after mixing of the complementary mating type cells. Three hrs after the mixing; Paroral union, 6 hr after mixing; crescent stage of the micronucleus, i. e., meiotic prophase, 9 hr after mixing; the first meiotic division, 11–12 hr after mixing; reciprocal exchange of gametic nuclei, 13 to 14 hr after mixing; separation of mating pairs, 15 hr after mixing; the first nuclear division, about 16 hr after mixing; the critical stage of nuclear differentiation, about 17 hr after mixing; recovery from shortening.

Exchange of migratory nuclei and formation of synkarya (fertilization nuclei) occurred at 12 to 13 hr after mixing. Then, the synkaryon divided three times. Mating pairs separated soon after the first division of the fertilization nucleus (Fig. 2).

At the third division of a fertilization nucleus, i. e., the critical division for macro- and micronuclear differentiation, the orientation of nuclear spindles lied parallel to the longitudinal axis of the cell and extend to opposite ends of the cell (Fig. 2). At the same time, a marked shortening of the cell length occurred. In consequence, the division products localized anteroposteriorly, that is, 4 nuclei positioned at the anterior part of the cell and the other 4 localized at the posterior part of the cell. The nuclei touched to the trichocysts layer at opposite ends of the cell. There were no difference in structure between anterior nuclei and posterior ones.

Effect of the macro- or micronuclear removal on the streaming during vegetative phase

The velocity in an amicronucleate clone 27aG3am was not different from that of micronucleates. That is, the mean value of amicronucleates 27aG3am around the macronucleus was 4.78±0.28 (n=9 cells) μm/sec, while that of micronucleates ranged from 4.37 to 4.46 μm/sec as mentioned above. This means that the injury brought by microinjection for nuclear removal did not affect on the velocity and that the absence of the micronucleus did not disrupt the streaming.

The macronucleus usually lied at the central area of the cell, though it sometimes moved back and forth during conjugation. When the macronucleus was removed, the streaming continued regardless the absence of the macronucleus. In the macronucleus free cell, the cytoplasm moved around the area where the macronucleus was and the direction of the flow was mostly the same as in macronucleate cells. At 1.5 to 3 hr after the removal of the macronucleus, the mean value measured decreased slightly to 3.66±0.7 4 (n=6 cells) mm/sec, though we can not conclude here whether the decrease has special means or not. The evidence suggests that the macronucleus is not necessary for the protoplasmic streaming.

Active streaming at the beginning of mating

Immediately after mixing the complementary types of G3-3508 and G3-9022, mating reaction occurred and then 3 to 5 min later the velocity of streaming increased (Figs. 3 and 4). Twenty to 30 min after mixing complementary types, the

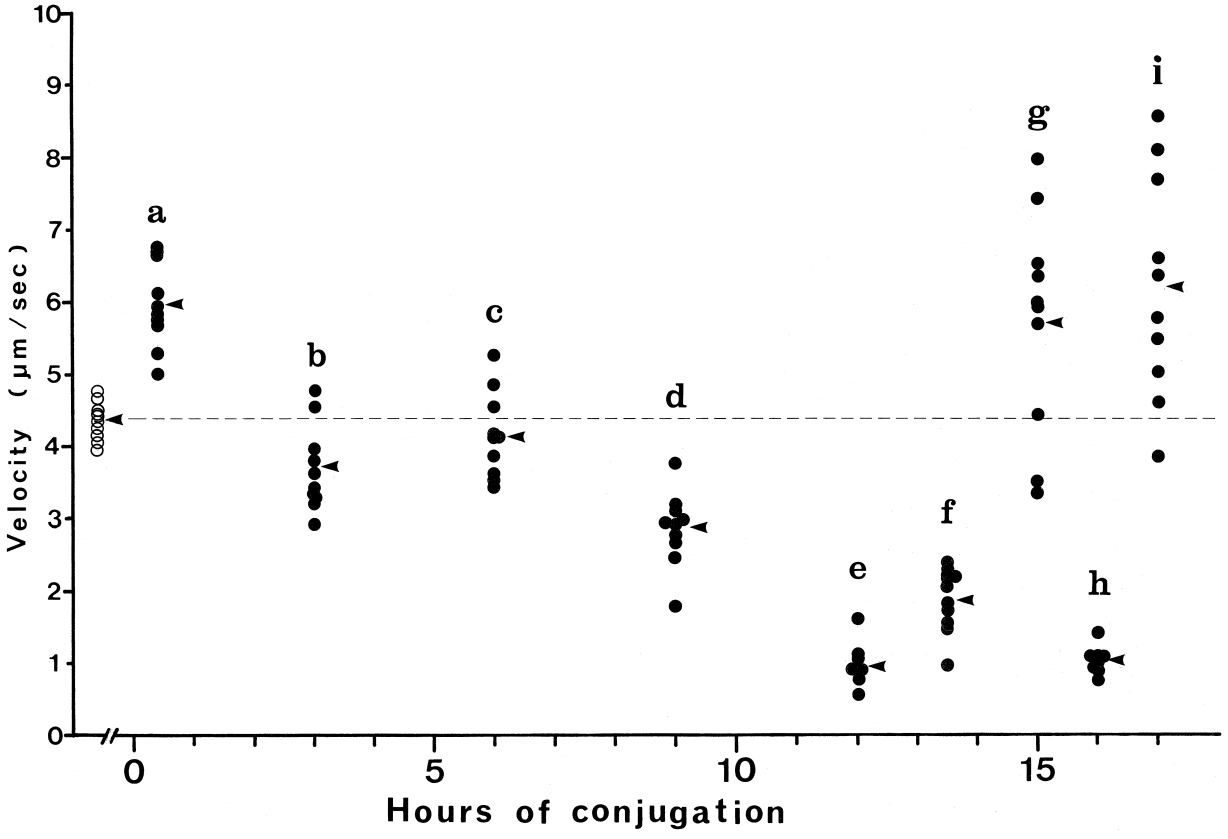

Fig. 3. Protoplasmic streaming around the macronucleus during conjugation. Open circles show the velocity of protoplasmic streaming in vegetative G1 cells. Each closed circle, (a) to (i), shows the mean value of the velocity at 5 different points in a cell during conjugation. Moreover, the arrow heads indicate mean values of closed circles. (a) 20–30 min after mixing complementary types, (b) paroral union 3 hr after the mixing, (c) at the crescent stage of the micronucleus, i. e., meiotic prophase 6 hrs after mixing, (d) 9 hr after mixing, which coincides with the metaphase of the first meiotic division, (e) at the stage of reciprocal exchange of gametic nuclei 11–12 hr after mixing, (f) soon after the separation of mating pairs, 13 to 14 hr after mixing, (g) about 30 min to 1 hr prior to the beginning of the cell shortening, 15 hr after mixing, (h) at the stage of cell shortening, i.e., the critical stage of nuclear differentiation about 16 hr after mixing, (i) recovery from shortening about 17 hr after mixing.

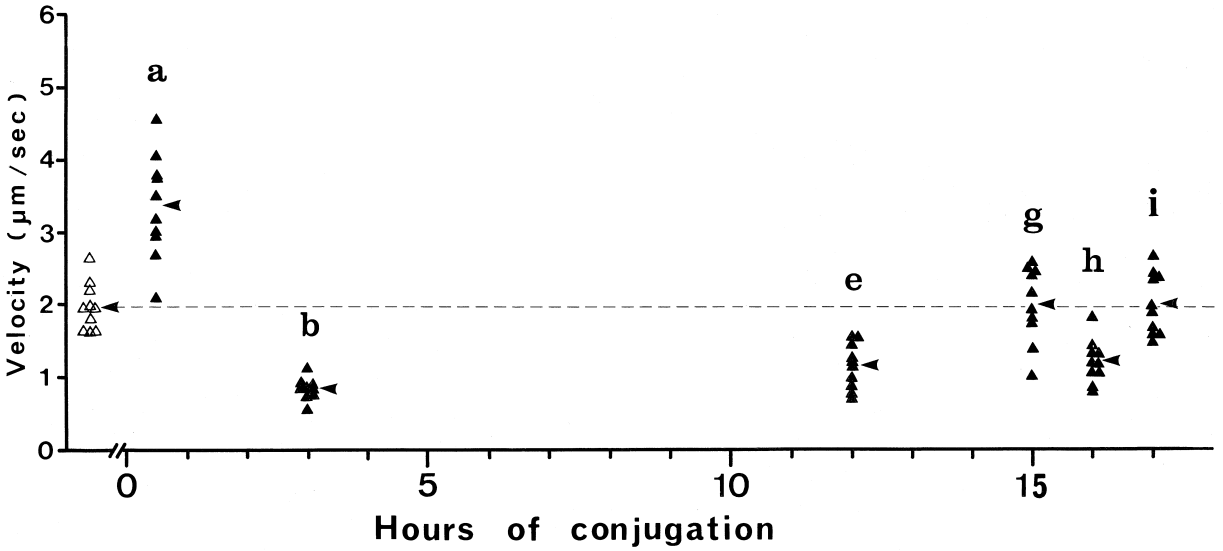

Fig. 4. Protoplasmic streaming under the trichocysts layer during conjugation. Open triangles show the velocity of protoplasmic streaming in vegetative G1 cells. Each closed triangle, (a) to (i), shows the mean value of the velocity at 5 different points in a cell during conjugation. The arrow heads indicate mean values of closed triangles. (a) to (i) are the same stages as those in Fig. 3.

velocity around the macronucleus increased to 5.97±0.53 μm/sec (n=9 cells), while the speed was 4.38±0.24 μm/sec before mixing the complementary types (Fig. 3). At the same time, the cytoplasm just under the trichocyst layer was also activated and its velocity was about 3.35±0.67 μm/sec (n=10 cells) (Fig. 3). The velocity decreased at around the stage of paroral union. The cytoplasm just under the trichocyst layer was very calm through all the mating stages except for this stage (Fig. 4). These results indicate that protoplasmic streaming at the beginning of conjugation is not only characterized by an increase in the velocity but also expansion of the streaming zone in a cell.

Is there any relationship between the increase in velocity and the micronucleus? When the amicronucleate clones of both mating types were mixed, the velocity showed very active value 5.23±0.4 7 (n=9 cells) that was equivalent to that of the micronucleates. When whole macronucleus was removed from each cell of mating type V about 1 to 3 hours before mixing with the complementary type, the velocity in the macronucleus free cells was also increased within 30 min after mixing the types, showing a mean value of 5.02±0.72 (n=5 cells) μm/sec. Though the mean value in macronucleus free cells is slightly lower than that of ordinary macronucleate cells but increased. These evidences show that the increase in the velocity at mating reaction was not mediated not only by the mediation of the micronucleus but by that of the macronucleus.

Injection of the anti-α-tubulin antibody prior to mating reaction

To probe the function of the microtubles on protoplasmic streaming, the anti-α-tubulin antibody (estimated to be 20 to 30 pl, 200 μg/ ml protein) was injected into the mating reactive cells of type V. This concentration and amount of the antibody inhibited the micronuclear migration to the paroral region, when it was injected soon after meiosis (Nakajima and Mikami, unpublished). After 30 min, the injected cells were mixed with the mating reactive cells of type VI that was cultured in CM. The mean value of the velocity was 4.79±0.48 (n=4 cells) μm/sec 20 to 30 min after the mixing. The value is slightly lower than those of control cells such as micronucleate cells (5.97 μm/sec), amicronucleate cells (5.23 μm/sec) and amacronucleate cells (5.02 μm/sec) as mentioned above. However, the value is slightly higher than that of vegetative cells (4.46 μm/sec). The results indicate that microtubules do not take main part in the protoplasmic streaming.

At the stages of disintegration of meiotic products and nuclear exchange

The values around the macronucleus at the paroral union (about 3 hr after mixing the complementary types) and at the crescent stage (about 6 hr after mixing the complementary types; the prophase of Meiosis I) of the micronucleus were nearly the same as or a little lower than that of vegetative G1 cells (Fig. 3-b and c). The velocity just under the trichocysts layer was lower than that in vegetative cells (Fig. 4-b), although there was no statistical significance between them. Thereafter, the flow around the macronucleus tended to decrease about 9 hours after conjugation, which coincided with the metaphase of the first meiotic division. Streaming almost ceased at about 12 hr after conjugation, which coincided with the degradation of meiotic products and the reciprocal exchange of gametic nuclei (Figs. 3 and 4).

At the stage of macro- and micronuclear differentiation

Mating pairs separated 13 to 14 hr after the mixing of the complementary mating types. Soon after the separation, the moving speed recovered and reached, or exceeded to the ordinary speed. The streaming just under the trichocysts layer

was not active and its velocity was the same as that in vegetative cells (Fig. 4-g). That around the macronucleus was 5.72±1.44 μm/sec (n=10 cells) 0.5 to 1 hr after the pair separation (Fig. 3-g), although the distribution of the values had a wider range than that of vegetative cells. About 1.5 hr after the separation (about 16 hours after mixing), the length of cell began to shorten, giving the cells a roundish appearance. This is the critical stage for determining nuclear differentiation. At this stage, however, the streaming almost ceased again, and was 1.01±0.17 μm/sec (n=10 cells) (Fig. 3-h). About 1 hr after the beginning of cell shortening, the length of cells began to recover, and protoplasmic streaming resumed again. The mean value was 6.19±1.47 μm/sec (n=10 cells) 0.5 to 1 hr after the most shortened stage (Fig. 3-i). The direction and speed of streaming were irregular during recovery. The reason for this was thought to be mainly due to the transformation of the ovoid macronucleus to a thick ribbonlike structure called a skein, which then fragmented into many pieces.

The streaming at gametogenesis and fertilization in conjugating pair of amicronucleate and micronucleate

The velocity around the macronucleus was measured at the stage of gametogenesis in the amicronucleate cells of heterotypic pair of an amicronucleate and a micronucleate. The streaming seemed to be mostly ceased both in micronucleate cells and amicronucleate cells, while the active steaming was observed in the amicronucleate cells during vegetative phase (Fig. 5). The mean value in the amicronucleate partners (27aG3am) was 1.24±0.25 μm/sec (n=7 cells) and that in micronucleates was 1.88±0.49 μm/sec (n=5 cells) (Fig. 5). The decrease of the velocity occurred regardless the presence of the micronucleus means that the decrease in the velocity at the gametogenesis and fertilization was under the control of other than micronucleus and that there is no strong correlation between micronuclear behavior and the streaming velocity.

The streaming in amicronucleate cells at the critical stage of nuclear differentiation

First to know the effect of injury brought by injecting a glass needle, the velocity of the protoplasmic steaming after micronuclear removal was compared with that of micronucleate at 7 to 9 hr of conjugation and found to be not much different between amicronucleate cell and micronucleate cell of each pair. The mean value of the former around the macronucleus was 3.02±0.27 μm/sec (n=5 cells) (Fig. 6, amic 7–9 hr of conj.) that is nearly as much as in micronucleate cells (Fig. 3-d). Thus, we can say that neither micromanipuration nor the absence of the micronucleus disturb the streaming.

As mentioned above, the streaming almost ceased at the critical stage of nuclear differentiation. Does the calm down of the streaming coupled with micronuclear behavior? To obtain the answer for this question, micronucleus was removed at 4.5 hr of conjugation and then 12 hr later the velocity was measured at the critical stage of nuclear differentiation. The mean value around the macronucleus was 1.41±0.72 μm/sec (n=5 cells) (Fig. 6, amic critical stage) that is mostly equivalent to that of micronucleate cells at the critical stage of nuclear differentiation as shown as in Fig 3-h. The micronuclear removal does not disorder the streaming during conjugation.

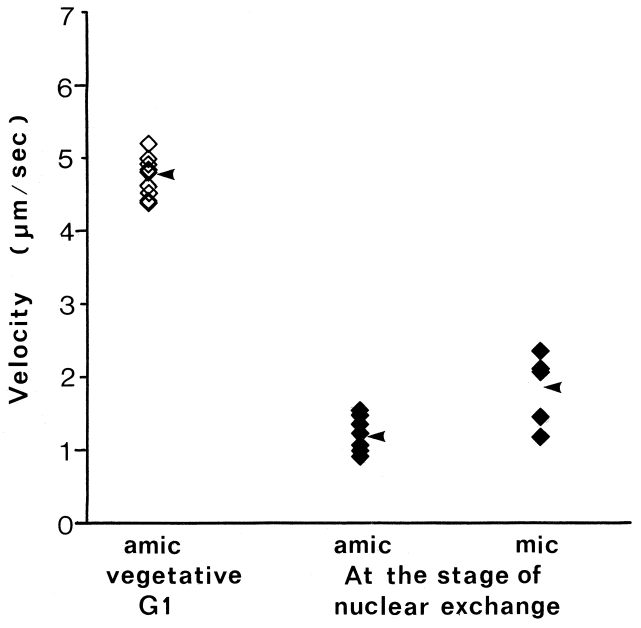

Fig. 5. The protoplasmic streaming at gametogenesis and fertilization in conjugants of amicronucleates and micronucleates.

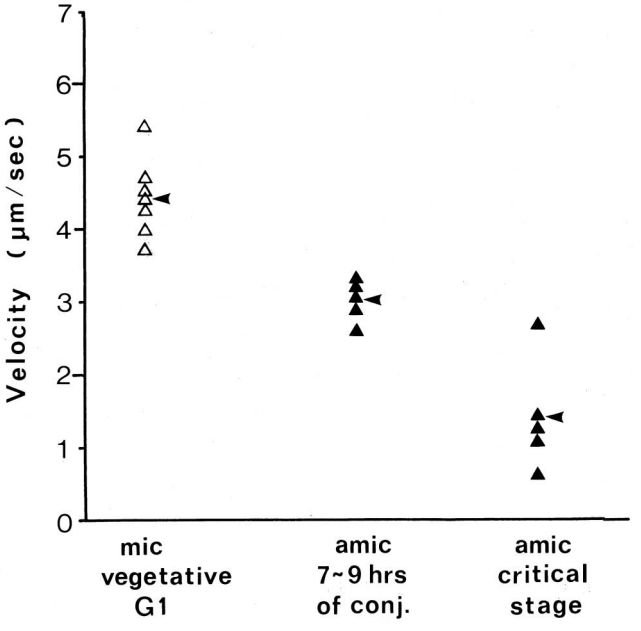

Fig. 6. The protoplasmic streaming at the critical stage of nuclear differentiation in the absence of the micronucleus.

DISCUSSION

In *Paramecium*, protoplasmic streaming known as cyclosis is observed during vegetative phase and flows constantly around the macronucleus in the same direction, forming a closed circuit channel. Removal of the macronucleus did not show apparent affect on the protoplasmic streaming. This means that the presence of the macronucleus is not indispensable for the cyclosis.

So far, we have no evidence to conclude whether certain proteins such as actin, myosin and microtubules take part in the occurrence of the protoplasmic streaming or not. We tried to inject an anti-α-tubulin antibody into some vegetative cells but did not succeed in measuring the velocity of the streaming after injection. Instead, we succeeded in recording the velocity at mating reaction soon after injection of the antibody. The result supports the possibility that microtubules do not take main part in the protoplasmic streaming.

The micronucleus usually touches and presses against the surface of the macronucleus during the vegetative phase except for dividing stage. At the beginning of conjugation, however, the micronucleus detaches from the macronucleus. In *P. aurelia* species complex, micronuclei are carried by cytoplasmic currents away from the side of the macronucleus after the beginning of conjugation (Diller, 1936). Wichterman (1940) reported that larger spherical micronuclei moved about in the cytoplasm quite actively, anteriorly and posteriorly, during the early stage of conjugation in *P. caudatum*. The micronuclear relocation called early micronuclear migration in *P. caudatum* takes place at about 10 to 15 min after the mating reaction (Fujishima and Hiwatashi, 1977). The increase in free cytosolic calcium concentration or its decrease from an increased level back to a normal level may trigger the migration (Cronkite, 1979). Later, this was supported by the evidence that the microinjection of Ca^{2+} induced the migration (Xianyu and Haga, 1993). However, the relationship between the intracellular Ca^{2+} concentration and protoplasmic streaming remains to be examined. The velocity of the protoplasmic streaming at mating reaction has never been measured, though an increase in velocity had been noticed after the onset of the mating reaction (unpublished observation by Fujishima and Hiwatashi, cited in Fujishima, 1988). In the present work, activation of protoplasmic streaming began 3 to 5 minutes after mixing the complementary mating type cells, and after about 20 minutes its mean value around the macronucleus increased from 4.38 to 5.97 μm/sec (Fig. 3). The most conspicuous feature of the streaming at this stage is the increase in velocity at the sub-trichocysts layer (Fig. 4). The active streaming at the beginning of conjugation was observed in the absence of the macronucleus. These evidences show that the increase in the velocity of the streaming has not direct relationship with the micronucleus and the macronucleus. We cannot obtained here any direct evidence to show a relationship between the activation of the streaming and early micronuclear migration, though it is likely that the activation of cytoplasmic streaming is one of the triggers of micronuclear migration at the beginning stage of conjugation.

The micronucleus undergoes meiosis after mating pair formation and gives rise to 4 haploid nuclei in each cell of the pair. The dividing micronuclei of meiosis move about constantly in the streaming cytoplasm during meiosis (Wichterman, 1940). In the present study, we ascertained that during meiotic divisions the streaming was active as well as in vegetative cells. After meiosis, only one of the 4 nuclei persisted and the other 3 disintegrated. The surviving nucleus laid in a bulge of the cell body, called the paroral cone, and attached to the surface membrane (Hertwig, 1889). The other three nuclei, which degenerated, were outside the paroral cone. Thus, the cytoplasm of the paroral cone or its surface membrane differs from the rest of the cytoplasm, and this difference determines nuclear survival or death (Sonneborn, 1954). In *Paramecium*, migration into the paroral region is essential for survival of the meiotic products of the micronucleus. Diller (1936) indicated the possibility that a vortex of cytoplasmic streaming may have swept them into this region. However, the involvement of the microtubules to the micronuclear migration was suggested by the treatment with anti-tubulin reagent (Yanagi and Hiwatashi, 1985). Moreover, intracytoplasmic microtubules are considered to be essential for the micronuclear selection mechanism after meiosis in *T. thermophila* (Gaertig and Fleury, 1992). Recently, connections of microtubular filaments between meiotic products and the paroral region were observed (Ishida *et al.*, 1999). This suggests that microtubules move the nucleus to the paroral cone. This possibility was proofed by the injection of anti-α-tubulin antibody. The antibody was injected into the cells soon after meiosis, all the four gametic micronuclei gathered each other at the central area, but their migration to the paroral region was prevented (Nakajima and Mikami, to be published).

The present study showed that the cytoplasm ceased streaming at the stages of disintegration of meiotic products and the exchange of gametic nuclei. In *P. tetraurelia*, it has been reported that a cessation of streaming occurred at the completion of binary fission and at the time of the presumable exchange of migratory pronuclei in conjugation, when cells were observed after immobilization with a diluted antiserum (Kuznicki *et al.*, 1972; Sikora and Kuznicki, 1976). In the present study, the cessation occurred even after removal of the micronucleus. Therefore, we can say that the micronucleus does not cause the decrease in the velocity of protoplasm.The differentiation of germ- and somatic nuclei was determined at the third mitosis of a fertilization nucleus in *P. caudatum* (Mikami, 1980). A fertilization nucleus that is formed by the reciprocal exchange of gametic nuclei between mating cells divides three times and gives rise to 8 nuclei. These division products localize for a moment anteroposteriorly. The nuclei positioned at the anterior part of the cell differentiate micronuclei and those localized at the posterior part differentiate into new somatic nuclei (reviewed by Hiwatashi and Mikami, 1989). Macro- and micronuclear differentiation seems to be determined by the cytoplasmic determinant which is localized anteroposteriorly, though morphological differences such as

germ plasm for example as in *Drosophila* has never been reported. Cytoplasmic streaming was very active after the separation of the mating pair (Fig. 3-g). Thereafter, cytoplasmic streaming ceased at the critical stage of macro- and micronuclear differentiation. This cessation occurred in the absence of the postzygotic micronuclei.

At the stage of macro- and micronuclear differentiation, nuclear positioning is achieved by elongation of the separation spindle of the micronuclei. In *Paramecium* as well as in *S. pombe*, the nuclear envelope does not break down during nuclear division and the mitotic spindle form inside the microtubules so that elongation of the spindles resulted in localizing the daughter nuclei anteroposteriorly. In *Drosophila*, the localization of some mRNAs to distinct intracellular regions is achieved through interactions of the Osker mRNA with cytoskeletal filaments (Bassel and Singer, 1997). We have to consider a possibility that a certain determinant for nuclear differentiation is localized by intracellular and intranuclear microtubules or microfilaments.

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