The ciliates contain two different types of nuclei in a cell, vegetative nucleus or macronucleus and germ nucleus or micronucleus. It is the latter that undergoes meiosis during conjugation. In holotrich ciliates, it has been known that spherical micronuclei elongate and change into spindle-like structure called crescent at meiotic prophase. It would be interesting to know how chromosomes arrange in such an elongated nucleus. Moreover, end-to-end chromosomal association is known in *Tetrahymena* which has the most prominent crescent (Ray 1956; Sugai and Hiwatashi 1974). In this case, a chromosome of genome size was observed in the pachytene crescent, while five bivalents were found at meiotic metaphase I. In *Paramecium* which belongs to the same order Hymenostomatida as *Tetrahymena*, however, such chromosomal association in the crescent has not yet been reported.

The purpose of this study is to know whether the chromosomal association is also present in the crescent of *Paramecium* and how the chromosomes arrange in it.

In the present paper, evidence of chromosomal association and the pattern of chromosomal arrangement in the crescents of three species of *Paramecium* were described.

**MATERIALS AND METHODS**

Three species of *Paramecium* were used in the present study. *Stocks: Paramecium caudatum;* The stocks of dm-1 and dm-3, which belong to mating types V and VI of syngen 3 respectively, were used. *P. tetraurelia;* Clones of mating types VII and VIII of stock 51 were used. Those were kindly supplied by Dr. S. Koizumi.

*P. multimicronucleatum;* Acycler strains CH312 and CH313, which belong to mating types III and IV of syngen 2, were used.

*Culture method:* The culture method for this experiment was the same as those of Hiwatashi (1968). Paramecia were grown at 25°C in the lettuce juice medium inoculated with *Enterobacter aerogenes* one day before use.

*Induction of conjugation:* Conjugation was induced by mixing starved cells of complementary mating types. For *P. tetraurelia*, which undergoes autogamy as well as conjugation, cells of about 10 fissions after conjugation were used. It is known
that autogamy does not occur during this period.

Cytological method: Preparation for cytological observation was made according to the method described by Sugai and Hiwatashi (1974). In brief, cells were fixed with Carnoy fixative using braking pipette and disrupted by spreading force of Carnoy leaving flattened nucleus and chromosome. The slide was treated by 0.02% RNase (Sigma, 5× recrystallized) for 2.5 hr at 35°C and stained with 2% acetic orcein.

RESULTS

The number of the micronucleus within a cell varies with different species of Paramecium. A cell of Paramecium caudatum has one micronucleus and P. tetraurelia has two micronuclei. P. multimicronucleatum generally has several micronuclei, while cells used in the present study have only two per cell. But all micronuclei undergo meiosis during conjugation.

(1) P. caudatum: Micronuclear changes at meiotic prophase in this species are shown in Figs. 1-9. Spherical micronucleus (Fig. 1) elongates and chromatin threads are not only polarized but also arrange parallel to the long axis of the micronucleus (Fig. 2). Fig. 2 shows early crescent with heavily stained portion (a), lightly stained parallel chromosomal threads (b) and the portion (c) in which no free end of the chromosomal thread was visible. Much thinner threads were recognized in Fig. 3 (arrow). Then, long threads protrude from the heavily stained portion (Fig. 4). For convenience, the direction of extension of fine threads was called 'anterior' of the crescent. This threads continue with its extension to the anterior tip where they form aggregate (Fig. 5 arrow). This is the most slender period. The crescent curves and contracts into typical form. Aggregation at the anterior tip becomes larger. This suggests that the chromatin mass of the anterior tip was caused by accumulation of the fine threads connecting the tip and the posterior part of the crescent. Fig. 6 shows the arrangement of chromosomes within the curved crescent. Long chromatin threads are recognized between the anterior chromatin mass and the posterior part (arrows). The outer thread was pull out by the spreading force of Carnoy's fixative. Mesh-like chromatin threads within the posterior part are also visible. In the later stage, this threads are no longer mesh-like, become thinner and converge to the posterior horn of the crescent (Fig. 7). Long thread on convex side condences and is

Figs. 1-9. Morphologic changes in micronucleus of P. caudatum. All figures, except Fig. 9, × 2800. 1. Spherical micronucleus not entering meiosis. Ma, macronucleus; Mi, micronucleus. 2. Early crescent with polarized chromatin threads. (a), heavily stained portion; (b) parallel arranged threads; (c), note no free end of threads. 3. Triangular stage, fine threads are visible but no free end at the pointer is visible. 4. Crescent with chromatin threads protruding to the anterior. 5. The most slender crescent. Note the anterior chromatin mass at the tip (arrow) and fine threads (pointers) connecting it to the posterior chromatin mass. 6. Curved crescent representing long chromatin threads (arrow) and mesh-like chromosome (pointer). 7. Note the thick, continuous chromatin threads on convex side (arrows). Chromatin threads of the other part are finer than those in Fig. 6. 8. Spindle-spaped crescent with condencing chromosomes. 9. Metaphase I. × 1100.
Fig. 1-5.
Figs. 6-9.
Figs. 10–13. Morphologic change in micronucleus of *P. tetraurelia*. ×2800. Fig. 10 shows fine threads (arrow) protruding from one side of the micronucleus. 11. Note the two chromatin mass connected by long, thin threads (arrow). 12. Shortened crescent. 13. Spindle-shaped crescent.

Figs. 14–16. Morphologic changes in micronucleus of *P. multimicronucleatum*. ×2800. Fig. 14 may be the same stage as Fig. 4, 10. 15. Note the paired threads at some part (arrows). 16. Note double strands and four strands (arrows).
found to be continuous throughout the length of the crescent. The crescent shortens gradually to spindle shape. Chromosomes become much thicker uniformly and the long thread is no longer present (Fig. 8). Many chromosomes of equal size appear at metaphase I (Fig. 9).

(2) *P. tetraurelia*: General outline of morphological changes in this species resembles those of *P. caudatum*. Spherical micronucleus elongates and fine threads are pulled out from one side of the nucleus (Fig. 10). At maximal extension of the crescent, anterior and posterior chromatin mass and the long threads connecting them are more conspicuous than those in *P. caudatum* (Fig. 11). Fig. 12 shows the shortened crescent in which thick threads are visible (arrow). Then the crescent becomes spindle shape and chromatin threads become much thinner (Fig. 13).

(3) *P. multimicronucleatum*: The crescent of this species is more slender than those of the other two species described above. Spherical micronucleus elongates and fine threads extend from the nucleus as the other *Paramecium* (Fig. 14). However, paired threads are recognized at the anterior and the posterior parts of the crescent (Fig. 15 arrows). The slender crescent with pointed ends is shown in Fig. 16. During this stage continuous chromatin threads are paired and each of paired chromatin threads appear to be double. It seems that this stage corresponds to diplotene.

**DISCUSSION**

In Calkins and Cull's study (1907) of the nuclear changes during conjugation (selfing) in *P. caudatum*, meiosis was also described in detail. This has been often cited and probably the most detailed observation on meiosis of *Paramecium* so far reported. In the paper were illustrated not only changes from the spherical micronucleus to the crescent but also the parallel arrangement of chromatin threads within the early crescent and the paired chromosomes.

In the present study on the same species, however, no chromosomal pairing was observed but the evidence of chromosomal association not found in Calkins and Cull's study using paraffine section method was obtained. It is conceivable that long chromosomes resulted from chromosomal association were cut and not observed in their sections.

The evidence of chromosomal association in *P. caudatum* was obtained from the following observations. First, the discrepancy between the number of the chromatin threads arranging parallel to the long axis of early crescent and that of chromosomes found at metaphase I, though exact number could not be identified. Moreover, no free end of chromatin threads at the portion (c) of the early crescent was found. Second, if the long chromatin threads found in the typical crescent as showed in Fig. 7 appeared in the metaphase plate with its integrity, long chromosomes might be observed. However, all chromosomes seen in the metaphase plate were of equal size.

The situation stated above appears to be the same in *P. tetraurelia*. Although metaphase was not observed in the present study, long chromatin threads also existed in the crescent. Jones observed meiosis in *P. tetraurelia* and described similar long
chromatin threads of the crescent and 84–90 chromosomes at metaphase (1956).

In *P. multimicronucleatum*, outline of the nuclear changes during conjugation was reported by Barnett (1964), but the chromatin threads in the crescent were not described. As revealed by the present study, this species has slender crescents with continuous chromatin threads, which may be arranged similarily as in the crescent of *Tetrahymena*. However, whether the long chromatin threads do fragment at metaphase is not clear because the chromosome number is not known.

Grell (1964) developed the theory of composite chromosome based on the observation of dividing nucleus of radiolarian *Aulacantha*. The composite chromosome corresponds to whole genome. Raikov (1969) applied this theory to the nuclei of ciliates. According to Raikov, the composite chromosome may be the morphological representation of macronuclear subnuclei, which previously postulated by Sonneborn (1947) from a genetic point of view. It has been said that the chromosomes in ciliates tend to aggregate at various nuclear stages. There is a possibility that the chromosomes are organized in a fashion similar to the composite chromosome in this aggregation. And the most appropriate stage to visualize that organization may be the crescent stage because of its slender feature. The long chromatin threads observed in the present study should have some relation to the composite chromosome. Examples of chromosomal aggregation in ciliates were reviewed by Raikov (1967, 1972). More detailed study to clarify the pattern and the order of chromosomal association is worthy of further study.

A number of chromosomal association were also known in higher plants (Wagenaar 1969) and even in mammals (DuPraw 1970; Budrick 1976). The significance of the chromosomal association has not been elucidated. The crescent in the ciliates provides useful material for the study of this phenomenon.

**SUMMARY**

Micronuclear changes at meiotic prophase were observed in three species of *Paramecium*, especially in detail in *P. caudatum*. Pattern of chromosomal arrangement in the deformed, meiotic micronucleus or crescent varies characteristically with meiotic stages. Long chromatin threads which represent chromosomal association were recognized in the crescent.

**LITERATURE CITED**


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