Short communication

Dynamic behaviour of stationary pronuclei during their positioning in Paramecium caudatum

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Abstract

During conjugation of Paramecium caudatum, there are two well-known stages when nuclear migration occurs. What happens to the nuclei is closely related to their localisations in cells. The first of these stages is the entrance of one meiotic product into the paroral region. This nucleus survives, while the remaining three outside this area degenerate. The second stage is the antero-posterior localisation of eight synkaryon division products. Four posterior nuclei are differentiated into macronuclear anlagen, whereas four anterior nuclei remain as the presumptive micronuclei. In this experiment, the process of the third prezygotic division of P. caudatum was studied with the help of protargol staining. Here, a third nuclear migration was discovered. By two spindle turnings and two spindle elongations, stationary pronuclei were positioned near migratory pronuclei. This positioning of stationary pronuclei could shorten the distance for transferred migratory pronuclei to recognise and reach the stationary pronuclei. This fosters the synkaryon formation of P. caudatum.

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Keywords: Conjugation; Stationary pronuclear positioning; Paramecium
Material and Methods

Chemicals

Silver protein from Merck KGaA (Germany) was used. Other chemicals were purchased from Hangzhou Dafang Chemical Reagent Inc. (China).

Cell culture and induction of conjugation

Two complementary mating types of *P. caudatum* Ehrenberg, 1833 collected from East Lake Campus of Zhejiang A & F University (China) were used. Cell culture and conjugation induction were performed, as described by Hiwatashi (1968). Concentrated conjugating pairs were obtained by the iron-dextran particle method (Yang and Takahashi 1999). All experiments were performed at room temperature (∼25 °C).

Protargol staining and observations

Cells were fixed with saturated HgCl₂ aqueous solution and stained by a modified protargol method, as briefly reported previously (Shi 1987; Shi and Frankel 1990; Yang and Shi 2007). The preparations were observed and photographed under a Nikon 50i microscope.

Results and Discussion

Prior to reciprocal MiP exchange during conjugation of *P. caudatum*, StP are always found close to MiP (Nakajima et al. 2001; Wichterman 1986). This kind of nuclear positioning has also been reported in other ciliates, including *Tetrahymena thermophila* (Orias et al. 1983), *P. multimicronucleatum* (Inaba et al. 1966), *P. aurelia* (Jurand 1976) and *P. polycaryum* (Yang and Shi 2007). What causes the close localisation between MiP and StP? Usually, it takes about 1 h to complete the mitotic process of the third prezygotic division at 25 °C (Yanagi 1987). In other words, this division occurs 13–14 h after mixing two complementary mating types. To clarify this issue, many conjugating pairs were stained by a modified protargol (Shi 1987). In 65 pairs, the third prezygotic nuclear division occurred.

![Fig. 1. Positioning of stationary pronuclei during the third prezygotic nuclear division of *P. caudatum*. Framed portions of A–L are magnified in A’–L’, respectively. (A) Soon after meiosis. (B–H) Different stages of anaphase. (B) The first spindle turning at early anaphase. (C–G) The first spindle elongation accompanying with the first spindle turning. (H) The second spindle turning at late anaphase. (I–L) Different stages of telophase. (I) The second spindle elongation at early telophase. (J–K) The second spindle elongation at late telophase. Solid arrows denote dividing nuclei in (A–C), and prospective StP in (D–L). Open arrows denote prospective MiP. Scale bars: 20 μm in (A–L) and 10 μm in (A’–L’).](image)
The prospective MiP kept their position in the paroral region and were tightly positioned against the conjugating pairs throughout the whole division process (open arrows in Fig. 1), whereas the prospective StP showed a different behaviour (solid arrows in Fig. 1). At the early anaphase, the end of prospective StP elongated into the cytoplasm with a turning at the spindle area (the 1st spindle turning) (Fig. 1(A), (A'), (B), (B')). Along with the proceeding of the division, the spindles elongated into the cytoplasm, moving continuously in the direction almost perpendicular to the longitudinal axis of cells at middle anaphase (the 1st spindle elongation) (Fig. 1 (C)–(G), (C')–(G')). At the late anaphase, a second turning occurred at the connecting area of prospective StP and spindles (the 2nd spindle turning) (Fig. 1(H), (H')). Continuous elongation of spindles occurred during the telophase (the 2nd spindle elongation), but the direction was towards the posterior end of cells, which was parallel to their longitudinal axis (Fig. 1(I)–(L), (I')–(L')). As soon as the spindles took the ‘V’-like shape during the late telophase, a new structure appeared at its jointing points (circles in Fig. 1(J)–(L), (J')–(L')). In short, during the third prezygotic division, the prospective MiP kept their stable positions in the paroral regions, whereas the prospective StP showed a dynamic movement first (two spindle turnings and two spindle elongations), then kept a position near the prospective MiP. This kind of nuclear division was observed for the first time and differed from all other five divisions during conjugation of *P. caudatum* (i.e., the first two prezygotic divisions and three post-zygotic divisions).

In fact, some events during the third prezygotic division observed in the current study (Fig. 1) could be found in some previous studies on *P. caudatum* and other species of *Paramecium*. For example, the 1st spindle turning and the 1st spindle elongation were observed in *P. caudatum* (Nakajima et al. 2002; Yanagi and Hiwatashi 1985) and *P. putrinum* (Jankowski 1972). ‘U’-shaped spindles were found in *P. polycaryum* (Yang and Shi 2007). Therefore, the events occurring in the course of the third prezygotic division that have been observed in the current study of *P. caudatum* can be assumed to be a common process during the conjugation of *Paramecium* species. The positioning of StP near the MiP could shorten the distance for the transferred MiP to recognise and reach the StP. Consequently, it could enhance the frequency of fertilisation (syncaryon formation) and benefit the propagation of new generations of *Paramecium*.

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**References**


