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Localization of stationary pronuclei during conjugation of *Paramecium* as indicated by immunofluorescence staining

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Abstract: After the third prezygotic division during conjugation of *Paramecium caudatum*, migratory and stationary pronuclei are produced. The migratory pronuclei remain in the paroral region tightly against the conjugating boundaries; while the stationary pronuclei are located beside the migratory pronuclei. To date, however, it is not clear what causes this close side-by-side localization between migratory and stationary pronuclei. In the current study, immunofluorescence staining with monoclonal antibody of anti-α tubulin indicated that "U" or "V" shaped spindles connected the migratory and stationary pronuclei during the third prezygotic division. This observation accounts for the close localization between these two types of pronuclei.

Key words: *Paramecium*; Conjugation; Connecting spindles; Pronucleus

免疫荧光染色揭示草履虫接合生殖过程中静止原核的空间位置

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摘要：在尾草履虫的接合生殖过程中, 共有三次配前核分裂。在配前第三次分裂结束后, 两个接合的细胞内均形成一个迁移原核和一个静止原核。迁移原核位于口旁锥内，而且紧贴于接合面, 静止原核则位于迁移原核的外侧, 两者呈左右排列, 距离接近。但是, 目前对导致两种原核近距离的原因尚不清楚。该文通过α-微管蛋白的单克隆抗体对受精核形成前的接合对进行了免疫荧光染色, 结果发现，配前第三次分裂不同于前两次分裂, 连接迁移原核和静止原核的核间连丝伸向细胞的后方, 呈“U”或“V”型, 结果导致两个原核左右排列, 而不是前后排列, 两者间的距离缩短。这个结果也阐明了造成两种原核近距离的原因。

关键词：草履虫; 接合生殖; 核间连丝; 原核
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Paramecium caudatum is a worldwide ciliate species with conjugation, one kind of sexual reproduction. Like other ciliates, *P. caudatum* has a polygenomic somatic macronucleus and a diploid germinal micronucleus, both of which are derived from synkaryon (fertilized nucleus) division products (Prescott, 1994; Wichterman, 1986). During conjugation, there are three micronuclear prezygotic divisions before synkaryon formation. The first two are meiotic, whereby the micronucleus divides twice successively to form four haploid nuclei. The third is mitotic, whereby one meiotic product divides once yielding a migratory pronucleus and a stationary pronucleus. The migratory pronuclei remain in the paroral region tightly against the conjugating boundaries. So far, however, there have been few detailed descriptions in regards to the stationary pronuclei, although previous studies have determined that stationary pronuclei are located near the migratory

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pronuclei (Nanney, 1980; Wichterman, 1986; Nakajima et al, 2001; Santos et al, 2000; Watanabe et al, 1996; Yang & Shi, 2007). This close localization between the two types of pronuclei should benefit the transferred migratory pronuclei to recognize and fuse with the stationary pronuclei to form synkarya. However, it is unknown what makes this close localization between two pronuclei, which is addressed in the current study.

1 Material and Methods

1.1 Chemicals and stock solutions

Monoclonal antibody of mouse anti-α tubulin, FITC-conjugated goat anti-mouse Ig G, propidium iodide (PI), 4% paraformaldehyde, 0.5 mol/L EGTA (pH 8.0), Triton-X 100, and RNase A were purchased from the Beyotime Institute of Biotechnology (Haimen Jiangsu, China). Other chemicals were obtained from the Hangzhou Dafang Chemical Reagent Inc (China).

1.2 Cell culture and induction of conjugation

Two complementary mating types of *P. caudatum* were collected from East Lake Campus of Zhejiang A & F University (China). Cell culture and conjugation induction followed previous descriptions (Hiwatashi, 1968). Conjugating pairs were isolated by iron-dextran particles (Yang & Takahashi, 1999).

1.3 Immunofluorescence staining

The protocol of immunofluorescence staining with monoclonal antibody of anti-α tubulin is as follows: 1) Cells were fixed in 2% paraformaldehyde diluted in 2 mmol/L phosphate buffer (pH 7.0) containing 25 mmol/L KCl (PBS). 2) Fixed cells were rinsed three times with washing buffer (PBS containing 5 mmol/L $MgSO_4$, 2 mmol/L EGTA, and 0.05% Triton-X 100). 3) Cells were blocked with 1% BSA dissolved in 5 mmol/L NH_4Cl. 4). Cells were incubated overnight in 1 000× diluted monoclonal antibody of anti-α tubulin containing 10 μg/mL RNase A. 5) Cells were incubated for 2 h in 500× diluted FITC-conjugated goat anti-mouse Ig G containing 2.5 μg /mL PI after three rinses. The stained cells were observed under a fluorescence microscope. All experiments were performed at room temperature (～25°C) (Yang & Takahashi, 2002).

2 Results

To determine the reason for the close localization between migratory and stationary pronuclei, conjugating pairs before synkaryon formation were immunostained with monoclonal antibody of anti-α tubulin, by which micronuclei and spindles were stained but macronuclei were not (Ishida et al, 1999). The spindles and nuclei on the telophase of three prezygotic divisions were compared.

2.1 The First and the second prezygotic divisions

At the first prezygotic division, the anti-α tubulin antibody recognized long and slender spindles (arrowheads in Fig. 1A), while the PI staining indicated two meiotic products distributed randomly in the cytoplasm (arrows in Fig. 1B). Merged pictures of Fig. 1A and B showed the co-localization of microtubules and micronuclei in orange (arrows in Fig. 1C). At early telophase of the second prezygotic division, as with the first prezygotic division, both slender spindles and four

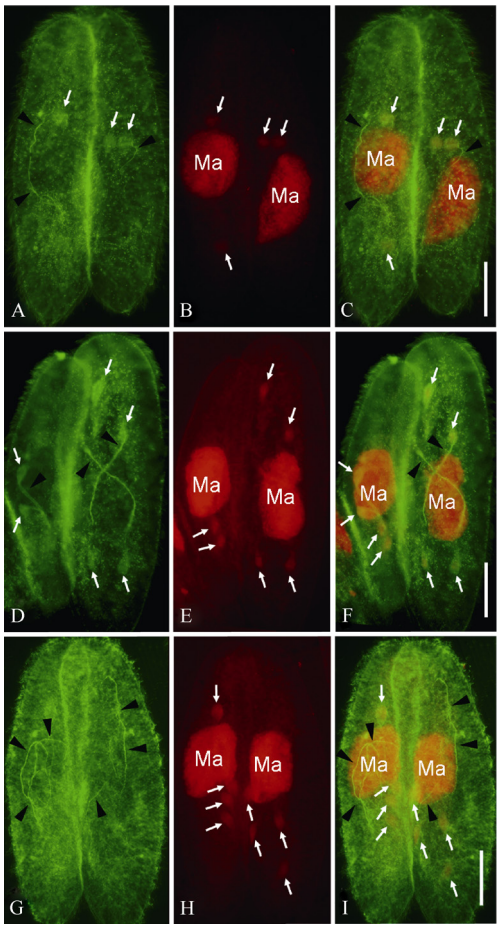

Fig. 1 Meiotic products and spindles during the meiosis of *Paramecium caudatum* as indicated by double staining of FITC and propidium iodide (PI)

(A, D, G) Images indicated by anti-α tubulin antibody followed by FITC-labeled goat antimouse Ig G. (B, E, H) Images of PI. (C, F, I) Merged images of FITC and PI. Three pictures in each row (A, B, C), (D, E, F) and (G, H, I) are the same cell. (A, B, C) Telophase of the first prezygotic division. (D, E, F) Early telophase of the second prezygotic division. (G, H, I) Late telophase of the second prezygotic division. Arrows: meiotic products. Arrowheads: spindles. Ma: old macronuclei. Scale bar = 20 μm.

randomly distributed meiotic products were observed (Fig. 1D-F). At late telophase of the second prezygotic division, slender and long spindles were observed and at least one meiotic product was observed in the paroral region (Fig. 1G-I).

2.2 The third prezygotic division

After meiosis, only one meiotic product survives in the paroral region, which undergoes mitotic division (the third prezygotic division) to produce migratory "male" and stationary "female" pronuclei, while the other three meiotic products undergo apoptotic degeneration (Gao et al, 2010; Hiwatashi & Mikami, 1989; Yang et al, 2007). The prospective migratory pronuclei (yellow arrows) and stationary pronuclei (blue arrows) were recognized by both the anti-α tubulin antibody and PI (Fig. 2), while the degenerating meiotic products were only recognized by PI (Fig. 2B, E, and H). The slender spindles connecting migratory and stationary pronuclei were recognized by the anti-α tubulin antibody and showed "U"- or "V"-like shapes (Circles in Fig. 2A, D, G). The prospective migratory pronuclei maintained their locations in the paroral region (yellow arrows in Fig. 2). The migratory pronuclei in the two cells of a conjugating pair almost overlapped in the paroral regions, while the prospective stationary pronuclei were located beside the migratory pronuclei (blue arrows in Fig. 2C, F and I). At late telophase of the third prezygotic division, migratory pronuclei and stationary pronuclei were detached from the spindles (Fig. 2G-I), while side-by-side localization between both pronuclei showed no obvious change (compare Fig. 2C, F with I)

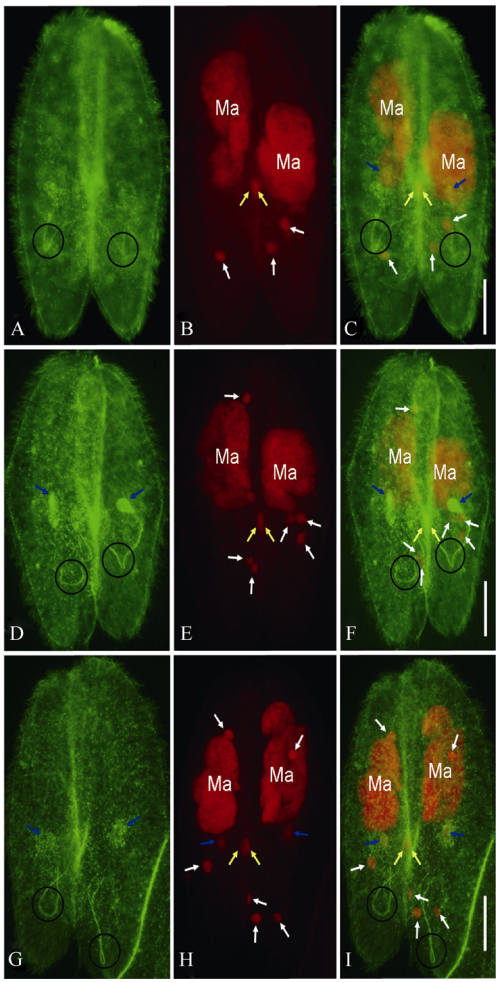

Fig. 2 Prospective migratory pronuclei, stationary pronuclei, and connecting spindles of *Paramecium caudatum* as indicated by double staining of FITC and PI

(A, D, G) Images indicated by anti-α tubulin antibody followed by FITC-labeled goat antimouse Ig G. (B, E, H) Images of PI. (C, F, I) Merged images of FITC and PI. Yellow arrows: prospective migratory pronuclei. Blue arrows: prospective stationary pronuclei. White arrows: degenerating meiotic products. Circles: "U"- or "V"-shaped connecting spindles between two pronuclei. Ma: old macronuclei. Scale bar = 20 μm.

3 Discussion

Many studies on conjugation of *P. caudatum* have been conducted since the discovery of the organism (Calkins & Cull, 1907; Hiwatashi & Mikami, 1989; Maupas, 1889). There have also been several studies by immunofluorescence staining with anti-α tubulin antibody (Ishida et al, 1999; Nakajima et al, 2001, 2002; Yang & Takahashi, 2002). Some new details were observed in the current study after conjugating pairs during the three prezygotic divisions of *P. caudatum* were immunostained with anti-α tubulin antibody. 1) Long and slender spindles during meiosis were observed (Fig. 1A, D, G). 2) At late telophase of the second prezygotic division, at least one meiotic product was located in paroral region (Fig. 1H, I). 3) At telophase of the third prezygotic division, the connecting spindles of migratory pronuclei and stationary pronuclei showed "U"- or "V"-like shapes (Fig. 2).

In fact, "U"-shaped spindles connecting migratory and stationary pronuclei during the third prezygotic division have also been observed in *P. polycaryum* (Yang & Shi, 2007), and "U"- or "V"-shaped spindles have been indicated by protargol in *P. caudatum* (Gao et al, 2011). If connecting spindles between prospective migratory and stationary pronuclei are stretched straightly, the two pronuclei will be located far from each other. However, "U"- or "V"- shaped spindles allows the two pronuclei to be located side-by-side, which should help transferred migratory pronuclei to

easily recognize and fuse with stationary pronuclei to complete synkaryon formation, the most important process during sexual reproduction. Side-by-side localization of the two pronuclei has been observed in *Tetrahymena* and other species of *Paramecium* (Snatos et al, 2000; Watanabe et al, 1996). Further studies are needed to determine if this phenomenon is common in ciliates and if these nuclear divisions exist in other organisms.

References:

Calkins GN, Cull SW. 1907. The conjugation of *Paramecium aurelia* (*caudatum*) [J]. *Arch Protistenkd*, **10:** 375-415.

Gao X, Zhang X, Yang X. 2010. Morphological apoptotic characteristics of the post-meiotic micronuclei in *Paramecium caudatum* [J]. *Eur J Protistol*, **46:** 243-250.

Gao X, Shi X, Yang X. 2011. Dynamic behaviour of stationary pronuclei during their positioning in *Paramecium caudatum* [J]. *Eur J Protistol*, **47:** 235-237.

Hiwatashi K. 1968. Determination and inheritance of mating types in *Paramecium caudatum* [J]. *Genetics*, **58:** 373-386.

Hiwatashi K, Mikami K. 1989. Fertilization in *Paramecium*: process of the nuclear reorganization [J]. *Int Rev Cytol*, **114:** 1-19.

Ishida M, Nakajima Y, Kurokawa K, Mikami K. 1999. Nuclear behavior and differentiation in *Paramecium caudatum* analyzed by immunofluorescence with anti-tubulin antibody [J]. *Zool Sci*, **16:** 915-926.

Maupas E. 1889. Le rajeunissement karyogamique chez les ciliés [J]. *Arch Zool Exp Gen*, **7:** 149-517.

Nakajima Y, Mikami K, Takahashi M. 2001. Role of the cytoplasmic and the intranuclear microtubules on the behavior of pronuclei during the conjugation in *Paramecium caudatum* [J]. *Proc Jpn Acad*: *Ser B*, **77:** 172-177.

Nakajima Y, Ishida M, Mikami K. 2002. Microtubules mediate germ-nuclear behavior after meiosis in conjugation of *Paramecium caudatum* [J]. *J Eukaryot Microbiol*, **49:** 74-81.

Nanney DL. 1980. Experimental Ciliatology [M]. New York: John Wiley and Sons.

Prescott DM. 1994. The DNA of ciliated protozoa [J]. *Microbiol Rev*, **58:** 233-267.

Santos ML, Lu E, Wolfe J. 2000. Nuclear death in living *Tetrahymena*: the case of the haploid nuclei [J]. *J Eukaryot Microbiol*, **47:** 493-498.

Watanabe T, Shi X, Liu G, Jin M. 1996. Cytological studies of conjugation and nuclear processes in *Paramecium duboscqui* Chatton & Brachon 1933 [J]. *Eur J Protistol*, **32** (Suppl)**:** 175-182.

Wichterman R. 1986. The Biology of *Paramecium* [M]. 2nd Ed. New York: Plenum Press.

Yang X, Takahashi M. 1999. Disturbance of determination of germinal and somatic nuclei by heat shock in *Paramecium caudatum* [J]. *J Eukaryot Microbiol*, **46:** 49-55.

Yang X, Takahashi M. 2002. Nuclei may anchor at specific locations during nuclear determination in *Paramecium caudatum* [J]. *EurJ Protistol*, **38:** 147-153.

Yang X, Shi X. 2007. Gametic nuclear exchange during the conjugation of *Paramecium polycaryum* [J]. *JPN J Protozool*, **40:** 113-121.

Yang X, Gao X, Shi X. 2007. Detection of haploid nuclear death in living *Paramecium caudatum*[J]. *JPN J Protozool*, **40:**123-130.

浙江农林大学动物学科杨仙玉课题组简介

浙江农林大学动物学科杨仙玉课题组目前主要从事纤毛虫发育学和蟾酥多肽类有效成分基因克隆两方面研究：以尾草履虫(*Paramecium caudatum*)为模式生物，重点研究核发育的详细过程，并借助分子生物学技术手段探讨其调控机制；已开展对蟾蜍皮肤 cDNA 质粒文库的筛选工作，克隆到多个与癌症的发生、转移等相关基因，正在进行重组蛋白表达及生理功能检测工作。在纤毛虫研究领域，曾获两次国家自然基金、教育部留学基金、浙江省教育厅项目、浙农林大发展基金项目资助；在蟾蜍研究方面获得浙江省留学人员择优资助项目及临安市科技发展项目资助。研究成果目前集中于纤毛虫的研究，在 *J Eukaryot Micorbiol*、*Europ J Protistol*、*J Exp Zool*、动物学研究、动物学杂志、四川动物等刊物发表论文多篇。

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