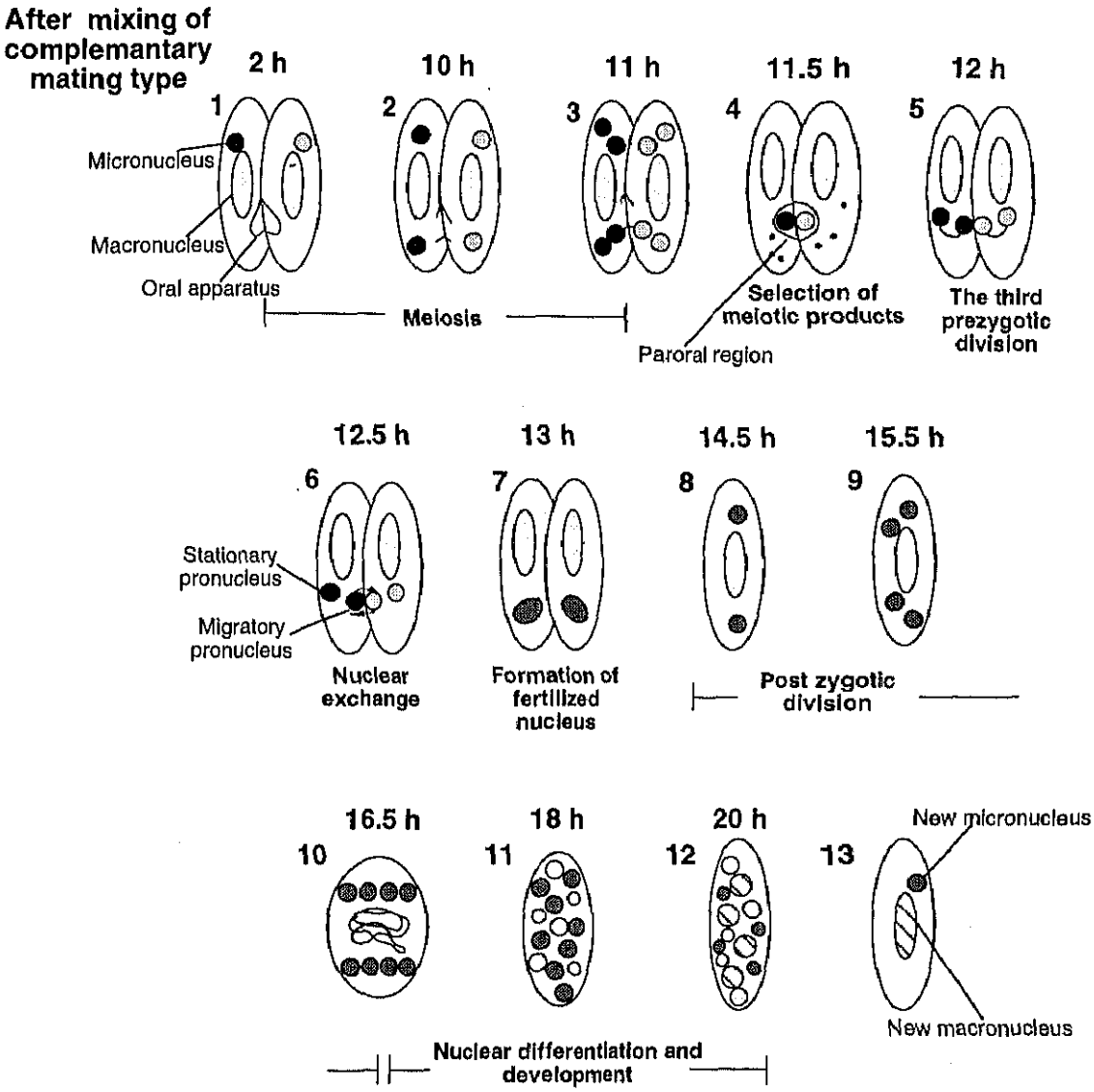
Figures and Figure Legends

Fig. 1 Conjugation process of *Paramecium caudatum*. Each cell has one polygenomic macronucleus and one diploid micronucleus. When mating reactive cells of complementary mating type are encountered, aggregation of cells occurs and then mating pairs appear. The germinal micronucleus undergoes meiosis and produces four haploid nuclei (1 - 3). The region around degenerated the oral apparatus develop to the paroral region (4). One meiotic product enters into the paroral region and survives there, while the other three out the region degenerate (4). Surviving meiotic product divides and produces a migratory pronucleus and a stationary pronucleus (5). After reciprocal exchange of migratory pronuclei between conjugating pairs, fusion of stationary and migratory pronuclei occurs to produce a fertilized nucleus (6 - 7). After the third postzygotic division (8 -9), the fate of nuclei is determined (10). Postzygotic nuclei localized in anterior part of cell become presumptive micronuclei and ones localized in posterior part become macronuclear anlagen (new macronuclei) (11 - 12). Old macronucleus become skein-form and then separates into about 50 fragments (11 - 12). At first and second cell division, the four macronuclear anlagens are distributed to each daughter cell. Only one of the four micronuclei divides and is distributed to daughter cells (13).


After mixing of complemantary mating type
2 h
10 h
11 h
11.5 h
12 h
1
2
3
4
5
Micronucleus
Macronucleus
Oral apparatus
Meiosis
Selection of meiotic products
Paroral region
The third prezygotic division
12.5 h
13 h
14.5 h
15.5 h
6
7
8
9
Stationary pronucleus
Migratory pronucleus
Nuclear exchange
Formation of fertilized nucleus
Post zygotic division
16.5 h
18 h
20 h
10
11
12
13
New micronucleus
New macronucleus
Nuclear differentiation and development

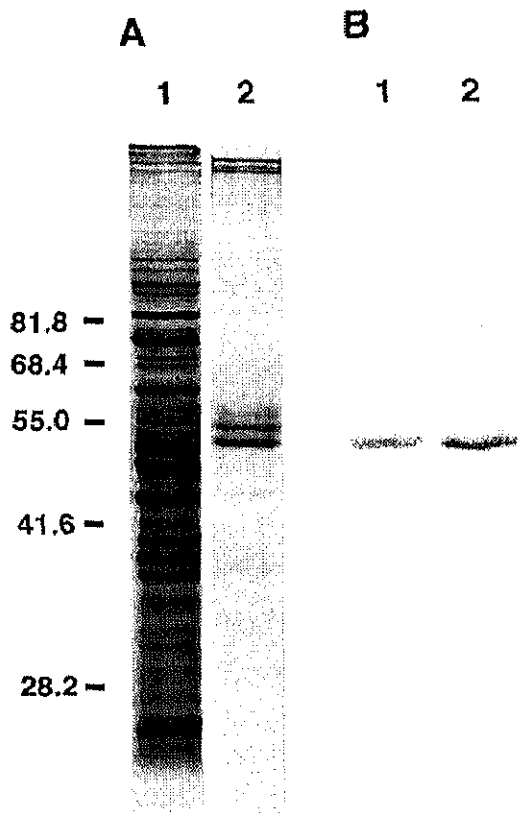
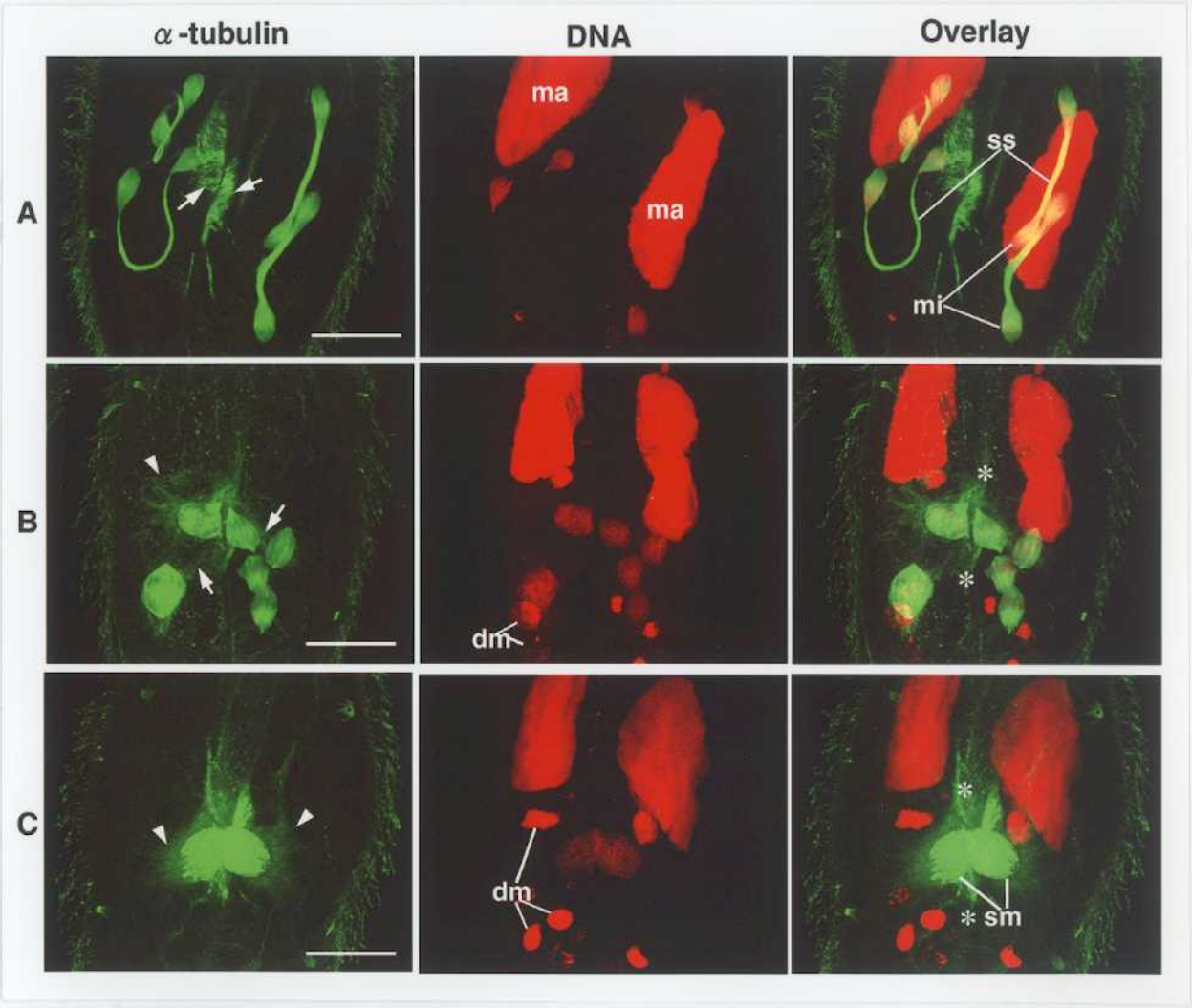


Fig. 2. Specificity of the monoclonal anti-α-tubulin antibody for tubulins of *Paramecium caudatum*. Extract of *P. caudatum* cells (Lane 1) and cilia (lane 2) were subjected to SDS-PAGE followed by silver staining (A) or immunoblotting with monoclonal anti-α-tubulin antibody (B). Bands at 50 kDa correspond to α-tubulin. Bars on the left represent molecular weights (kDa).

Fig. 3. Immunofluorescent images of microtubules in conjugating cells of meiosis and post-meiotic process. The mating pairs were stained with a monoclonal anti-α-tubulin antibody and co-stained with PI. **A**: Telophase of the second meiotic division. The separation spindles have elongated (ss). α-tubulin is detectable in the micronuclei (mi), but not in the macronuclei (ma). The oral apparatus are degenerating (arrows). **B**: Meiotic products after the second meiotic division. Almost all the meiotic products gather around the paroral region (between asterisks) in right conjugating cell. Cytoplasmic microtubules connect the paroral region and the meiotic products (arrow). In left conjugating cell, two meiotic products are degenerating (dm). Cytoplasmic microtubules assemble around the prospective surviving meiotic product (arrowhead) in the paroral region (between asterisks), and connect between meiotic product labeled by anti-α-tubulin antibody and the paroral region (arrow). **C**: One meiotic product survived in the paroral region (sm). One of the four meiotic products moved into the paroral region is surrounded by cytoplasmic microtubules (arrowheads). While the other three degenerated products (dm) are not surrounded by cytoplasmic microtubules. Bars = 25 μm.


α-tubulin
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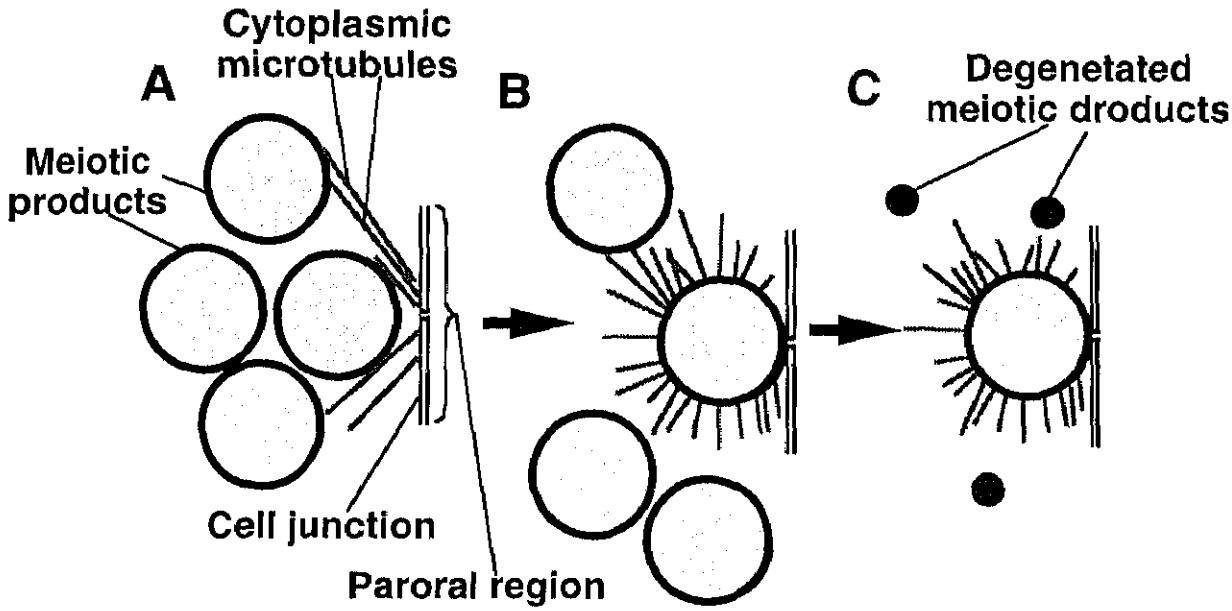


Fig. 4. Schematic representation of selection of meiotic products during conjugation in *Paramecium caudatum.* **A:** After meiosis, the cytoplasmic microtubules appear between some meiotic products and the paroral region. **B:** Only one meiotic product enters into the paroral region and is surrounded by the cytoplasmic microtubules. **C:** One meiotic product surrounded by the cytoplasmic microtubules survives in the paroral region, while the other three not surrounded by the cytoplasmic microtubules out the region degenerate.

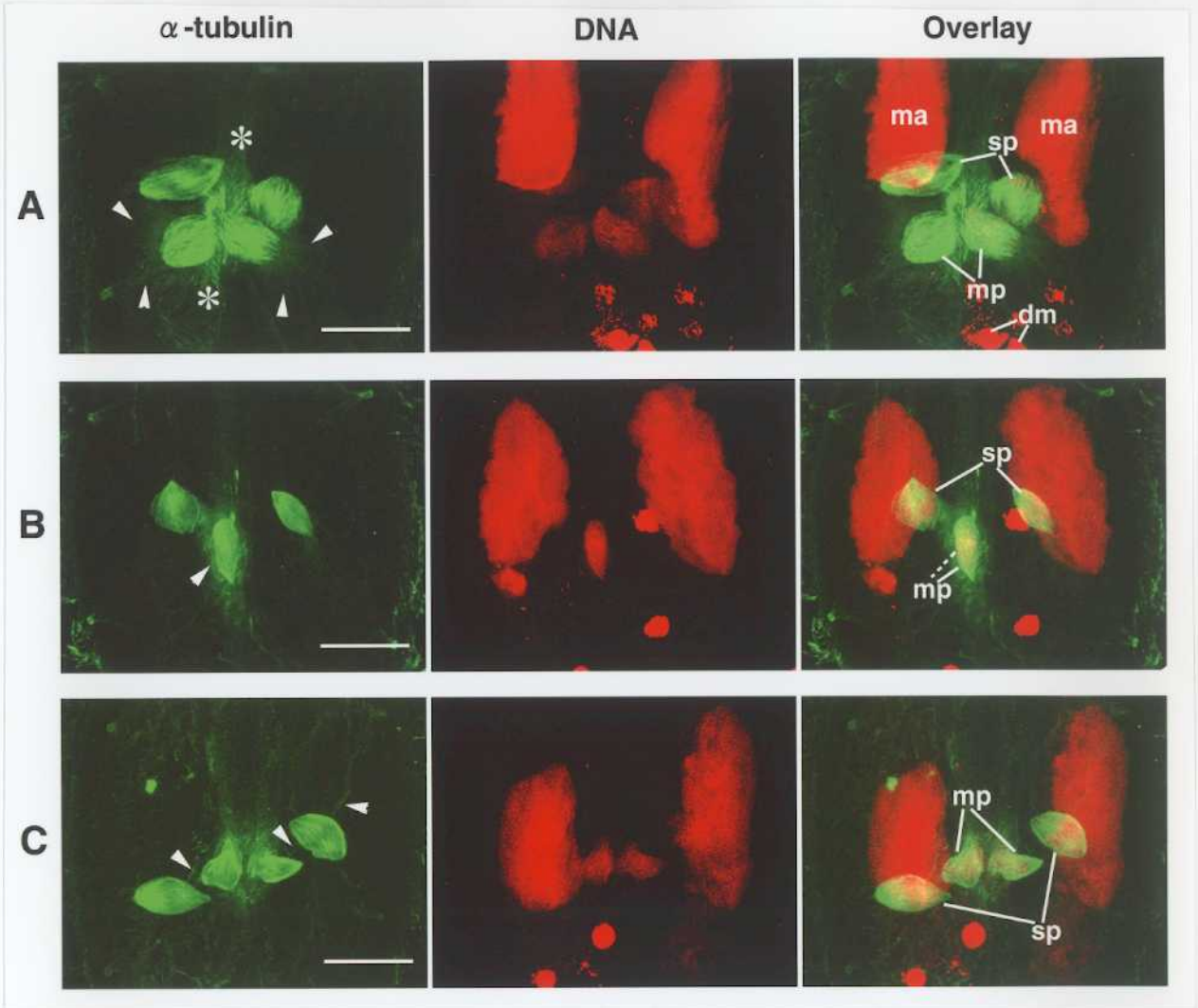


Fig. 5. Immunofluorescent images of microtubules in conjugating cells during the pronuclear exchange. The mating pairs were stained with a monoclonal anti-α-tubulin antibody and co-stained with PI. **A**: Pronuclei after the third prezygotic division to form migratory (mp) and stationary pronuclei (sp). Cytoplasmic microtubules (arrowheads) assemble around migratory pronucleus in the paroral region (between asterisks). ma; macronucleus, dm; degenerated micronuclei. **B**: Pronuclear transfer. The migratory pronucleus (mp) assumes a biconvex lens shape and are surrounded with cytoplasmic microtubules (arrowhead). sp; stationary pronuclei. **C**: Pronuclei shortly after entering the partner cell. Migratory pronuclei (mp) stretch themselves to the stationary pronucleus (sp) of the partner cells. The microtubules (arrowheads) appear in the vicinity of stationary and migratory pronuclei. Bars = 25 μm.

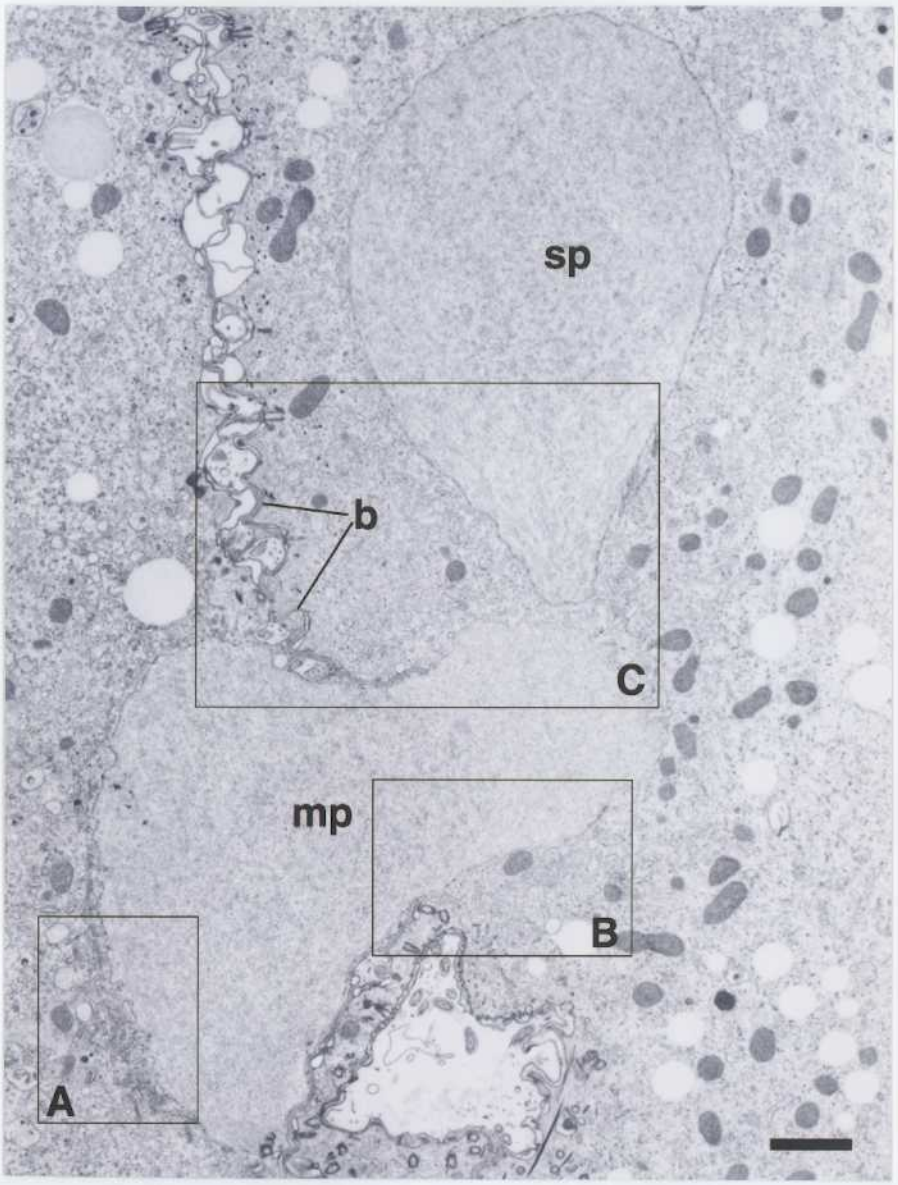


Fig. 6. Electron micrograph of a vertical section through the region of pronuclear exchange. A migratory pronucleus (mp) in the left conjugant is migrating into the right conjugant through the junction of the boundary membrane (b) and approaching the stationary pronucleus (sp). Bar = 2 μm.

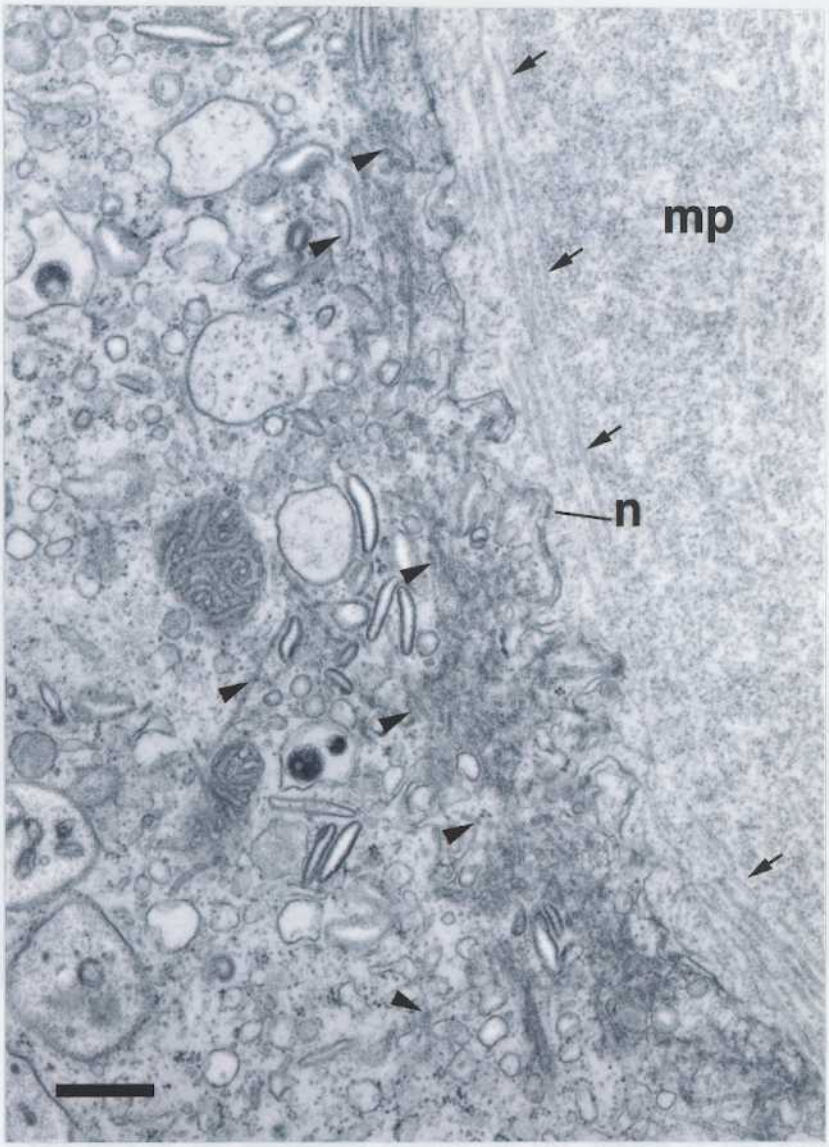


Fig. 7. Higher magnification of the same section as a framed portion **A** of Fig. 6. The cytoplasmic microtubules (arrowheads) surround the posterior hemisphere of the migratory pronucleus (mp). Bar = 500 nm.

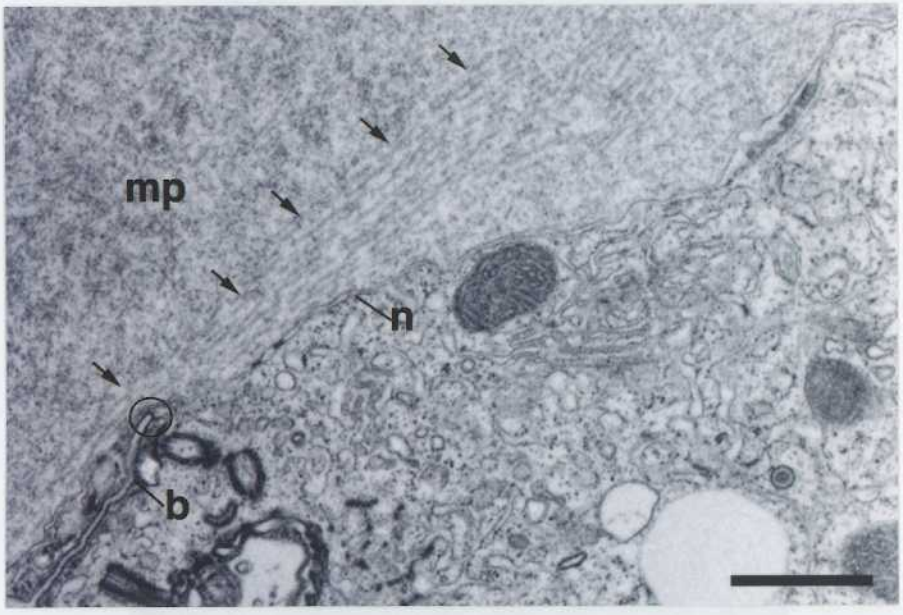


Fig. 8. Higher magnification of the same section as a framed portion **B** of Fig. 6. Intranuclear microtubules (arrows) are aligned in a parallel array of pronuclear movement. The end of the boundary membrane (b), which is the passage of the migratory pronucleus (mp), is marked by a circle. n; nuclear membrane. Bar = 1 μ m.

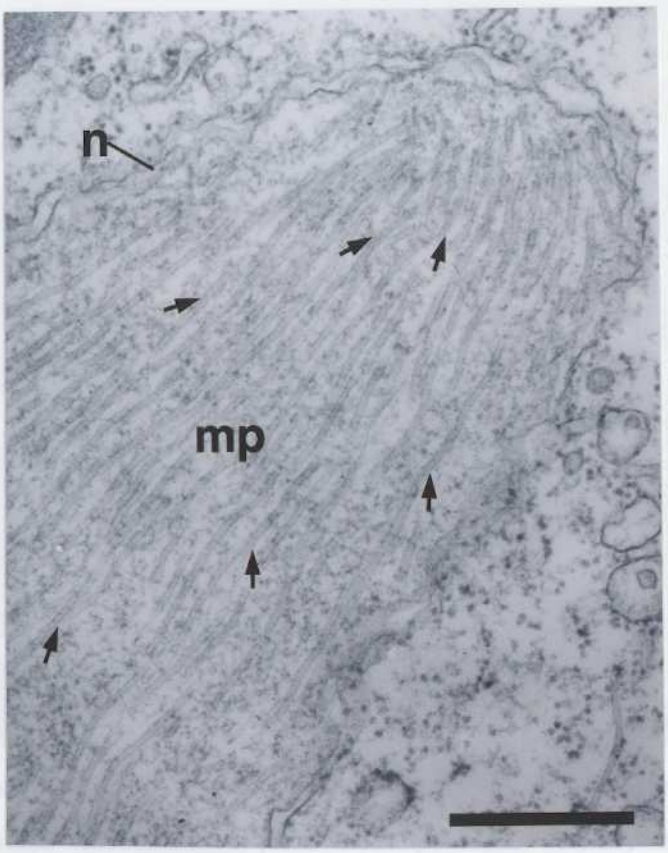


Fig. 9. An upper section of Fig. 6 at higher magnification. Tip of the migratory pronucleus (mp) with intranuclear microtubules (arrows), which are aligned in a direction of movement. n; nuclear membrane. Bar = 500 nm.

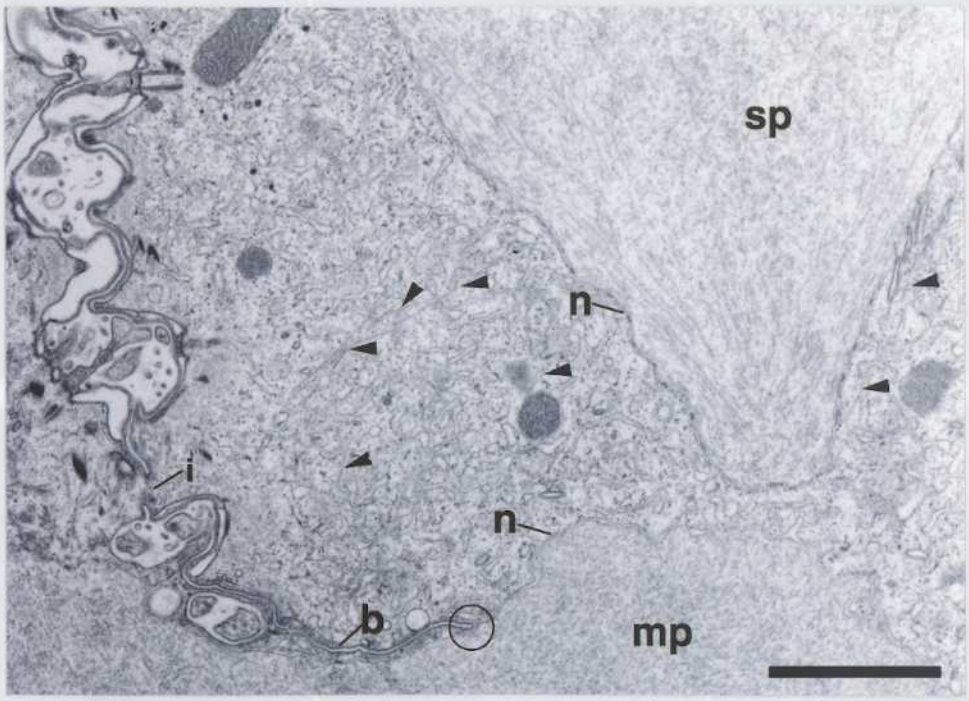


Fig. 10. Higher magnification of the same section as a framed portion **C** of Fig. 6. There are cytoplasmic microtubules (arrowheads) in the vicinity of the stationary pronucleus (sp). The opposite end of the boundary membrane (b), which is shown in Fig. 6, is marked by a circle. n; nuclear membrane, i; interruption of the boundary membrane, mp; migratory pronucleus. Bar = 2 μ m.

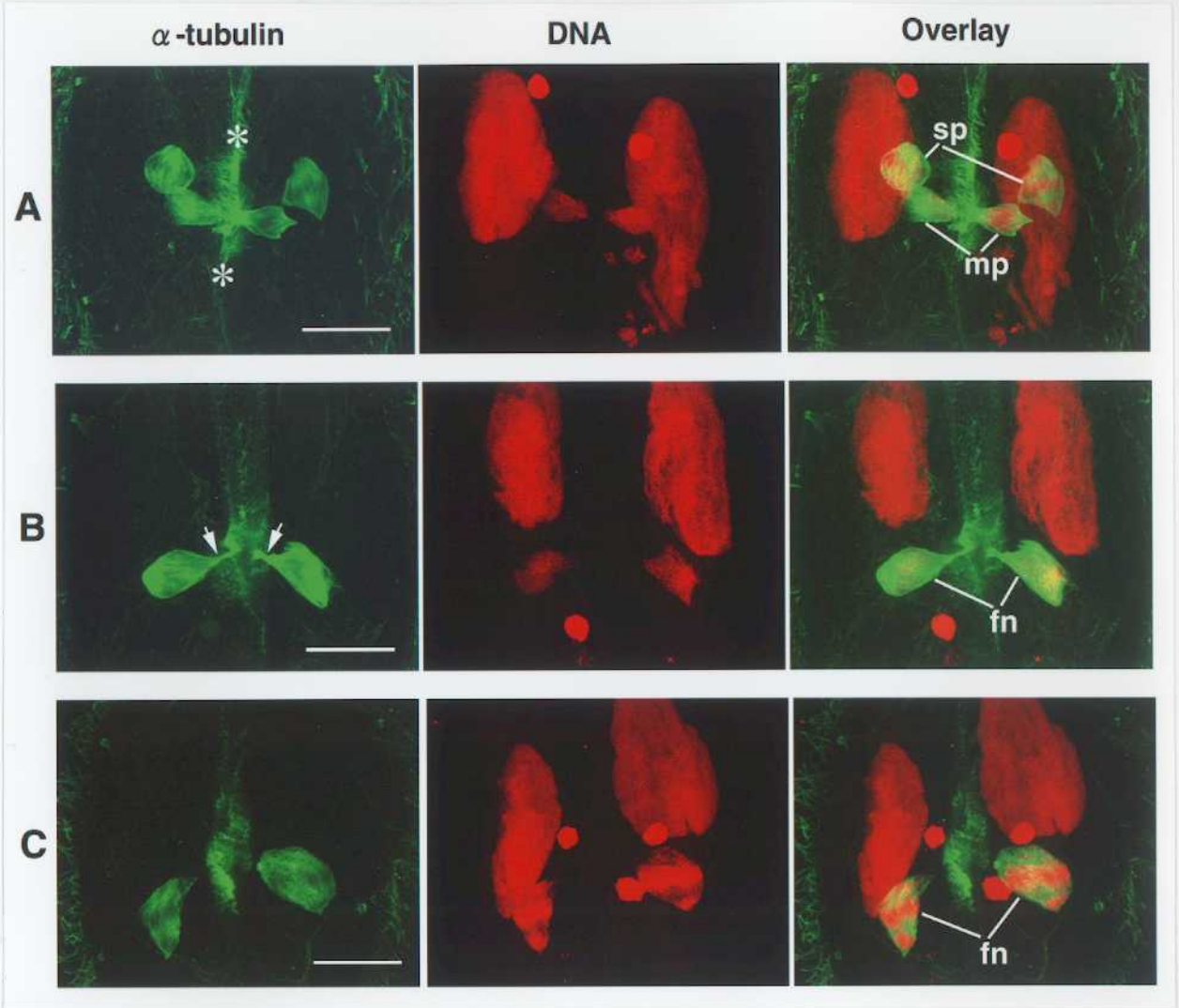


Fig. 11. Immunofluorescent images of microtubules in conjugating cells after entering the partner cell. The mating pairs were stained with a monoclonal anti-α-tubulin antibody and co-stained with PI. **A**: Migratory pronucleus (mp) is approaching stationary pronucleus (sp) after entering the partner cell. Microtubules are oriented parallel to the long axis to the migratory pronuclear direction of movement in the paroral region (between asterisks). **B**: Pronuclei just after the pronuclear fusion. The fertilized nuclei (fn) are still interconnected with the cell junction (arrowheads). **C**: Formation of the fertilized nucleus. The connection between the fertilized nuclei (fn) and the cell junction disappears before post-zygotic division. Bars = 25 μm.

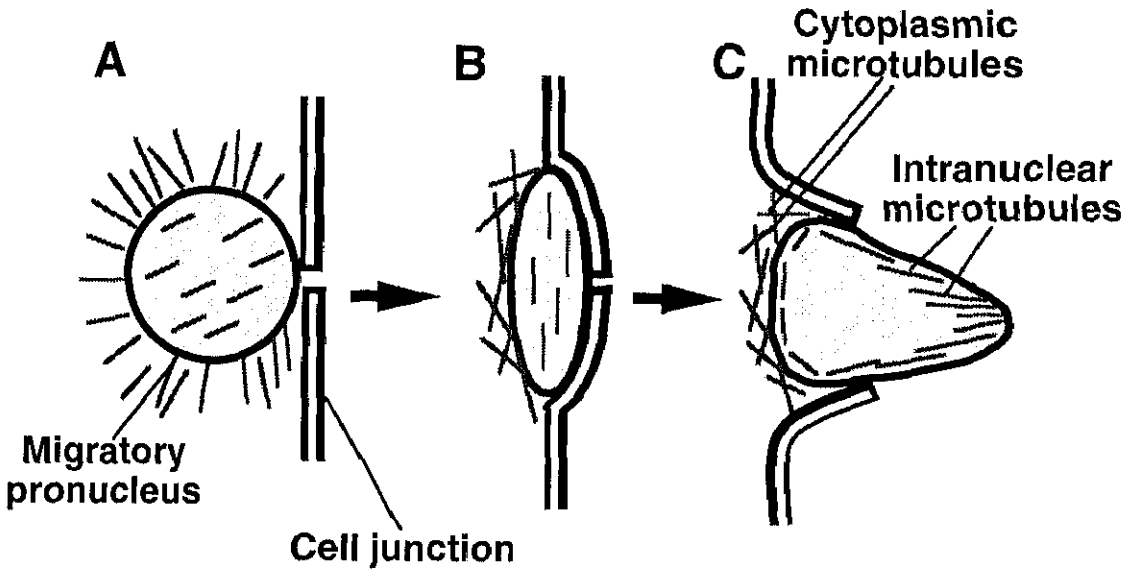


Fig. 12. Schematic representation of nuclear exchange during conjugation in *Paramecium caudatum.* **A:** After the third prezygotic division, the migratory pronucleus is surrounded with the cytoplasmic microtubules. **B:** The migratory pronucleus seems to push by the cytoplasmic microtubules, and changes from a round shape to a biconvex lens shape. **C:** At the moment of nuclear exchange, the migratory pronucleus changes from the biconvex lens shape to an extended shape. The numerous intranuclear microtubules are aligned the direction of extension. The cytoplasmic microtubules surround the posterior hemisphere of the migratory pronucleus at that time.

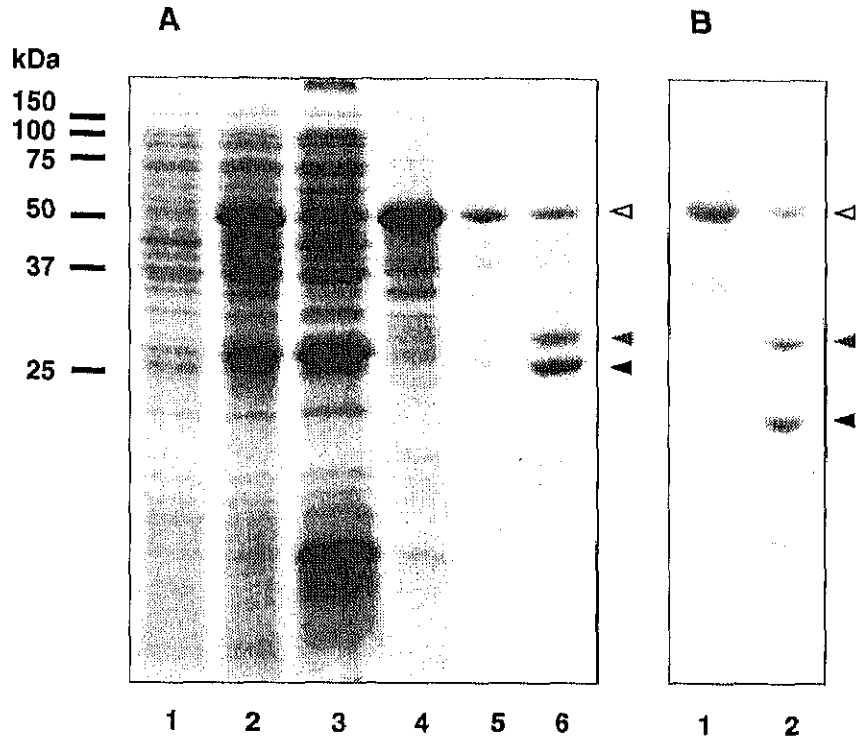


Fig. 13. Purification of recombinant *P. caudatum* C-terminal γ-tubulin. Proteins were resolved by 15% acrylamide (A) or 17% acrylamide-4M Urea (B) SDS-PAGE and visualized by Coomassie brilliant blue staining. Whole-cell lysates of bacteria transformed with pGEX-6p-2-γ-tubulin before (A, lane 1) and after (A, lane 2) IPTG induction. After sonication, total lysate was subjected to centrifugation, yielding a soluble supernatant (A, lane 3) and an unsoluble pellet (A, lane 4). After lysis the pellet with 0.4% sarkosyl, the soluble fraction incubate with glutatione-Sepharose 4B (A, lane 5 and B, lane 1). After incubation with PreScission Protease, the fusion protein was recovered by centrifugation. The resulting pellet included C-terminal γ-tubulin (A, lane 6 and B, lane2). The open arrowheads indicate the position of GST-γ-tubulin fusion protein, the gray-arrowheads indicate the position of C-terminal γ-tubulin, and the closed arrowheads indicate the position of GST. Bars on the left represent molecular weights.

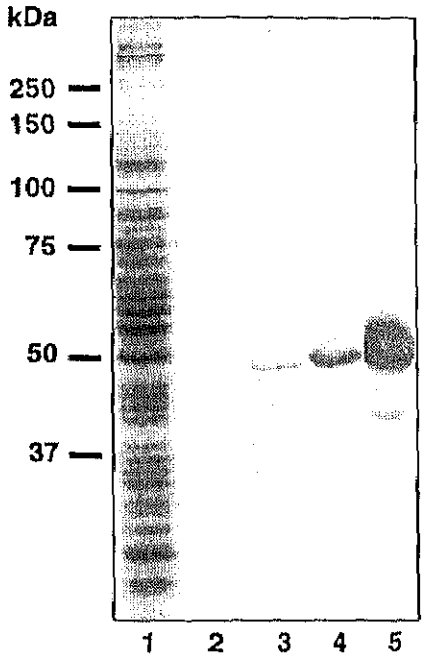


Fig. 14. Characterization of the immune serum Pcg3 raised against the C-terminal amino acids region of *P. caudatum* γ-tubulin is shown. Immunoblot analysis of cell extract from *P. caudatum* is shown. Extract of *P. caudatum* cells were resolved by 10% SDS-PAGE followed by staining with Coomassie brilliant blue (lane 1) or immunoblotting with a preimmune serum (lane 2), an anti-γ-tubulin antibody (Pcg 3, lane 3), an anti-α-tubulin antibody (lane 4) or an antiserum against microtubules (lane 5). The Pcg3 revealed one polypeptide of molecular mass of about 50 kDa (lane 3). The labeling was prevented by preimmune serum (lanr 2). Apparently Pcg 3 recognizes different polypeptides from α- and β-tubulin. Bars on the left represent molecular weights.

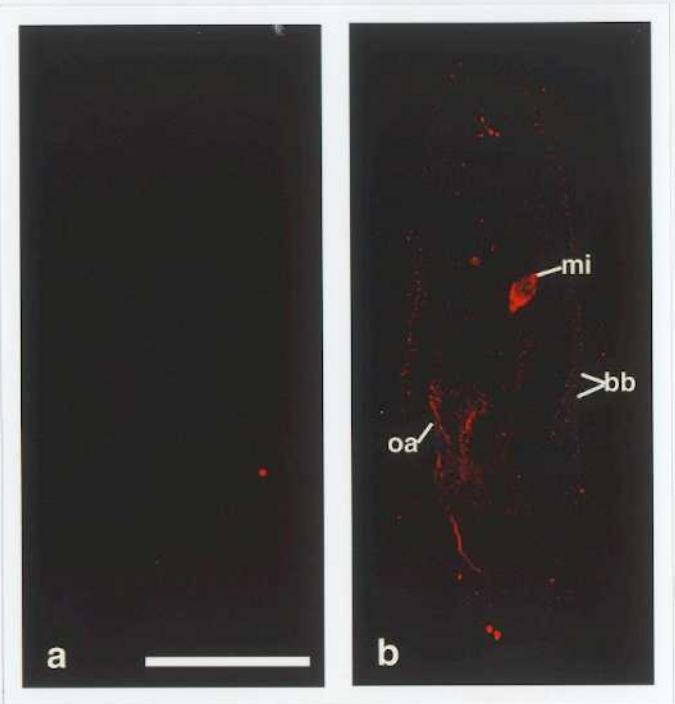
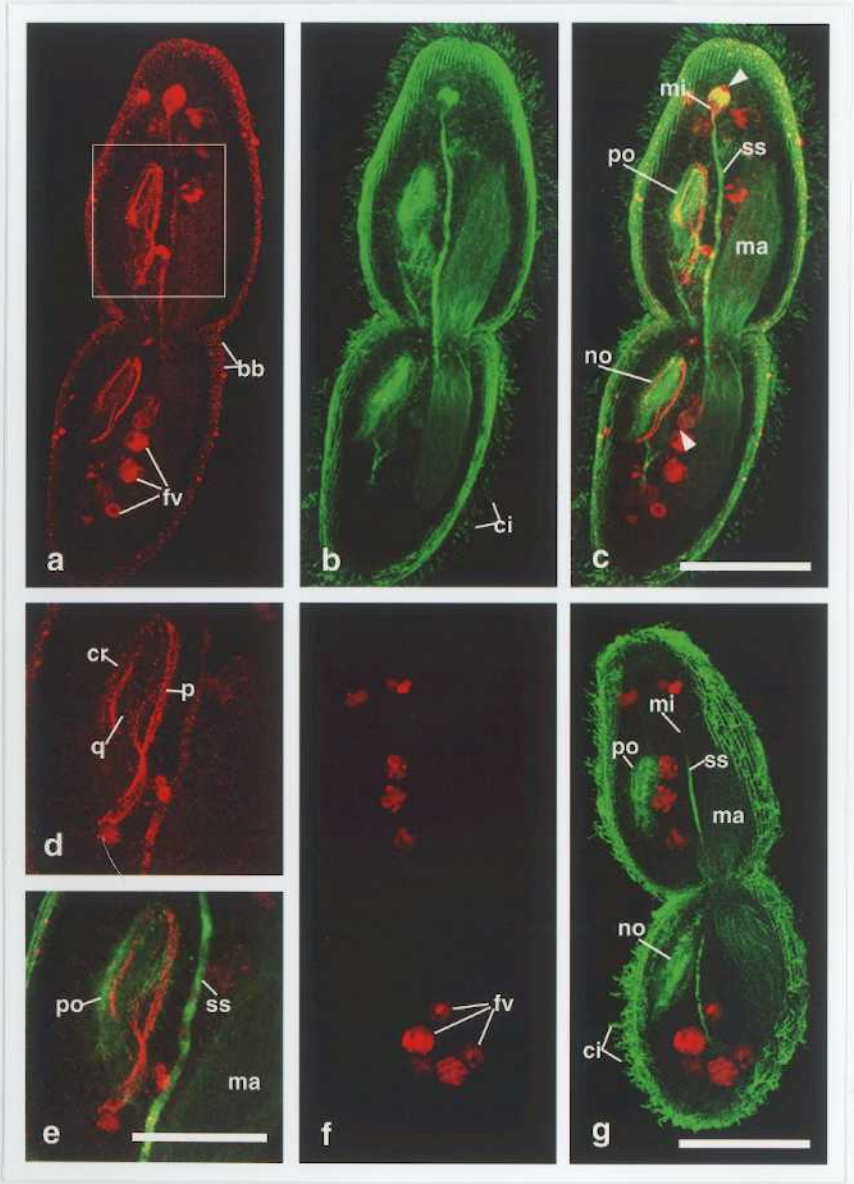


Fig. 15. Localization of γ-tubulin in interphase cell of *P. caudatum*. The cells of *P. caudatum* were stained with either an anti γ-tubulin antibody, Pcg3 (**b**) or a preimmune serum (**a**). **a**: Use of the preimmune serum is negative. **b**: Pcg 3 recognize micronucleus (mi), oral apparatus (oa) and basal bodies (bb). Note Pcg 3 does not recognize the macronucleus. Bar = 50 μm.

Fig. 16. Localization of γ-tubulin in dividing cells of *P. caudatum*. The cells of *P. caudatum* were stained with an anti-α-tubulin antibody and co-stained with either an anti γ-tubulin antibody, Pcg 3 (**a** and **d**) or a preimmune serum (**f**). Images (**c** and **e**) represent overlay image of γ-tubulin (red) and α-tubulin (green). (**g**) represents overlay image of preimmune serum (red) and α-tubulin (green).

a - c: The polar parts of the micronucleus (mi) and separation spindle (ss) at telophase are stained by Pcg 3 (arrowheads). The separation spindle which separated the daughter micronucrei and longitudial microtubules of the macronucleus (ma) are stained by the anti-α-tubulin antibody. bb, basal body; fv, food vacuole; ci, cilia; po, parental oral apparatus; no, new oral apparatus. **d** and **e**: Higher-magnification image of separation spindle (ss) of telophase micronucleus, macronucleus (ma) and parental oral apparatus (po). In the parental oral appatus, somatic ciliary rows (cr), quodrulus (q) and two peniculi (p) are strongly labeled by Pcg 3. **f** and **g**: Fluorescence localized in food vacuoles (fv). These fluorescence may be self-fluorescence of the chloroplast including the food organisms Bars represent 50 μm in **c** and **g**, and 20 μm in **e**.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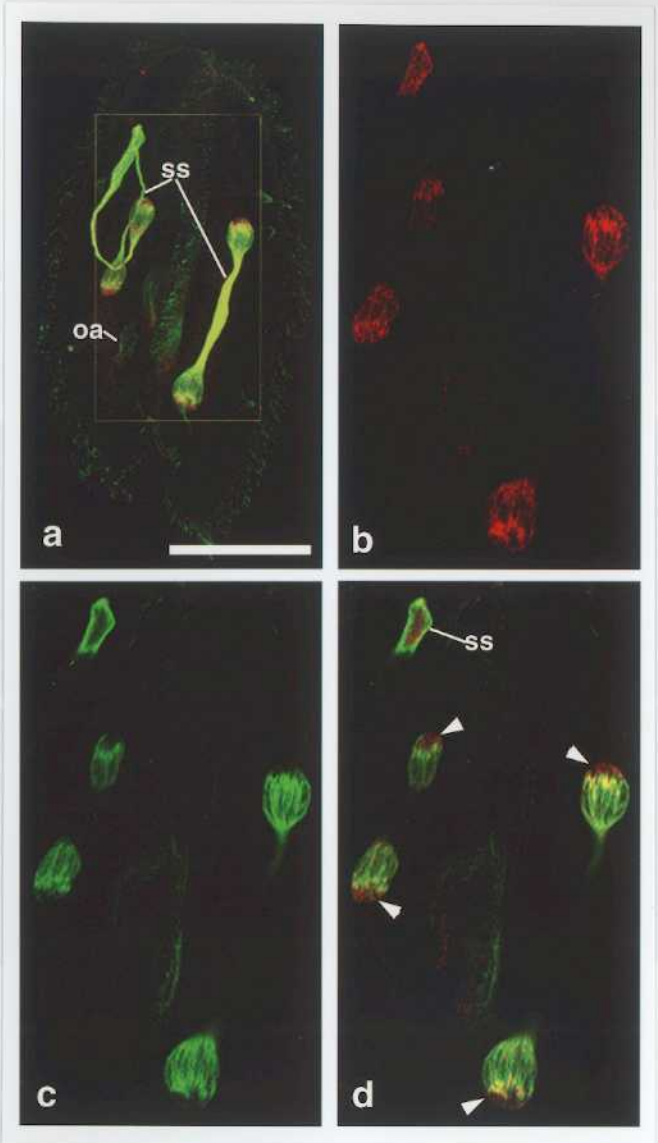


Fig. 17. Localization of γ-tubulin at telophase of the first meiotic division. The mating pair of *P. caudatum* was stained with an anti-γ-tubulin antibody, Pcg 3 (**b**) and co-stained with an anti-α-tubulin antibody (**c**). Images (**a** and **d**) represent overlay image of γ-tubulin (red) and α-tubulin (green). Reconstruction from optical sections (**a**) and single optical sections (**b** - **d**). **b** - **d**; Higher-magnification images of framed portion in **a**. γ-tubulin localizes in the spindle poles (arrowheads). α-tubulin localize in the edge of the cross section of the separation spindle (ss), in contrast, dots of γ-tubulin locate throughout the section. oa, degenerating oral apparatus. Bar = 50 μm.

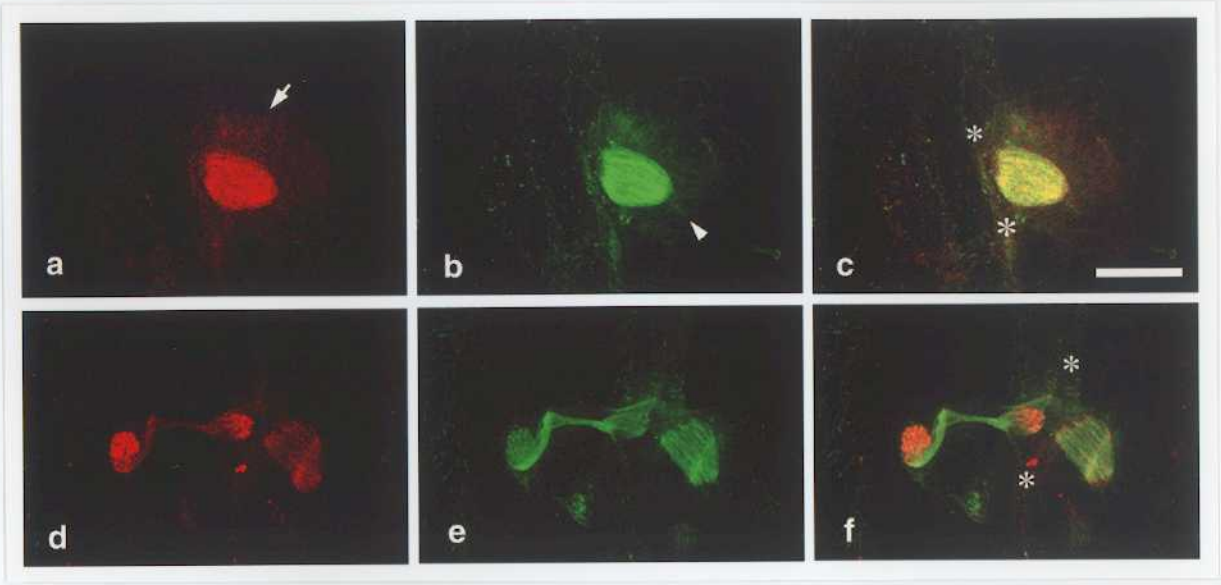
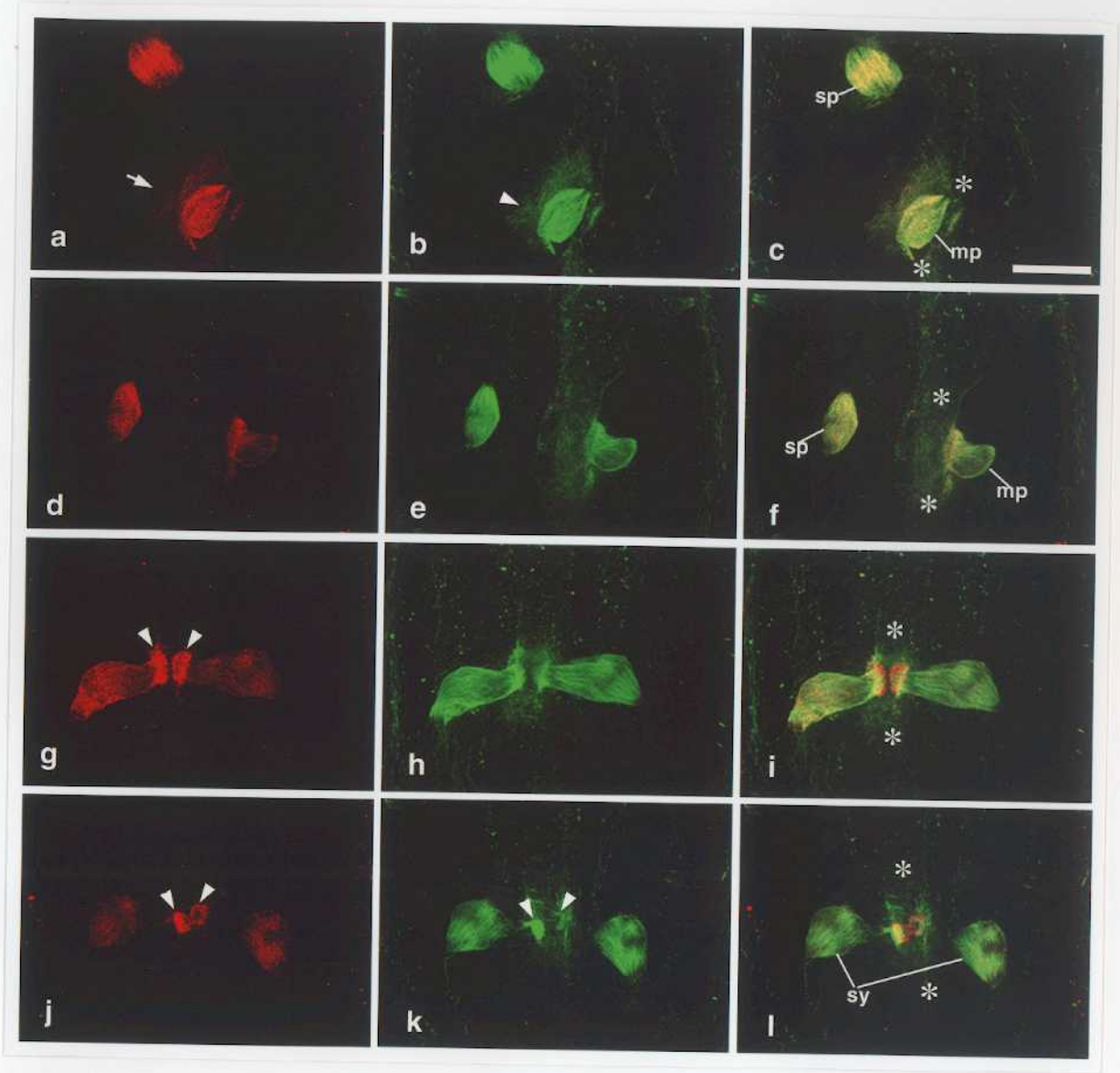


Fig. 18. Localization of γ-tubulin at the postmeiotic stage and the third prezygotic division. The mating pairs of *P. caudatum* were stained with an anti-γ-tubulin antibody, Pcg 3 (**a** and **d**) and co-stained with an anti-α-tubulin antibody (**b** and **e**). Images (**c** and **f**) represent overlay image of γ-tubulin (red) and α-tubulin (green). **a** - **c**: One of the four meiotic product moved into the paroral region and survived. The mating pair between a micronucleate (right) and an amicronucleate cells (left). γ-tubulin is accumulated around the nucleus (arrow), which are surrounded by cytoplasmic microtubules (arrowhead). **d** - **e**: Survived meiotic product undergoes the third prezygotic division. Fluorescence of γ-tubulin slightly locate the end of the nucleus at anaphase (right), and is accumulated at the spindle poles of telophase (left). Bar = 20 μm.

Fig. 19. Localization of γ-tubulin during pronuclear exchange and formation of fertilized nucleus. The mating pairs of *P. caudatum* were stained with Pcg 3 (**a**, **d**, **g** and **i**) and co-stained with an anti-α-tubulin antibody (**b**, **e**, **h** and **k**). Images (**c**, **f**, **i** and **l**) represent overlay image of γ-tubulin (red) and α-tubulin (green). In **a** - **f**, the mating pairs between a micronucleate cell (left) and an amicronucleate cell (right). The other mating pairs in **g** - **l** are between micronucleate cells. **a** - **c**: Pronuclei after the third meiotic division. γ-tubulin accumulates around the migratory pronucleus (mp, arrow) and not around the stationary pronucleus (sp). Arrowhead; cytoplasmic microtubules. **d** - **f**: Pronuclei shortly after the nuclear exchange. Fluorescence of γ-tubulin is slightly localized at the end or back of the migratory pronucleus (mp). sp; stationary pronucleus. **g** - **i**: Pronuclei just before (right) or after (left) the pronuclear fusion. γ-tubulin accumulates at the end of or back of the migratory pronucleus. **j** - **l**: Formation of the fertilized nucleus. Fluorescence of γ- and α-tubulin remains at the cell junction (arrowheads). The space between asterisks is the paroral region. Bar = 20 μm.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