STUDIES ON CORTICAL CYTOSKELETAL PROTEINS AT THE LATE STAGE OF THE CONJUGATION OF *TETRAHYMENA THERMOPHILA*

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Abstract: Colchicine and cytochalasin B were used to investigate their influence on the conjugation of *Tetrahymena thermophila*, especially at the late stage. In the experimental group that was treated with colchicine, the percent of 34KD、37KD、46KD and 57KD cortical cytoskeletal proteins changed a lot, while in the experimental group treated with cytochalasin B, the percent of 40KD and 74KD proteins changed numeraely. According to some relevant papers, we inferred that 34KD、37KD、46KD and 57KD may be tubulin, while 40KD and 74KD may be microfilamental proteins, furthermore, these proteins are all important to the morphogenesis of *Tetrahymena thermophila* during conjugation, and it needs further investigation.

Key words: Colchicines; Cytochalasin B; Microfilamental protein

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The ciliated protozoa have provided good material for investigation of various cellular phenomena for many decades. The rule of cytoskeletal elements in vegetative cortical morphogenetic mechanisms has been investigated by several workers[1—4]. A detailed study of the cortical and cellular membranes of *Euplotes* during conjugation was undertaken[5], and a thorough study of cortical cytoskeletal proteins of *Tetrahymena* during conjugation was also investigated[6], and the influence of cytochalasin B and colchicine on *Tetrahymena thermophila* during the initial stage of infusion membrane formation and the stage of pronuclei exchange was also considered[7,8]. Our work involved studying the influence of colchicine and Cytochalasin B(CB) on the major cortical cytoskeletal proteins during conjugation, especially at the late stage of conjugation.

1 Materials and methods

1.1 Strains Complementary mating types of *Tetrahymena thermophila* BF_1 and BF_5, presented by Prof. Mihoko Takahashi in Japan, were used.

1.2 Nutrient medium 0.25% proteose peptone, 0.25% yeast extract paste, 3.5% glucose, pH7.2.

1.3 Maintenance of stocks Sterile stock cultures were grown at the temperature of 30℃ and the density of cells can reach 6×10^5 cell/mL.

1.4 Starvation treatment The cells at the logarithmic growth stage were washed into starvation medium-PBS buffer, pH7.2 and starved about 23hr without shaking.

1.5 Treatment of CB Experimental group: The rough equal numbers of the two clones were mixed in the starving medium. Colchicine culture (0.5%) was put in, the final density of colchicine is 10^{-4}mol/L, and in the other experimental group, CB culture (50mg/L) was put into the starving medium, and the final density of CB is 1 mg/L.

Control group: The rough equal numbers of the two clones were mixed without adding colchicine or CB culture.

1.6 Biochemical extraction of cortical cytoskeletal proteins The mixed cells were treated with 1% TritonX-100, the methods were according to Vaudaux[9].

1.7 Gel electrophoresis SDS-polyacrylamide gel electrophoresis was carried out in a discontinuous SDS system[10] on gel slabs 1.5mm thick with 29% acrylamide and 1% bisacrylamide in the separating gel[11].

1.8 Gel scanning and analyzing The slabs were sta-

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ined with 0.05% coomassie blue in methanol: water: acetic acid(5∶5∶1, v/v) and 0.7% glycerol. After the gels were dried, they were scanned with reflection/transmission medium scanner and analyzed with the SmartView software.

2 Results

2.1 The content of cortical cytoskeletal proteins

From the above form, the authors know that the content of cortical cytoskeletal proteins at vegetative stage is higher than other stages and compared with those of the control group at the same stage, the protein contents of experimental group were decreased. The authors inferred that during the conjugation, some proteins were gradually decomposed and the new proteins were not formed, and this verified that colchicine and CB could have an influence on some cortical cytoskeletal proteins, but the mechanism needs further investigation.

Stages	Vegetative stage	Starving stage	Late stage of conjugation: control group	Late stage of conjugation treated by colchicine	Late stage of conjugation treated by CB
Wave length: 280nm	0.137	0.123	0.102	0.087	0.122
Wave length: 260nm	0.161	0.156	0.133	0.105	0.183
Protein content(mg/mL)	0.795	0.629	0.495	0.484	0.415

2.2 Comparative studies on cortical cytoskeletal proteins at different stages of the mixed cells

2.3 Gel atlas are as follows

From the gel atlas, The authors know that the percent of cortical cytoskeletal proteins of vegetative stage is different from that of the other stages, and compared with the control group, the content of proteins is different from those of the experimental group. This provides for us the further verification that colchicine and CB can influence the cortical cytoskeletal proteins of *Tetrahymena thermophila* during conjugation, especially at the late stage of conjugation.

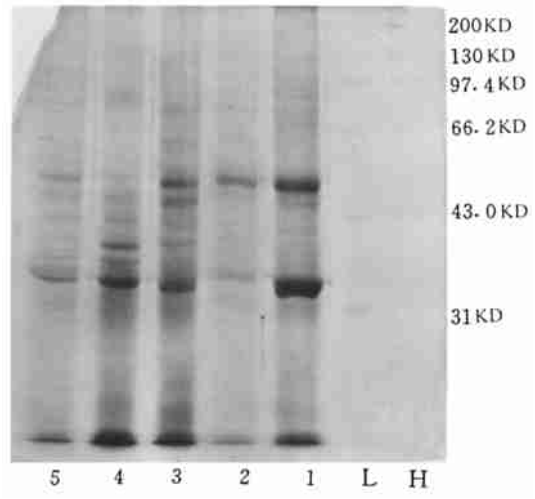

Fig.1 Comparison of cortical cytoskeletal proteins of different stages of *T. thermophila*

H: high standard proteins: 200KD, 130KD, 97.4KD, 66.2KD, 43KD

L: low standard proteins: 97.4KD, 66.2KD, 43KD, 31KD

1. Cortical cytoskeletal proteins at vegetative stage; 2. Cortical cytoskeletal proteins at the starving stage; 3. Cortical cytoskeletal proteins at late stage of conjugation(control group); 4. Cortical cytoskeletal proteins at the late stage of conjugation treated by colchicine; 5. Cortical cytoskeletal proteins at the late stage of conjugation treated by CB

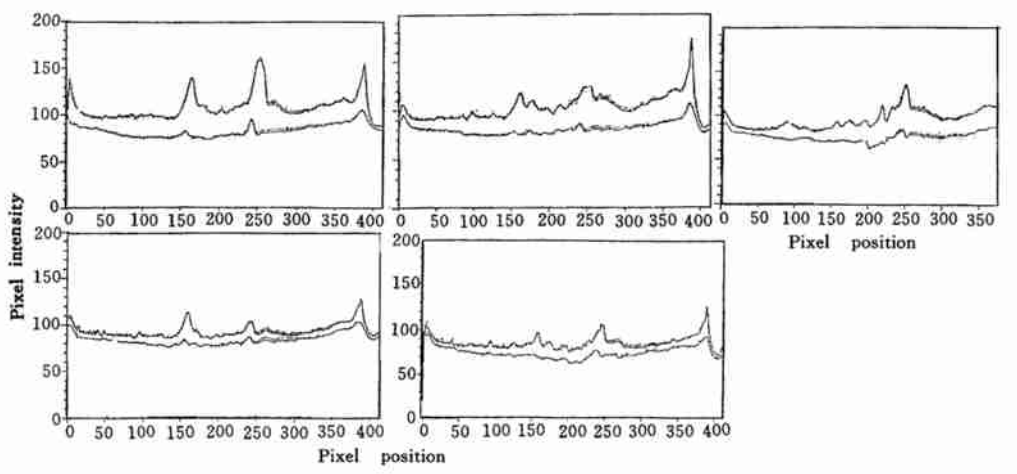

Fig.2 Comparative absorbance profiles of different stages of *T. thermophila*

3 Discussion

After the treatment of CB, The authors found that the period of conjugation is longer than that of the control group, this is similar to what Kloetzel[12] and James[13] found in *Euplotes*. Thus we concluded that CB could have some influence on the morphogenesis of *Tetrahymena thermophila*. From the result of gel atlas, we found that colchicine has more influence on 34KD、37KD、46KD and 57KD cortical cytoskeletal proteins, while CB has more influence on 40KD and 74KD proteins; and in the experimental group treated by starving, the percent of 37KD and 46KD proteins changed a lot. According to what Wang Kangle and Pang Yanbin found in *Tetrahymena Shanghaiensis*[6] and what James J. Geyer and John A. Kloetzel found in *Euplotes aediculatus*[13], the authors inferred that 34KD、37KD、46KD and 57KD may be tubulin, while 40KD and 74KD may be microfilamental proteins. Through determining the nature of cortical cytoskeletal proteins in the process of late conjugation of *Tetrahymena thermophila*, we can carry out a more thorough study on such proteins in the morphorgenesis of *Tetrahymena thermophila*.

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嗜热四膜虫接合生殖后期皮层细胞骨架蛋白的研究

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摘要: 用秋水仙素和细胞松弛素 B 处理接合期的嗜热四膜虫, 以观察其对接合生殖期, 尤其是接合后期的嗜热四膜虫皮层细胞骨架蛋白的影响。用秋水仙素处理的试验组的皮层细胞骨架蛋白组分中 34KD、37KD、46KD 和 57KD 蛋白的含量有明显改变, 而用细胞松弛素 B 处理的试验组中 40KD 和 74KD 蛋白的含量改变较大。根据相关文献, 作者推测 34KD、37KD、46KD、57KD 蛋白是微管蛋白, 而 40KD 和 74KD 可能是微丝蛋白。这些蛋白对嗜热四膜虫接合过程中的形态发生的重要作用有待进一步研究。

关键词: 秋水仙素; 细胞松弛素 B; 微丝蛋白