STUDIES ON THE EFFECT OF CYTOCHALASIN B ON MAJOR CORTICAL CYTOSKELETAL PROTEINS OF *TETRAHYMENA THERMOPHILA* DURING CONJUGATION

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Abstract: The effect of cytochalasin B on cortical cytoskeletal proteins of the complementary mating types of *Tetrahymena thermophila* BF_1 and BF_5 during their conjugation was carried out, especially during the initial stage of fusion membrane formation stage and the stage of pronuclei exchange. We found that after the treatment of CB, 146KD cortical cytoskeletal protein disappeared, while it existed in the control group, and the percent of 27KD、43KD、47KD and 174KD declined, but the percent of 32KD、41KD、51KD and 54KD kept almost unchanged. Thus we concluded that 27KD、43KD、47KD、146KD and 174KD may be the microfilament proteins.

Key words: Cytochalain B; *Tetrahymena thermophila;* Conjugation cortical cytoskeletal protein
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The fate and possible roles of the cytoskeleton in the process of conjugation in the hypotrich ciliate *Euplotes aediculatus* were investigated by James J. Geyer etc., and they inferred that the treatment of conjugants for 6 hours with cytochalasin B prevented separation, possibly through inhibition of the actin-like microfilament assembly in the fusion zone.[1] Compared with the number of fine structural and morphogenetic studies on ciliates, the formation of cortical cytoskeletal proteins of *Tetrahymena thermophila* received scant attention. We investigated the influence of cytochalasin B on major cortical cytoskeletal proteins of *Tetrahymena thermophila* during conjugation, especially at the stage of fusion zone formation and the stage of migratory pronuclei exchange, a detailed study of the influence of CB on cortical cytoskeletal proteins at the late stage of conjugation was also undertaken, and is presented in an accompanying report.[10]

1 Materials and methods

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

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

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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:** CB culture (50mg/L) was put into starvation medium, and then the rough equal number of the two clones were mixed, the final density of CB in starving medium is 1mg/L. **control group:** The rough equal number of the two clones were mixed without adding 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.[2]

1.7 Gel electrophoresis SDS-polyacrylamide gel electrophoresis was carried out in a discontinuous SDS system[3] on gel slabs 1.5mm thick with 29% acrylamide and 1% bisacrylamide in the separating gel[4].

1.8 Gel scanning and analyzing The slabs were stained 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

stages	Vegetative stage	Fusion membrane formation stage: control group	Fusion membrane formation stage: experimental group	Pronuclei exchanging stage: control group	Pronuclei exchanging: stage: experimental group
Wave length: 280nm	0.137	0.127	0.135	0.095	0.105
Wave length: 260nm	0.161	0.175	0.198	0.119	0.144
Protein content (mg/mL)	0.795	0.547	0.492	0.497	0.457

From the above form, we know that the content of proteins at the vegetative stage is higher than other stages and compared with the control group at the same stage, the protein contents of experimental group were decreased. We concluded that during the conjugation, some proteins were gradually decomposed and the new proteins were not formed, and this verified that CB could have an influence on some microfilamental proteins.

2.2 Comparative study on cortical cytoskeletal proteins at different stages of the mixed cells (Fig. 1)

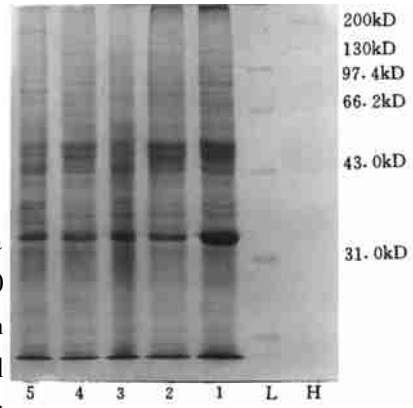

Fig 1 Comparison of cortical cytoskeletal proteins of different stages of *Tetrahymena thermophila*

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

L: low standard proteins: 97.4KD, 66.2KD, 43KD, 31KD, 20.1KD

1. cortical cytoskeletal proteins at the vegetative stage 2. cortical cytoskeletal proteins at the stage of fusion membrane formation (control group) 3. cortical cytoskeletal proteins at the stage of fusion membrane formation (experimental group) 4 cortical cytoskeletal proteins at pronuclei exchanging stage(control group) 5. cortical cytoskeletal proteins at pronuclei exchanging stage (experimental group)

2.3 Gel atlas are as follows (Fig. 2)

3 Discussion

Hamilton etc concluded that migratory pronucleus transfer in *Tetrahymena* was not affected by cytochalasin D, a specific inhibitor of actin function, even at concentrations 40 times higher than those required to inhibit phagocytosis in similarly treated vegetative cells[6], and they also inferred that microtubules must play an important role in pronuclear fusion in *Tetrahymena*, because anti-microtubule agents can block this process[7]. Numata etc. found that in *Tetrahymena*, the 14-nm filament immunofluorescence becomes detectable around the stationary pronucleus just before fusion[8]. Orias etc. thought that a microtubular basket is to assist transfer in *Tetrahymena*[9]. During our experiment, after the treatment of CB, we found that the period of conjugation is longer than that of the control group, this is similar to what Kloetzel and James found in *Euplotes*, thus we concluded that CB can have some influence on the morphogenesis of *Tetrahymena thermophila*. From the result of the gel atlas, we know that in the experimental group, during the stage of fusion zone formation and pronuclei exchange, the

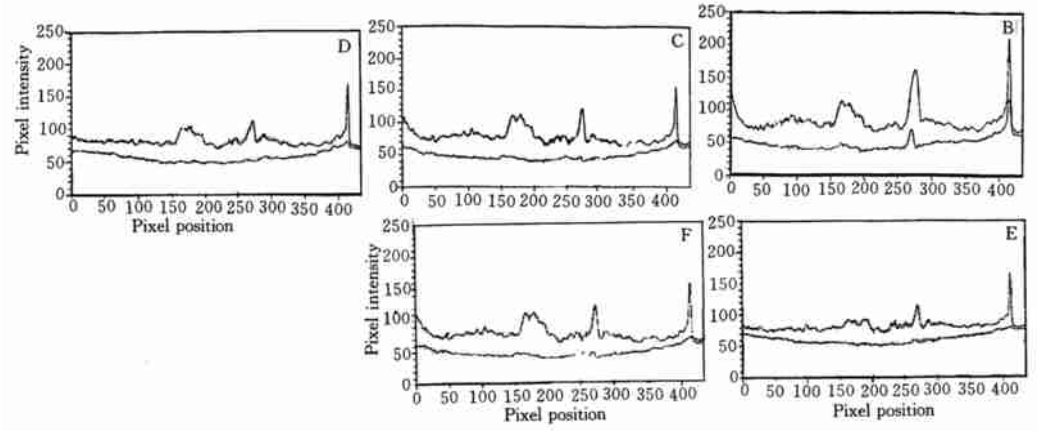

Fig. 2 Comparative absorbance profiles of different stages of *Tetrahymena thermophila*

146KD cortical cytoskeletal protein disappeared, and the percent of 27KD、43KD、47KD and 174KD decreased, while the content of 32KD、41KD、51KD and 54KD kept almost unchanged. We concluded that 27KD、43KD、47KD、146KD and 174KD may be microfilamental proteins, this is significant for us to make a further investigation on their importance in the morphogenesis of *Tetrahymena thermophila*.

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细胞松弛素对嗜热四膜虫皮层细胞骨架蛋白的影响

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摘要:测定了细胞松弛素 B 对嗜热四膜虫 BF_1 株和 BF_5 在生殖接合期的皮层骨架蛋白,尤其是在接合子接合膜形成和原核交换阶段影响甚大。作者发现 CB 处理后,与对照组相比较,皮层骨架蛋白 146KD 消失,27KD、43KD、47KD 和 174KD 含量下降,32KD、41KD、51KD 和 54KD 保持不变。结果显示,松弛素 B 对微纤毛蛋白 27KD、43KD、47KD、146KD 和 174KD 有影响。

关键词:细胞松弛素 B;嗜热四膜虫;皮层细胞骨架蛋白