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The Mutant Gene Product of a *Tetrahymena* Cell-Division-Arrest Mutant *cdaA* Is Localized in the Accessory Structure of Specialized Basal Body Close to the Division Furrow

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ABSTRACT—A division arrest mutant, *cdaA*, of *Tetrahymena thermophila* is known to have a temperature sensitive-defect in the determination of the division plane, and its gene product had been shown to be a protein designated as p85 (Mr=85,000; pI=4.7). Here the localization of p85 was shown to be the accessary structure of specialized basal body close to the division furrow by immunoelectron microscopy using anti-p85 antiserum.

INTRODUCTION

We have been studying the molecular mechanisms of the determination of the division plane, using a *Tetrahymena* temperature-sensitive mutant, *cdaA1*. Frankel *et al.* isolated this mutant and demonstrated that a restrictive temperature prevented *cdaA1* cells from forming the fission zone which corresponded to the position of the fission plane [1, 2]. Consequently, *cdaA1* fails to perform the subsequent furrowing and exhibits a long-term cell division arrest. In our previous immunofluorescent microscopic study, p85 was found to be localized at the equatorial basal bodies just before the formation of the fission zone at a permissive temperature [3]. On the other hand, at the restrictive temperature, *cdaA1* cells showed neither equatorial localization of p85 nor formation of the fission zone [3].

In *Tetrahymena* cells, the contractile ring microfilaments (actin filaments) which play a central role in the constriction of the division furrow run along the equatorial basal bodies [4]. Skeletal muscle actin can copolymerize with *Tetrahymena* actin but has properties different from those of *Tetrahymena* actin [5, 6]. When skeletal muscle actin was injected into *Tetrahymena* just before the onset of furrow constriction, it polymerized near the equatorial division plane, but this non-functional contractile ring microfilament caused long-term arrest of cell division [7].

These observations suggest that p85 is a crucial factor for determing the cell division plane and that it also functions as a polymerization nucleus for contractile ring microfilaments. To further scrutinize the relationship between the division plane and the localization of p85, the detailed localization of p85 must be demonstrated and we used immunoelectron microscopy, using an antiserum against p85. In this report, we show that p85 is localized in a certain accessory structure associated with distal end of the specialized basal body closest to the division plane.

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MATERIALS AND METHODS

Cell culture

Inbred wild-type strain (B1868) of *Tetrahymena thermophila* used in this study was kindly provided by Dr. D. L. Nanney. Cells were grown axenically in a medium containing 1% proteose peptone, 0.5% yeast extract, and 0.87% glucose and maintained at 26°C [8].

Antiserum

Polyclonal antiserum against p85 was the same as that used in our previous paper [3].

Electron microscopy

Fixation of *Tetrahymena* cells was performed by freeze-substitution as described by Chida and Ueda [9]. To prevent formation of ice crystal in cytoplasm, the suspension of *Tetrahymena* cells was mixed with an equal volume of a 20% v/v solution of dimethyl sulfoxide in NKC solution (0.2% NaCl, 0.008% KCl and 0.012% $CaCl_2$) [10]. Cells were harvested by centrifugation and spread on the formvar membrane stretched on the loop of copper wire. The cells on the membrane were quickly immersed in liquid propane. Frozen samples were transferred to a substitution fluid, anhydrous acetone, and kept for 36 hr at −85°C. Samples were incrementally warmed to 4°C, then washed with anhydrous acetone. The samples were infiltrated and embedded in Lowicryl K4M and

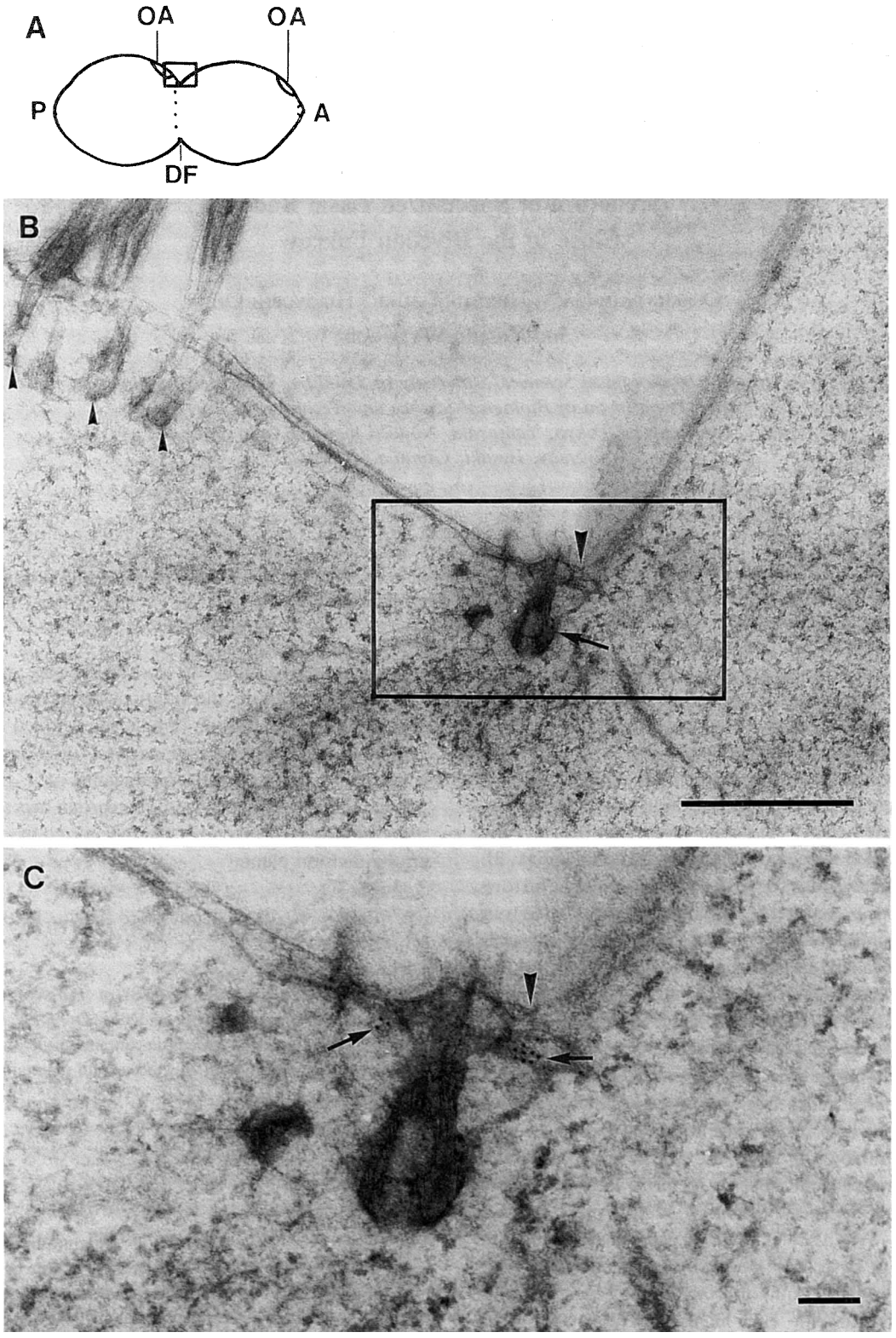

FIG. 1. Immunoelectron micrographs of a dividing *Tetrahymena* cell with anti-p85 antiserum and colloidal gold-conjugated secondary antibody. (A) Schematic illustration of a dividing *Tetrahymena* cell. OA: Oral apparatus, DF: division furrow, A and P: anterior and posterior parts of the cell. (B) Immunoelectron micrograph of the division furrow region which corresponds to the rectangle shown in A. The basal body (arrow) close to division furrow lacks cilium, whereas three basal bodies (arrowheads) with cilia are those constituting the oral apparatus. The large arrowhead indicates the division furrow. The bar represents 1 μm. (C) Enlarged micrograph of the division furrow region corresponding to the rectangle shown in B. The gold particles (arrows) are localized predominantly at the distal end of a certain accessory of the basal body close to the division furrow. An arrowhead indicates the division furrow. The bar represents 100 nm.

polymerized at −20°C for 24 hr and at 26°C for 36 hr by ultraviolet irradiation. Thin-sectioned specimens were mounted onto nickel grids and blocked with phosphate-buffered saline (PBS) containing 1% bovine serum albumin for 15 min. They were then incubated with anti-p85 antiserum (diluted 1 : 800 in PBS containing 0.1% BSA) for 2 hr at room temperature. After thoroughly washing the specimens with PBS, the second antibody (goat anti-guinea pig IgG coupled to 10-nm gold particles; diluted 1 : 20 in PBS containing 0.1% BSA) was applied for 30 min at room temperature. They were again washed in PBS to remove nonreacted colloidal gold particles. As a control, preimmune serum was used in the same way. The specimens were then stained with uranylacetate and lead citrate and examined with a JEM 100CXII(JEOL) electron microscope.

RESULTS AND DISCUSSION

The localization of p85 was firstly examined by ordinary immunoelectron microscopy but we found that antigenicity of p85 was unusually sensitive to chemical fixation, dehydration with acetone or ethanol, and heat treatment. Therefore, *Tetrahymena* cells were fixed in freeze-substitution fluid, and embedded within Lowicryl K4M at a low temperature. When the thin sections of freeze-substituted cells embedded in Lowicryl K4M were incubated with specific polyclonal anti-p85 antiserum followed by second antibody conjugated with 10-nm colloidal gold particles, a heavy gold labeling at an accessory structure of a basal body closest to the division plane could be demonstrated (Fig. 1C). The gold particle labeling is predominantly associated with a certain accessory structure of the basal body on the side of the division furrow and this labelled portion may correspond to the position of contractile ring microfilaments. On the other hand, any accessory structures of basal bodies situated far from the division plane did not show gold particle deposit (Fig. 1B).

The basal body closest to the division furrow lacks cilium, while those far from the division furrow possess cilia. The fission zone is bounded posteriorly by a unique basal-body couplet that is destined to participate in the formation of the apical crown of the posterior daughter cell [11–13]. The anterior basal body of this couplet remains parmanently unciliated and was labeled with gold particles (Fig. 1C). Only a small part of the posterior basal body of the couplet is seen in Figure 1B, so Figure 1B may be a somewhat oblique longitudinal section of the cell.

A kinetodesmal fiber, a band of posterior microtubules and a band of transverse microtubules are all started from the proximal end of an usual basal body. The transverse microtubules and the kinetodesmal fiber are seen anterior to the usual basal body [14, 15]. However, the specialized anterior basal body of the couplet lacks both cilia and such basal body-associated microtubular structures [13]. Immunoelectron micrograph shows that p85 is contained in a certain specialized accessory structure on the distal end of the anterior basal body and that the structure is situated just beneath the epiplasmic layer (Fig. 1C). In a control micrograph using preimmune serum, specific localization of gold particles was not observed (data not shown). This ultrastructural finding reinforces our notion that p85 is a crucial factor for both division plane determination and contractile ring microfilament formation.

Buzanska and Wheatley reported that Okadaic acid promotes cell division in the *cda A1* mutant at the restrictive temperature and suggested that the defect of this mutant is related to some impairment in protein phosphorylation rather than change in the structural element itself [16]. Although p85 is suggested to be a cytoskeletal component [3], a function of p85 might be controlled by phosphorylation and/or dephosphorylation. To explore this further, we are now trying to clone and sequence a full length genomic DNA encoding p85.

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REFERENCES

1 Frankel J, Jenkins LM, Doerder RP, Nelsen EM (1976) Genetics 83: 489–506
2 Frankel J, Nelsen EM, Jenkins LM (1977) Dev Biol 58: 255–275
3 Ohba H, Ohmori I, Numata O, Watanabe Y (1986) J Biochem 100: 797–808
4 Hirono M, Nakamura M, Tsunemoto M, Yasuda T, Ohba H, Numata O, Watanabe Y (1987) J Biochem 102: 537–545
5 Hirono M, Kumagai Y, Numata O, Watanabe Y (1989) Proc Natl Acad Sci USA 86: 75–79
6 Hirono M, Tanaka R, Watanabe Y (1990) J Biochem 102: 32–36
7 Ohba M, Hirono M, Edamatsu M, Watanabe Y (1992) Dev Genetics 13: 210–215
8 Watanabe Y, Ikeda M (1965) Exp Cell Res 39: 443–452
9 Chida Y, Ueda K (1991) Ann Bot 67: 435–442
10 Simon EM (1972) Cryobiology 9: 75–81
11 McCoy JW (1974) Acta Protozool 8: 155–159
12 Ng SF, Williams RJ (1977) J Protozool 24: 257–263
13 Jerka-Dziadosz M (1981) Acta Protozool 20: 337–356
14 Allen RD (1967) J Protozool 14: 553–565
15 Allen RD (1969) J Cell Biol 40: 716–733
16 Buzanska L, Wheatley DN (1994) Eur J Cell Biol 63: 149–158