Acta Protozool. (2004) 43: 291 - 301

ACTA
PROTOZOOLOGICA

# Syndrome of the Failure to Turn off Mitotic Activity in *Tetrahymena thermophila*: in *cdaA1* Phenotypes

**Ewa JOACHIMIAK, Janina KACZANOWSKA, Mauryla KIERSNOWSKA and Andrzej KACZANOWSKI**

**Department of Cytophysiology, Institute of Zoology, Warsaw University, Warsaw, Poland**

**Summary.** During early micronuclear mitosis of a wild type *Tetrahymena thermophila,* basal body proliferation and cortical growth are localized in the equatorial region of the pre-dividing cell. These processes are arrested prior to cytokinesis when the fission line gaps appear in ciliary rows. Then a putative marker of cellular polarity, the fenestrin antigen, appears in the apical zone of the dividing cell and around the old oral apparatus (OA1) and in the cortex localized posterior to the fission line gaps and around the new oral apparatus (OA2) i.e. in the apical cortex of the prospective posterior daughter cell. Prior to cytokinesis, the membranelles within OA1 and OA2 oral apparatuses are strongly labeled with the MPM2 antibody against mitotic phosphoproteins. The transition to cytokinesis is correlated with disappearance of both the polar fenestrin staining and of the phosphoprotein antigens in OA1 and OA2. *cdaA1* (cell division arrest) mutant cells grown at the restrictive temperature do not produce a fission line and they do not undergo cytokinesis thereby generating irregular chains. The *cdaA1* phenotypes continue elongation of their ciliary rows in equatorial regions, mostly without formation of the fission line gaps, accompanied with repetitive micronuclear mitoses and repetitive formation of the defective oral structures. In *cdaA1* cells at restrictive temperature, the fenestrin antigen was recruited and then permanently found in the apical regions and around all oral apparatuses, and was always absent in equatorial regions, in spite of variability of immunostaining patterns, sizes and advancement of organization of OAs in different specimens of the same sample. The MPM2- tagged phosphoproteins were retained in all oral apparatuses in different *cdaA 1* phenotypes. We suggest that the *cdaA1* phenotypes produced at restrictive temperature behave as cells trapped in a metastable phase with a syndrome of an arrest of the mechanism required to regain the morphostatic stage of a non-dividing cell.

**Key words:** *cdaA1* mutation, cell cycle, fenestrin, MPM2 phosphoproteins, *Tetrahymena thermophila.*

## INTRODUCTION

In yeasts many regulators define the spatial pattern of cell growth, positioning of the fission line, cytokinesis (Ayscough *et al*. 1997, Finger *et al*. 1998) and pattern the of acquisition of cell polarity during the cell cycle (Casamayor and Snyder 2002, Niccoli and Nurse 2002). The cell cycle is ended with an activation of the mitotic exit network (MEN) of events stimulated by an activation of the specific phosphatase (Cdc 14) and then turned off by an activation of the specific inhibitor of G protein signaling involved in acquisition of cell polarity to reset the whole cell cycle (Visintin *et al*. 1998, Wang *et al*. 2003).

The regulators corresponding to yeast's regulators of the patterned growth, mitotic exit and resetting of the cell cycle remain unknown in the ciliate cells. Biometrical

Address for correspondence: Janina Kaczanowska, Department of Cytophysiology, Institute of Zoology, Warsaw University, Miecznikowa str. 1, 02-096 Warszawa, Poland; Fax: (0-48) 22 554 1203; E-mail: kaczan@biol.uw.edu.pl

analysis showed that localization of the oral primordium for the prospective posterior cell division product in *T. thermophila* (OA2), is specified by two gradients of ciliary basal body proliferation within ciliary rows: an antero-posterior gradient and a dorso-ventral gradient (Kaczanowski 1978). In the later stages of divisional morphogenesis, the proliferation of the basal bodies is arrested, resulting in fission zone gaps within ciliary meridians that localizes the equatorial position of a fission line (Kaczanowska *et al*. 1992, 1993). The fission line separates nascent posterior and anterior poles of prospective daughter cells (Frankel *et al*. 1981, Kaczanowska *et al*. 1999). The metamery of divisional morphogenesis and the asymmetry of the fission zone correlates with a transient appearance of the fenestrin antigen in the apical region of the parental dividing cell and in a belt localized posterior to its fission line (Nelsen *et al*. 1994). Thus the fenestrin antigen is a transient cortical marker of an anterior pole character (Kaczanowska *et al*. 2003), and this patterned cortical growth during divisional morphogenesis is also associated with stage-dependent patterns of phosphorylation of proteins in oral membranelles (Kaczanowska *et al*. 1999). Subdivision of the anterior-posterior axis of the mother cell into two axes of prospective daughter cells is correlated with a transient appearance of the anterior marker of the submembrane protein - fenestrin (Nelsen *et al*. 1994) and with a transient decrease of the B antigen against the submembrane cytoskeletal epiplasm (Williams *et al*.1987,1995; Kaczanowska *et al*. 1993, 1999; Honts and Williams 2003). The apical staining with the anti-fenestrin antibody (Nelsen *et al*. 1994) appears at specific stages of oral development both in dividing and in reorganizing cells (Kaczanowska *et al*. 2003).

The MPM2 antibody. directed against some mitotic phosphoproteins (Davies *et al*. 1983, Westendorf *et al*. 1994, Ding *et al*. 1997) permanently labeled basal bodies of ciliary rows and other cortical organelles (like the contractile vacuole pores and cytoproct), but during morphogenesis this label only transiently appears in the oral membranelles of the developing or reorganizing oral apparatus and increases in surrounding cortical regions, then disappears during the transition to a morphostatic state (Kiersnowska and Golinska1996, Kaczanowska *et al*. 1999). Thus both the fenestrin and MPM2 antibodies may be used as markers of the exit of a cell from a metastable phase to the morphostatic condition of the quiescent cell.

To address this problem, we used the *cdaA1* mutant. At the restrictive temperature, the *cdaA1* mutant of *T. thermophila* (Frankel *et al*. 1976, 1977) shows complete arrest in cytokinesis, general increase of cell size, consecutive rounds of incomplete oral morphogenesis, repetitive micronuclear divisions, repetitive macronuclear DNA synthesis leading to a 2-4 fold increase of DNA content in the macronucleus (Frankel *et al*. 1976, 1980; Cleffmann and Frankel 1978). This resulted in formation of an array of *cdaA1* phenotypes with: disturbed cortical polarity of ciliary rows (Ng and Frankel 1977), which are folded in irregular cortical protrusions within the cortex of the prospective fission zone (Buzanska *et al*. 1989), and with repetitive but curtailed and defective stomatogenesis (Frankel *et al*. 1977, 1980; Kaczanowska 1990).

The aim of this report is to characterize the *cdaA1* phenotypes (Frankel *et al*. 1976) by an analysis of the patterns of immunofluorescence with the anti-fenestrin marker (Nelsen *et al*. 1994) and with the MPM-2 antibody against the phosphorylated epitopes of cytoskeletal mitotic phosphoproteins (Davies *et al*. 1983) in the *cdaA1* cells. Therefore the following questions were asked in this study: (1) whether in cytokinesis - arrested *cdaA1,* the fenestrin antigen will appear in the putative fission line zones and in the anterior apical region, (2) whether the variability of phenotypes correlates with the defined anti-fenestrin immunostained internal oral patterns, or with the advancement of development achieved by particular oral structures in the specimen, and (3) whether parallel correlations affect MPM2 immunostaining of oral patterns in particular specimens in the array of phenotypes.

## MATERIALS AND METHODS

**Material**. *Tetrahymena thermophila cdaA1* (previously *mol$^a$/mol$^a$*, Frankel *et al.* 1976) strain IA104 was kindly provided by Dr J. Frankel, Iowa University, U.S.A. For inducing expression of the *cdaA1* mutation 300 ml Erhlenmeyer flasks containing 50 ml of the growth medium (1% PPY supplemented with iron and antibiotics, Nelsen *et al*. 1981) were inoculated with the mutant strain and incubated overnight at the 28°C to yield densities about 1000 cells/ml. Then some flasks were shifted to the 36°C restrictive temperature and other (controls) were left at the 28°C. The cell samples were immunostained at different times after shifting the cells to the restrictive temp (2, 2.5, 4 and 7 h). In one control experiment, the wild type conjugants of two complementary strains of *T. thermophila* CU427 (mt VI) and CU428 (mt VII) mixed for 4.5 h at 28°C were used. These strains were derived from Cornell University Athens, U.S.A and obtained from Dr J. Gaertig.

**Antibodies**. The monoclonal antibodies: FXIX-9A7 (against 64 kDa polypeptide - fenestrin) and MPM-2 (against phosphopro-

teins) were generously supplied by Drs: N. Williams, J. Frankel, and M. Nelsen, and by Dr P. Rao, Houston, U.S.A respectively. The secondary antibody was FITC - conjugated goat anti-mouse IgG (SIGMA).

**Indirect immunofluorescence**. The fenestrin epitopes were labeled according to Williams *et al*. (1990). The cells were collected by the low speed centrifugation , washed with 10 mM Tris - HCl (pH 7.4), permeabilized and fixed for 5-10 min on ice, with a solution of 0.2% Triton X-100 in 50% ethanol. The extracted cells were washed 2 × 10 min with blocking solution: of 0.2% BSA/TBS (bovine serum albumin - 20 mM Tris-HCl, 150 mM NaCl, pH 8.2). After 2 h of incubation with the primary antibody diluted 1:50 with 0.2% BSA/TBS at 30°C the pellet of cells was washed 3 × 10 min with the blocking buffer, and secondary antibody (diluted 1:200 with 0.2% BSA/TBS) was added for 2 h. Then the cells were washed 3-4 times and suspended on a slide in DAKO fluorescent mounting medium (anti quenching of the fluorescent label agent) and observed in a NIKON ECLYPSE E - 600 microscope.

The cells were labeled with the MPM2 antibody according to Kiersnowska and Golinska (1996). The growing cells were collected by low speed centrifugation and after washing with the 10 mM TRIS - HCl (pH 7.4) they were extracted for 3-5 min with 0.5% Triton X-100 in PHEM buffer, pH 6.9 (Schliwa and Van Blerkom 1981), fixed for 30 min with 2% paraformaldehyde, washed 4 × 10 min with 0.1% BSA / PBS (bovine serum albumin /phosphate buffer) and incubated overnight in 4°C with primary antibody at dilution 1: 200 in1% BSA/PBS. Then the cell samples were incubated with the secondary antibody and prepared for microscopic examination in the same way as those cells labeled with the anti-fenestrin antibody, except that PBS buffer solution was applied for washing.

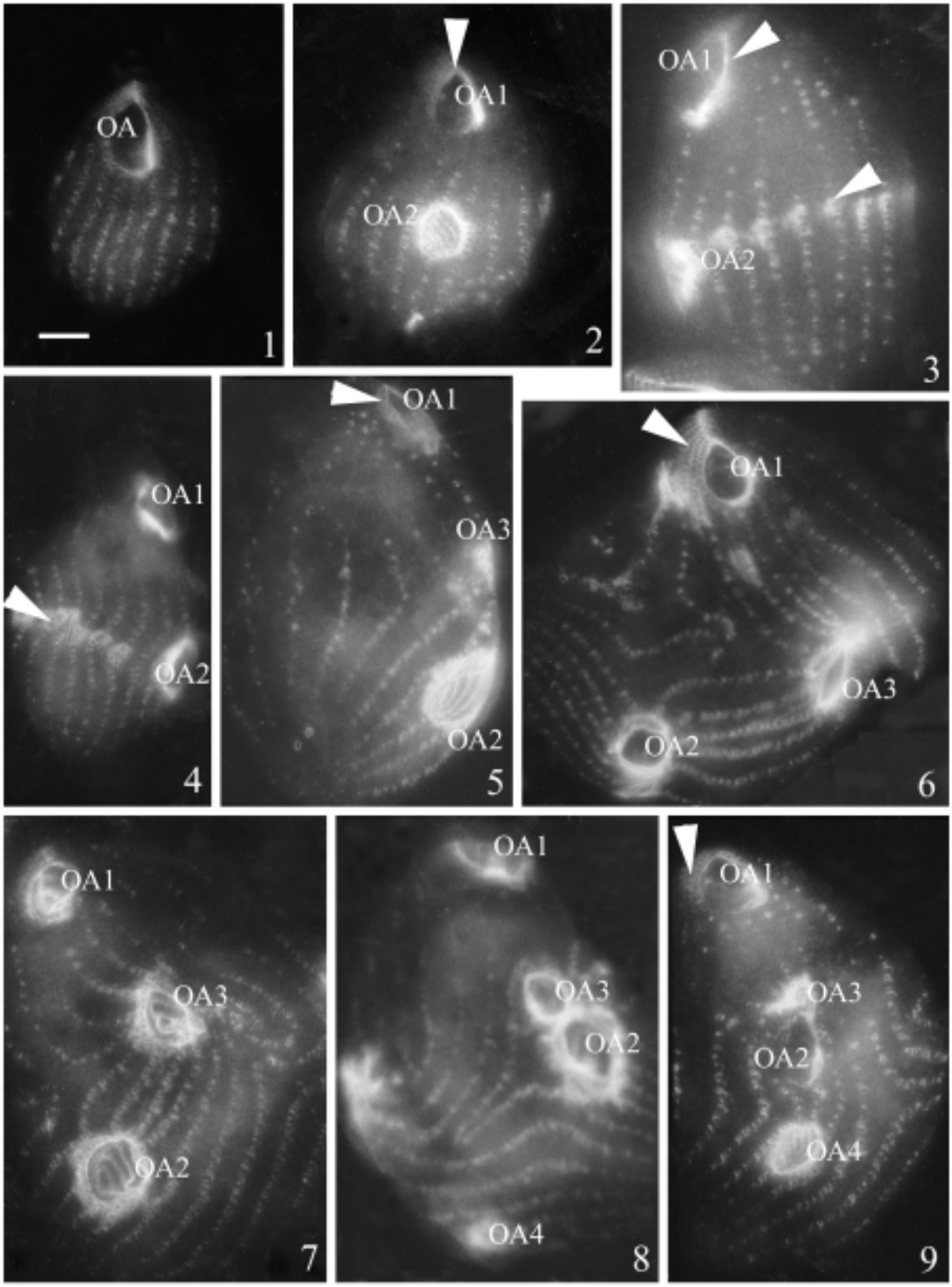
**Western blotting.** Microtubule-free cortical residues were isolated from *cdaA1 Tetrahymena thermophila* grown at permissive temperature 28°C (as described above), and from cell samples shifted to the restrictive temperature 36°C for 2.5, 4 and 7 h. The samples were washed with 10 mM Tris-HCl, pH 7.4 and extracted with a cold solution containing of Triton X-100 in a high salt solution, with a cocktail of proteolysis inhibitors as described by Williams *et al*. (1990). SDS-PAGE electrophoresis and immunoblotting were carried out with the anti-fenestrin antibody diluted 1:200 incubated overnight at 4°C, and then the second anti-body the anti-mouse IgG- AP conjugated was developed with BCIP/NBT (Sigma) protocol according to producer's instruction. Three independent experiments were performed and one was supplemented with the sample of the wild type conjugating cells kept at 28°C for 4.5 h as the case of *wt* starved cells but nevertheless involved in morphogenetic activity.

## RESULTS

### Cortical patterns of anti- fenestrin immunofluorescence in *cdaA1* phenotypes

**Cells incubated at permissive temperature (28°C).** The morphostatic *cdaA1* cells showed slight fluorescence of the collar of the oral apparatus (OA) and a weak fluorescence around basal body domains in the middle segments of ciliary rows (Fig. 1). In dividing cells, a weak apical fluorescence intensified (Fig. 2, arrowhead) and the newly developing oral primordium (OA2) gradually differentiates a "negative pattern of oral membranelles" in which the three membranelles are not immunostained, while the oral matrix around them is stained (Fig. 2, OA2). Thereafter, with some delay, this "negative pattern of membranelles" also appears in the parental OA1 (not shown). In the next stage, the fluorescence of the apical cortex and of the belt localized posterior to the fission line appears (Fig. 3, arrowheads), while the negative labeling of the membranelles gradually disappears in both oral apparatuses (Fig. 3, OA1 and OA2), whereas, the residual labeling outlines the OAs. During cytokinesis, the polar immunostaining of both offspring gradually diminishes while residual labeling outlining the OAs persists (not shown). Thus at the permissive temperature, the patterns of cortical labeling of morphostatic and dividing *cdaA1* cells with the anti-fenestrin antibody were the same as those in the w*t* cells (Kaczanowska *et al*. 2003).

**Cells incubated 2h or more at restrictive temperature.** Growing c*daA1* cells expressed cell division arrest (their mutant phenotypes) as soon as 2 h after shifting to the restrictive temperature, what was monitored under the microscopy, and became majority in a sample incubated for 2.5 h. Practically all specimens expressed *cdaA1* phenotypes after 4 h of incubation, whereas after 7 h of incubation some cells became immobile (possibly dying cells). *cdaA1* phenotypes taken from the after 2 h of incubation at 36°C sample are very variable. Some of them (for instance Fig. 4) keep a normal size and shape, whereas others exhibit very different sizes and shapes. All *cda A1* cells kept at the restrictive temperature show an apical labeling of the fenestrin marker (Figs 4-9) and absence of this marker in the equatorial regions (Figs 5-9). The first sign of an abnormality in cells shifted to restrictive temperature was found in the cell of Fig. 4, with an incomplete fluorescent belt localized posterior to the fission line (arrowhead) and with two oral apparatuses with greatly reduced internal fluorescence (as on Fig. 3). A cell (Fig. 5) from the same sample with three oral apparatuses OA1, OA2 and OA3 shows a "negative pattern of membranelles" (like in Fig. 2) only in the OA2. All these cells manifested less or more extended apical fluorescence as focused in Figs 5 and 6 (arrowheads) together with a total absence of this fluorescence in vicinity of the fission zone, in spite of gaps of some ciliary rows (Fig. 6). In contrast to Fig. 5, in Fig. 6, all three oral


OA
1
OA1
OA2
2
OA1
OA2
3
OA1
OA2
4
OA1
OA3
OA2
5
OA1
OA3
OA2
6
OA1
OA3
OA2
7
OA1
OA3
OA2
8
OA1
OA3
OA2
OA4
9
OA1
OA3
OA2
OA4

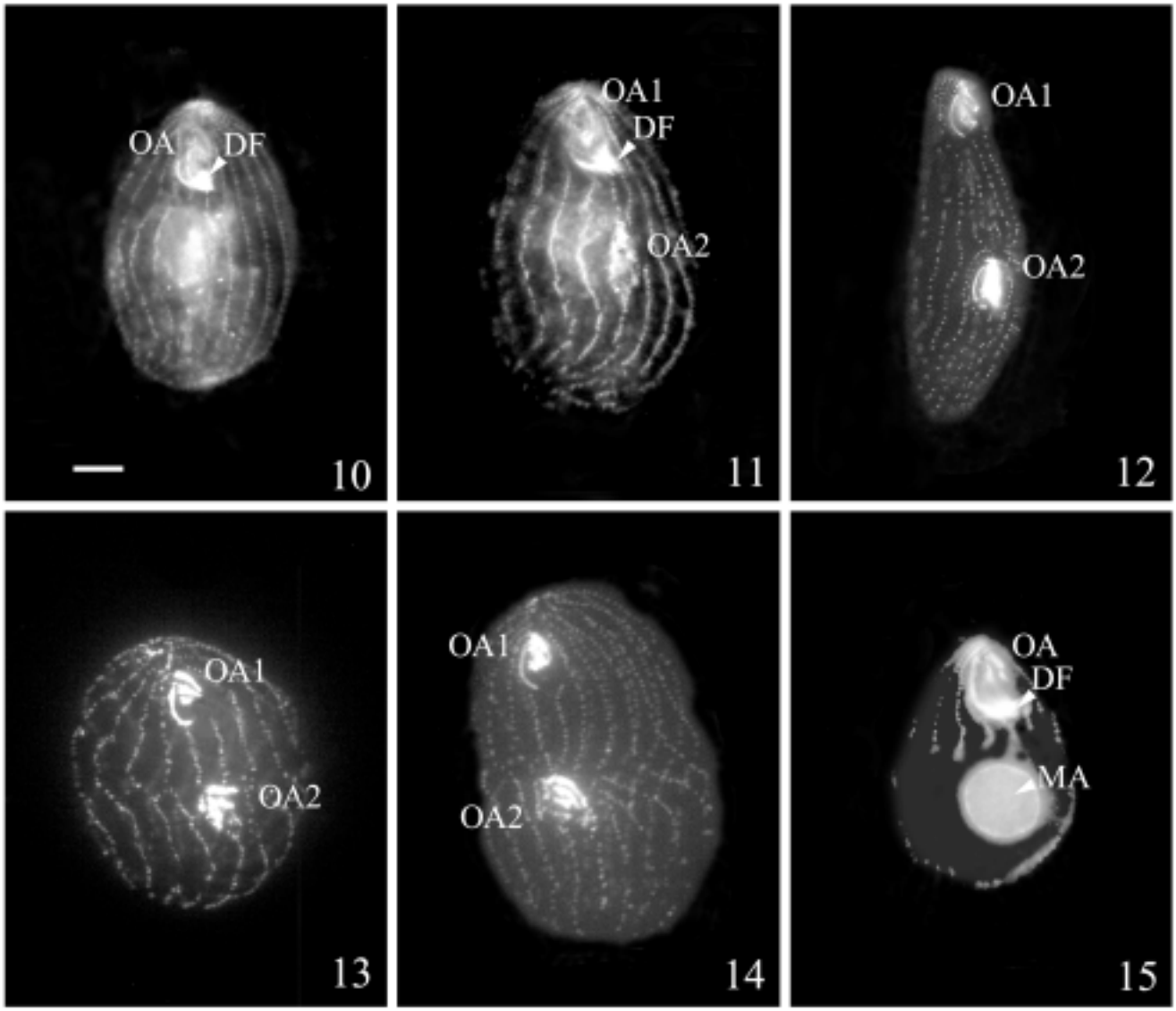
apparatuses, OA1, OA2 and OA3 lack internal fluorescence. In the Fig. 7 another cell from the same sample shows fenestrin labeling around oral structures, which represents the same stage of a "negative pattern of membranelles" in all three oral apparatuses (OA1, OA2, and OA3). In Fig. 8, collars surround the four oral apparatuses that lack fluorescence within oral pouches of variable sizes. Finally, a cell with two apparent generations of oral apparatuses is presented in Fig. 9; OA1 and OA2 are at the stage of nearly total disappearance of fluorescence within the oral pouches, whereas the OA3 and OA4 are in stage of a "negative pattern of membranelles" (Fig. 9, OA3 and OA4). In this cell, the apical staining with fenestrin is preserved (Fig. 9, arrowhead) as in other specimens. Hence, in *cdaA1* mutants, perturbations in the pattern of ciliary rows are associated with an absence of the fenestrin marker of polarity in the prospective posterior daughter cell, whereas this marker is preserved in the apical region of phenotypes and in collars outlining all oral apparatuses. Nevertheless, the size and number of oral apparatuses, presence or absence of labeling by anti-fenestrin antibody of the "negative pattern of membranelles" are variable. The same variability is observed in samples incubated at restrictive temperature for more than 2 h (in samples incubated 4 and 7 h).

## Cortical patterns of MPM2 immunofluorescence in cdaA1 phenotypes

**Cells kept at permissive temperature.** In non-dividing wild type *T. thermophila* as well as in *cdaA1* cells kept at a permissive condition, the MPM2- tagged phosphoproteins are detected in the inner pouch of the oral apparatus (OA), with a very strong fluorescence of the deep fiber at its bottom (Fig. 10, DF), with an absence of fluorescence of the oral membranelles, and a weak fluorescence of the oral collar and the basal bodies of ciliary rows (Fig. 10). In early dividing cells very strong fluorescence appears of the newly produced primordium of the second oral apparatus (Fig. 11, OA2), whereas the pattern of immunostaining of OA1 roughly corresponds to that of OA of a non-dividing cell (Fig. 11, DF). In this cell some apical fluorescence, and intense staining of basal bodies in middle segments of ciliary rows are observed (Fig. 11). In more advanced stage of cell division, a disappearance of the fluorescence in the bottom of the OA1 (corresponding to a site of the deep fiber in Figs 10, 11) and a start of transient immunostaining of its membranelles (Fig. 12, OA1) sharply contrasts with a very strong fluorescence of the oral region of OA2 (Fig. 12, OA2). At more advanced stage, the membranelles in both oral apparatuses (Fig. 13, OA1 and OA2) are heavily immunostained in equivalent intensities. Prior to cytokinesis, a gradual disappearance of fluorescence of membranelles in OA1 and OA2 (Fig. 14 *vs.* Fig. 13) is observed. It results in a weak "negative pattern of membranelles" in both oral apparatuses with a nearly total disappearance of fluorescence within oral apparatuses (OA1 and OA2) (not shown). After division, in quiescent daughter cells some apical fluorescence gradually diminishes, whereas the deep fiber gradually re-appears in the bottom of OA which strongly binds MPM2 antibody (Fig. 15, DF). This cell also shows a transiently appearing fluorescence of the macronucleus (Fig. 15, MA). Thus at the permissive temperature, the patterns of cortical labeling of morphostatic and dividing *cdaA1* cells with the MPM2 antibody are the same as those in the w*t* cells (Kiersnowska and Golinska 1996, Kaczanowska *et al*.1999).

**Cells kept 2 h, 4 h and 7 h at restrictive temperature.** A major fraction in a sample incubated at 2 h are the *cdaA1* cells arrested in cell division of a size of cells

**Figs 1-9.** Cortical patterns of anti-fenestrin immunostaining in *cdaA1 Tetrahymena thermophila*. **1-3** - cells grown at permissive temperature; **4-9** - cells incubated 2 h at the restrictive temperature. **1** - a nondividing cells with fluorescence of the collar around the oral apparatus (OA), and some fluorescence in the inner part of the oral pouch and of basal body domains of ciliary rows; **2** - the early dividing cell with the morphostatic pattern of the parental oral apparatus (OA1) and some apical fluorescence (arrowhead) and with a "negative pattern of tetrahymenium" of the developing OA2; **3** - an advanced dividing cell prior to cytokinesis with decreasing fluorescence. The fluorescence diminishes inside the oral apparatuses (OA1 and OA2) and outlines the collars. The apical fluorescence (out of focus) and strong belt of fluorescence posterior to the fission line are marked by arrowheads; **4** - an unusual pattern of a nearly normal-looking cell with the outlines of two apparatuses OA1 and OA2 and with incomplete belt of fluorescence (arrowhead) localized posterior to the fission line (more explanation in text); **5** - a *cdaA1* phenotype with a parental oral apparatus (OA1) and two developing oral apparatuses (OA2 and OA3) at different stages of development. The enlarged OA2 is at stage of a "negative pattern of tetrahymenium" and with apical fluorescence (out of focus, arrowhead); **6** - another cell with three oral apparatuses (OA1, OA2 and OA3) without oral internal fluorescence. The cell is focused on the apical fluorescence (arrowhead). Some ciliary rows show a fission zone discontinuities; **7** - a cell from the same sample showing three oral apparatuses (OA1, OA2 and OA3) at the same stage of a "negative pattern of tetrahymenium"; **8** - a cell with four outlined oral apparatuses (OA1, OA2, OA3 and OA4) of different sizes with some looped ciliary rows. **9** - a specimen with the four oral apparatuses of two generations; the OA1 and OA2 at a very advanced morphogenesis with diminishing internal fluorescence, OA4 at a stage of a "negative pattern of tetrahymenium" and OA3 at early primordial stage. All cells at the same magnification as indicated by the bar 10 μm in Fig.1.




**Figs 10-15.** Cortical patterns of phosphoproteins immunostained with the MPM2 antibody in *cdaA1 Tetrahymena thermophila.* Cells kept at permissive temperature. **10 -** a morphostatic pattern of a *cdaA1* cell with a weak fluorescence of the oral apparatus (OA) except an intense fluorescence within the bottom of the oral pouch co-localized with a deep fiber (DF), and in the basal body domain in meridional ciliary rows; **11** - an early dividing cell with a weak fluorescence of parental oral apparatus (OA1) showing phosphorylated deep fiber (DF) with a strong fluorescence of the developing oral primordiun (OA2) and of middle segments of meridional ciliary rows; **12 -** a cell in more advanced cell division than Fig. 11. The parental oral apparatus (OA1) gains fluorescence of membranelles whereas a deep fiber is absent, the new oral apparatus (OA2) shows maximal strong fluorescence of a whole oral region; **13 -** a cell in advanced cell division showing strong fluorescence of patterns of oral membranelles of both oral apparatuses (OA1 and OA2), however lacking fluorescence of a matrix of oral regions; **14 -** a cell at beginning of cytokinesis with diminishing fluorescence of membranelles of both oral apparatuses (OA1 and OA2); **15 -** an early post-dividing cell with remnant of apical fluorescence and weak fluorescence of oral apparatus (OA1) except the re-appearing deep fiber (DF). Some fluorescence of a macronucleus (MA) is observed in early post-dividing cells. Figs 1-9 are printed at the same magnification as in and indicated by the bar 10 μm on Fig.10.

dividing in permissive temperature (Fig. 16 *vs*. Fig. 14), with two strongly fluorescent oral apparatuses (Fig. 16, OA1 and OA2) and with a more or less meridional disposition of ciliary rows of strongly fluorescent basal bodies. Nevertheless, some cells show an increase of a total dimensions, irregularities in pattern of ciliary meridians and the abnormally intensive immunostaining of both oral regions (Figs 17-20, OA1 and OA2). In the same sample, some specimens with the very bright both oral regions (Figs 18, 19; OA1 and OA2) are supplemented

with the new oral primordium. Two localizations of these primordia are discerned, either the OA3 primordium appears localized between OA1 and OA2 (Fig. 18, OA3), or in rare cases, the OA4 primordium appears at the rear end of the cell (Fig. 19, OA4). Some specimens yield three oral apparatuses of the same dimensions and brightness that seems to be equivalent (Fig. 20, OA1, OA2 and OA3). Finally, some specimens shows four oral apparatuses of different sizes and locations (Fig. 21) apparently representing two rounds of stomatogenesis; the enlarged OA1 and OA2 are accompanied with the minor ones OA3 and OA4 (Fig. 21, OA1, OA2, OA3 and OA4). In all these phenotypes all oral apparatuses kept intense fluorescence. Very few cells (except of 2% of morphostatic cells) with two or more oral apparatuses showed submaximal fluorescence of the oral regions.

In the sample taken from cells incubated 4 h, the fraction of unmodified cells with the pattern presented in Fig. 10 concerns about 2-4% and most of the specimens correspond to cortical patterns of fluorescence presented in Figs 17-21. In these phenotypes all oral apparatuses kept intense fluorescence. Apparently, the depletion of the MPM2-tagged phosphoproteins in the oral membranelles that usually occurs prior to cytokinesis is inhibited in practically all *cdaA1* cells.

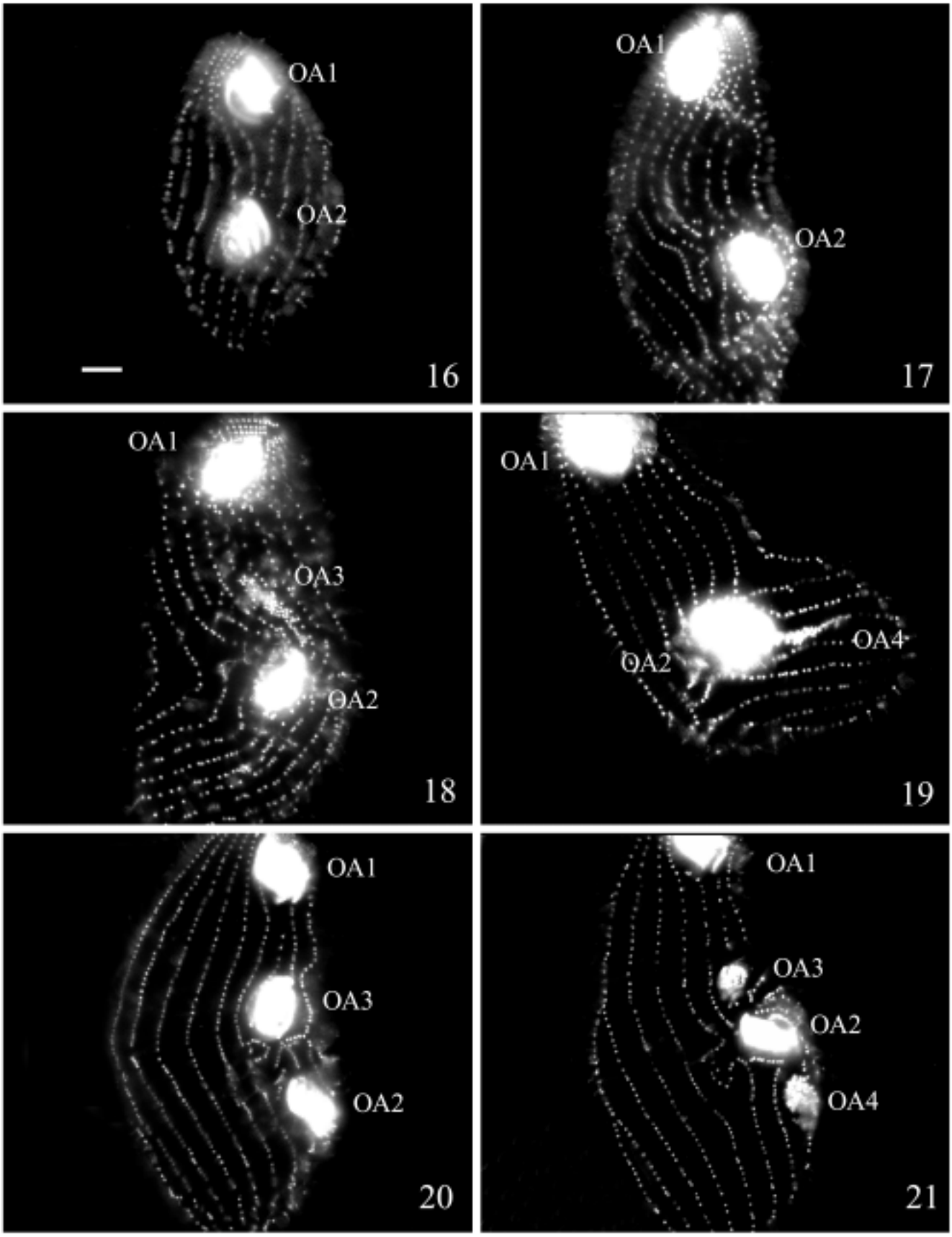
### The amounts of the fenestrin in cytoskeletal fraction of *cdaA1* cells kept at permissive temperature and at restrictive temperature during sequent hours of incubation

The Western blots for the presence of fenestrin antigen in the cytoskeletal fraction of the *cdaA1* cells were performed in three independent experiments according to Material and Methods. In these experiments, the growing cell cultures were shifted to a restrictive temperature and cell samples were collected after 2.5, 4, and 7 h of incubation and from a control culture that was not shifted to 36°C as well as a sample of wild type conjugating cells (see Materials and Methods). The results are presented in Fig. 22. Nearly the same amounts of the fenestrin protein are found in cells either from the culture kept at permissive temperature (first lane, 0 h of incubation), or after 2.5, 4 and 7 h of incubation. Apparently, the major fraction of non-dividing well fed control cells (first lane) and these that entered into defective divisional morphogenesis (second lane) demonstrated an amount of fenestrin protein slightly lower than the amounts found in other lanes. This means that from 2.5 h of incubation roughly the same amounts of the fenestrin are detected in all samples and they roughly correspond to amount of the fenestrin found in starved wild type conjugating for 4.5 h. It follows from the above that absence of the fenestrin within the putative fission zones of *cdaA1* phenotypes is not due to a decreased amounts of this protein in the cytoskeletal fraction.

## DISCUSSION

In *cdaA1* cells kept at permissive temperature, as in *wild type* cells, the co-ordinated spatial cortical patterns of cell growth and stages of divisional morphogenesis were correlated with changes in patterns of immunofluorescence with both antibodies (Kaczanowska *et al*. 1999, 2003). In early dividing cells, the MPM-2 and fenestrin antigens appeared in the OA2 primordium and next in developing membranelles of the newly formed OA2. Prior to cytokinesis, this staining of membranelles disappeared, replaced by formation of a ”negative patterns of membranelles” of both parental OA1 and in newly formed OA2 followed by a nearly total disappearance (except of weak oral outlines and of the developing inner pouch) of fluorescence in OAs of daughter cells. Additionally, prior to cell division the fenestrin antigen appeared around the proliferating basal body domains of meridional ciliary rows, and as fluorescence of the anterior cortex pole and of the newly produced anterior pole of the posterior daughter cell. Dual metameric cortical patterns for prospective daughter cells are transiently marked by the fenestrin immunostaining of the apical cortex and of the belt of cortex localized posterior to the fission line. This labeling disappears during cytokinesis. The MPM2 phosphoproteins associated with *Tetrahymena* oral apparatuses during morphogenesis disappear prior to cytokinesis, and this labeling only partly re-appears in daughter cells reduced to a site co-localized to a developing deep fiber.

At a restrictive temperature, the *cdaA1* cells were arrested in fission line formation. Failure of the arrest of cortical growth in this equatorial region results in irregular lengthening and looping of meridional ciliary rows, and in consecutive attempts to develop a new oral apparatuses. In these *cdaA1* phenotypes, the fenestrin marker is found on the anterior pole of cells indicating that they have reached an advanced stage of divisional morphogenesis, with residual or no labeling in the fission zone. The results of Western blotting show that the amount of fenestrin in cortical residues do not decrease


OA1
OA2
16
OA1
OA2
17
OA1
OA3
OA2
18
OA1
OA4
OA2
19
OA1
OA3
OA2
20
OA1
OA3
OA2
OA4
21

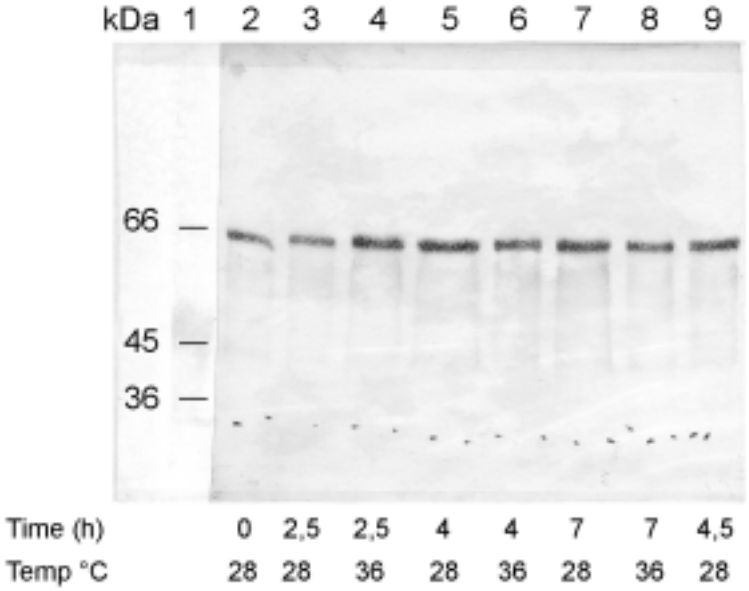



**Fig. 22.** A Western blot of electrophoresed proteins of cytoskeletal cortices of *cdaA1* cells marked with the anti-fenestrin antibody. The 1st lane shows the distribution of the commercial cortical markers (Sigma) of proteins of 36, 45 and 66 kDa. The 2nd lane detects the fenestrin from cells grown at permissive temperature,3rd and 4th lanes- from the cells incubated 2.5 h, 5th and 6th lanes-from the cells incubated 4 h, and 7th and 8th lanes from the cells incubated 7 h at permissive and restrictive temperatures, respectively. The 9th lane - conjugants (CU427 × CU428) after 4.5 h from mixing of mates.

in these cells. Thus the failure of cytokinesis and of immunostaining of the putative fission zone are not correlated with a decrease of the amount of cortical fenestrin. On the other hand, the fenestrin antigen appears at the anterior pole in cells in different stages of oral morphogenesis and with a variable number of oral apparatuses suggesting that *cdaA1* cells do not turn off their morphogenetic activity while they undergo successive rounds of cortical development (Kaczanowska 1990). This is consistent with data about other activities, such as repetitive micronuclear mitoses and DNA replication in macronucleus (Frankel *et al*. 1976, Cleffmann and Frankel 1978) and progressing morphological anomalies (Frankel *et al*. 1977) which may reflect the sustaining of a metastable phase in *cdaA1* cells. In support of this interpretation, the programmed depletion of the MPM2-tagged phosphoproteins found in oral apparatuses of wild type cells during cytokinesis was arrested in practically all *cdaA1* cells irrespective to the advancement of oral morphogenesis and of the internal oral patterns of the anti-fenestrin immunostaining, or of the duration of incubation at restrictive temperature. The defined and short-lasting temperature-sensitive period in the cell cycle of *cdaA1* cells for expression of abnormal phenotypes (Frankel *et al*. 1980) is taken as evidence that *cdaA1* mutation concerns fundamental failure of some transition(s) in progress of the cell cycle of *Tetrahymena*. Thus an absence of dephosphorylation of phosphoproteins within the oral apparatuses and the retention of apical fenestrin immunostaining in *cdaA1* phenotypes

**Figs 16-21.** *cdaA1* cells incubated 2 h at restrictive temperature. **16** - a normally looking cell with heavily stained membranelles of both oral apparatuses (OA1 and OA2); **17** - a specimen with a slight disorder in polarity of ciliary rows, and with some apical fluorescence, showing maximal, heavy fluorescence of whole oral regions of both oral apparatuses (OA1 and OA2); **18** - a specimen with the same pattern of fluorescence as shown in oral regions (OA1 and OA2) in Fig. 17 but with an additional bright primordium of next oral apparatus (OA3); **19** - a specimen with the same pattern of fluorescence as shown in oral regions (OA1 and OA2) in Figs 17 and 18 with an additional primordium of posterior oral apparatus (OA4); **20** - a cell with the three heavily labeled oral apparatuses (OA1, OA2 and OA3) with one interrupted ciliary row and with some folding of ciliary rows in area between OA3 and OA2; **21** - an enlarged undivided cell with four strongly labeled oral apparatuses of two generations (OA1 and OA2 of the first generation and OA3 and OA4 of the second generation) With some ciliary rows looped towards the oral apparatus (OA2). Figs 1-15 are printed at the same magnification as in with the bar 10 μm shown on Fig. 16.

are correlated with a failure in cytokinesis and an arrest in the transition from morphogenetic activities of dividing cell to morphostasis of divided daughter cells. Apparently, the mitotic exit network in *Tetrahymena thermophila* wild type and *cdaA1* concerns both dephosphorylating and phosphorylating events (Buzanska and Wheatley 1994).

In budding yeast, the mechanisms of transition from the metastable state of a budding cell into the quiescent state of the daughter cells by the Amn1 inhibitor was recently identified (Wang *et al*. 2003). An important step in this pathway is the release and activation of Cdc 14 serine phosphatase which inhibits Cdk1 activity (Visintin *et al*. 1998). These findings raise the question whether a mechanism corresponding to the mechanism of the mitotic exit network (MEN) of yeast, with activation of some dephosphorylation and of Amn 1 inhibitor, is present in *T. thermophila* and is affected by the *cdaA1* mutation, and whether in particular this exit depends upon the localization of protein p85 in the fission line of the *wt* cells that does not appear in equatorial regions of the *cdaA1* cells (Gonda *et al*. 1999a). The p85 is a protein partly homologous to a cdc2 kinase (Gonda *et al*. 1999b) which is sensitive to $Ca^{++}$/CaM signaling pathway and has the same aminoacid sequence in *wt* and *cdaA1* phenotypes (Gonda *et al*. 1999a, b; Numata and Gonda 2001). Gonda *et al*. (1999a) found a slight difference of molecular weights between p85 in the *wt* and *cdaA1* phenotypes, which is due to some unknown posttranslational modification pertinent to its proper activity at the end of the cell cycle. Recently, as many as three cyclin-dependent kinases were found in *T. thermophila,* but the regulation of their activities in the cell cycle remains unknown (Zhang *et al*. 2002).

**Acknowledgements.** The authors thank Dr Joseph Frankel (Iowa Univ., U.S.A.) for supplying *cdaA1 Tetrahymena thermophila.* We are very thankful to Drs J. Frankel, N.E. Williams, M Nelsen and P. Rao for generous gifts of antibodies used in this study. Assistance and technical aid in preparation of western blot by Dr Dorota Wloga is also acknowledged. We are very grateful for technical assistance of MSc Marzena Kwiatkowska-Dąbrowska. This work was supported by the grant from Polish Science Committee No 6PO4C 012 19 to Dr Andrzej Kaczanowski.

## REFERENCES

Ayscough K. R., Stryker J., Pokala N., Sanders M., Crews P., Drubin D. G. (1997) High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. *J. Cell Biol*. **137:** 399-416

Buzanska L., Wheatley D. N. (1994) Okadaic acid promotes cell division in synchronized *Tetrahymena pyriformis* and in the cell division-arrested (*cdaA1*) temperature sensitive mutant of *T. thermophila*. *Eur. J. Cell Biol*. **63:** 149-158

Buzanska L., Gregory D. W., Wheatley D. N. (1989) Protrusion formation in the cell division-arrested mutant *Tetrahymena thermophila cdaA1*, and the elucidation of some rules governing cytoskeletal growth. *J. exp. Zool*. **251:** 27-36

Casamayor A., Snyder M. (2002) Bud-site selection and cell polarity in budding yeast. *Curr. Opin. Microbiol.* **5:** 179-186

Cleffmann G., Frankel J. (1978) The DNA replication schedule is not affected in a division blocked mutant of *Tetrahymena thermophila. Exp. Cell Res.* **117:** 191-194

Davies F. M., Tsao T. Y., Fowler S. K., Rao P. N. (1983) Monoclonal antibodies to mitotic cells. *Proc. Natl. Acad. Sci. USA* **80:** 2926-2930

Ding M., Feng Y., Vandre D. D. (1997) Partial characterization of the MPM-2 phosphoepitope. *Exp. Cell Res*. **231:** 3-13

Finger E. P., Hughes T. E., Novick P. (1998) Sec3p is a spatial landmark for polarized secretion in budding yeast. *Cell* **92:** 559-571

Frankel J., Jenkins L. M., DeBault L. E. (1976) Causal relations among cell cycle processes in *Tetrahymena pyriformis*. An analysis employing temperature-sensitive mutants. *J. Cell Biol*. **71:** 242-260

Frankel J., Nelsen E. M., Jenkins I. M.. (1977) Mutations affecting cell division in *Tetrahymena pyriformis Syngen I*. II. Phenotypes of single and double homozygotes. *Devel. Biol*. **58:** 255-275

Frankel J., Mohler J., Frankel A. W. K. (1980) Temperature - sensitive periods of mutations affecting cell division in *Tetrahymena thermophila*. *J. Cell Sci*. **43:** 75-91

Frankel J., Nelsen E. M., Martel E. (1981) Development of a ciliature of *Tetrahymena thermophila* II. Spatial subdivision prior to cytokinesis. *Devel. Biol*. **88:** 349-354

Gonda K., Nishibori K., Ohba H., Watanabe A., Numata O. (1999a) Molecular cloning of the gene for 85 that regulates the initiation of cytokinesis in *Tetrahymena. Biophys. Biochem. Res. Com.* **264:** 1112-118

Gonda K., Katoh M., Hanyu K., Watanabe A., Numata O. (1999b) $Ca^{++}$/calmodulin and p85 cooperatively regulate the initiation of cytokinesis in *Tetrahymena*. *J. Cell Sci*. **112:** 3619-3626

Honts J., Williams N. E. (2003) Novel cytoskeletal proteins in the cortex of *Tetrahymena*. *J. Eukaryot. Microbiol.* **50:** 9-14

Kaczanowska J. (1990) Integration of oral structures in *cdaA1* mutant of *Tetrahymena thermophila*. *Acta Protozool*. **29:** 275-290

Kaczanowska J., Buzanska L., Frontczak M. (1992) The influence of fission line expression on the number and positioning of oral primordia in the *cdaA1* mutant of *Tetrahymena thermophila*. *Devel. Genetics* **13:** 216-222

Kaczanowska J., Buzanska L., Ostrowski M. (1993) Relationship between spatial patterning of basal bodies and membrane cytoskeleton (epiplasm) during the cell cycle of: *cdaA* mutant and anti-membrane immunostaining. *J. Eukaryot. Microbiol*. **40:** 747-754

Kaczanowska J., Joachimiak E., Buzanska L., Krawczynska W., Wheatley D. N, Kaczanowski A. (1999) Molecular subdivision of the cortex of dividing *Tetrahymena* is coupled with the formation of the fission zone. *Devel. Biol*. **212:** 150-164

Kaczanowska J., Joachimiak E., Kiersnowska M., Krzywicka A., Golinska K., Kaczanowski A. (2003) The fenestrin antigen in submembrane skeleton of the ciliate *Tetrahymena thermophila* is proposed as a marker of cell polarity during cell division and in oral replacement. *Protist* **154:** 251-264

Kaczanowski A. (1978) Gradients of proliferation of ciliary basal bodies and the determination of the oral primordium in *Tetrahymena*. *J. exp. Zool*. **204:** 417-430

Kiersnowska M., Golinska K. (1996) Pattern of phosphorylated structures in the morphostatic ciliate *Tetrahymena thermophila*: MPM-2 immunogold labeling. *Acta Protozool*. **35:** 297-308

Nelsen F. M., Frankel J., Martel E. (1981) Development of the ciliature of *Tetrahymena thermophila*.I. Temporal co-ordination with oral development. *Devel. Biol*. **88:** 27-38

Nelsen E. M., Williams N. E., Hong Y., Knaak J., Frankel J. (1994) „Fenestrin" and conjugation in *Tetrahymena thermophila*. *J. Eukaryot. Microbiol*. **41:** 483-495

Ng S. F., Frankel J. (1977) 180° rotation of ciliary rows and its morphogenetic implications in *Tetrahymena pyriformis*. *Proc. Natl. Acad. Sci. USA* **74:** 1115-1119

Niccoli T., Nurse P. (2002) Different mechanisms of cell polarization in vegetative and shmooing growth in fission yeast. *J. Cell Sci*. **115:** 1651-1662

Numata O., Gonda K. (2001) Determination of division plane and organization of contactile ring in *Tetrahymena. Cell Struct. Func.* **26:** 591-601

Schliwa M., Van Blerkom J. (1981) Structural interaction of cytoskeletal components. *J. Cell Biol.* **90:** 222-235

Visintin R., Craig K., Hwang E.S., Prinz S., Tyers M., Amon A. (1998) The phosphatase Cdc 14 trigers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol. Cell* **2:** 709-718

Wang Y., Shirogane T., Liu D., Harper J.W., Elledge S. J. (2003) Exit from exit: resetting the cell cycle through AMN1 inhibition of G protein signaling. *Cell* **112:** 697-709

Westendorf J. M., Rao P. N., Gerace L. (1994) Clonning of cDNA for M-phase phosphoproteins recognized by the MPM2 monoclonal antibody and determination of the phosphorylated epitope. *Proc. Natl. Acad. Sci. USA* **91:** 714-718

Williams N. E., Honts J. E., Jaeckel-Williams R. F. (1987) Regional differentiation of the membrane in *Tetrahymena. J. Cell Sci*. **87:** 457-463

Williams N. E., Honts J. E, Kaczanowska J. (1990) The formation of basal body domains in the membrane skeleton of *Tetrahymena*. *Development* **109:** 935-942

Williams N. E., Honts J. E., Dress V. M, Nelsen E. M., Frankel J. (1995) Monoclonal antibodies reveal complex structure in the membrane skeleton of *Tetrahymena. J. Eukaryot. Microbiol*. **42:** 422-427

Zhang H., Huang X., Tang L., Zhang Q-J, Frankel J., Berger J. D. (2002). A cyclin dependent protein kinase homologue asssociated with the basal body domains in the ciliate. *Biochim. Biophys. Acta* **1591:** 119-128

Received on 21st January, 2004; revised version on 28th May, 2004; accepted on 3rd June, 2004