

Acquisition of cell polarity during cell cycle and oral replacement in *Tetrahymena*

JANINA KACZANOWSKA*, SZYMON KACZANOWSKI¹, MAURYLA KIERSNOWSKA, HANNA FABCZAK²,
KAROLINA TULODZIECKA and ANDRZEJ KACZANOWSKI

Department of Cytophysiology, Institute of Zoology, Warsaw University, ¹Institute of Biophysics and Biochemistry P A N and
²Nencki Institute of Experimental Biology P A N, Warsaw, Poland

ABSTRACT The aim of this study was to search for a mechanism responsible for the acquisition of cell polarity in a ciliate *Tetrahymena*. Homologs of the mammalian genes coding for CDC42-GSK3 β - MARK/PAR1-MAPs proteins were found in the *Tetrahymena* genome (Eisen *et al.*, 2006, and this study). These proteins belong to a pathway which controls assembly and disassembly of microtubule bundles and cell polarity in neural cells. In *Tetrahymena*, there are two types of morphogenesis: divisional and oral replacement (OR). In divisional morphogenesis, an elongation of longitudinal microtubule bundles (LMs) takes place during cell division. In contrast, in OR type morphogenesis, which occurs in starved non-dividing cells, a polar retraction of LMs occurs. In *T. pyriformis*, the frequency of developmental switch to OR morphogenesis increases in the presence of wortmannin, an inhibitor of the CDC42-GSK3 β -MARK pathway. In contrast, wortmannin when applied to dividing cells does not affect divisional morphogenesis. Using immunostaining with the antibody against mammalian mitotic phosphoproteins (MPM-2) we show that these proteins co-localize with the LMs and are distributed along the anterior-posterior gradient. In addition, we show that during OR type morphogenesis, the fate of LMs correlates with the anterior-posterior gradient of instability of the cortical structures. We used the conditional mouthless mutant of *T. thermophila* (Tiedtke *et al.*, 1988) to test if the presence of the oral apparatus is required for the maintenance of cell polarity. We discuss our results in relation to the hypothesis of GSK3 β -MARK pathway involvement in the acquisition of cell polarity in *Tetrahymena*.

KEY WORDS: *Tetrahymena*, polarity, cytoskeleton, MARK, armadillo

The *Tetrahymena* (like other ciliates) is a permanently polarized cell. Longitudinal ciliary rows (CR) define its anterior-posterior axis and a sub-apically located oral apparatus (OA) marks its ventral side. The position of other cortical structures depends on the position of the oral apparatus (Frankel 1999) (Fig. 1A and Fig. 7A). In *Tetrahymena* the spatial deployment of the cytoskeletal proteins is under genetic control of a mechanism of global positioning (Kaczanowski 1975, Williams *et al.*, 1987, Jerka-Dziadosz *et al.*, 1995, Kaczanowska *et al.*, 2003, Strzyzewska-Jowko *et al.*, 2003). The aim of the present study was to gain an insight into the mechanism responsible for the acquisition and maintenance of cell polarity, during morphogenesis of *Tetrahymena*.

In *Tetrahymena* there are two types of morphogenesis: divisional and oral replacement (OR). The cortex of *Tetrahymena* contains a regular array of polarized longitudinal microtubular

bands (LMs) running next to meridians of the rows of basal bodies. The assembly of LM-bands is unidirectional (Allen 1967; Ng 1978, 1979; Ng and Frankel, 1977) and they play an important role in divisional morphogenesis as a putative cytoskeleton (Jerka-Dziadosz *et al.*, 1995). The anterior-posterior axis is preserved during cell division that results in anterior and posterior offspring (Frankel *et al.*, 1981; Frankel 1989; Kaczanowska *et al.*, 1993;

Abbreviations used in this paper: AF, anarchic field of oral primordium; CR, ciliary rows of basal body units; CVP, the contractile vacuole pore located at the posterior area of the cell to the right of the ventral side; EHT, end of heat treatment; LM, Longitudinal microtubule bands; nOA, newly formed oral apparatus that appears during oral replacement morphogenesis; OA, oral apparatus; OA1, parental oral apparatus; OA2, new oral apparatus for posterior daughter cell; OR, oral replacement; RM, remnant of engulfed and regressed anterior oral apparatus.

*Address correspondence to: Janina Kaczanowska. Dept. of Cytophysiology, Institute of Zoology, Warsaw University, Warsaw, Poland.
e-mail: kaczan@biol.uw.edu.pl

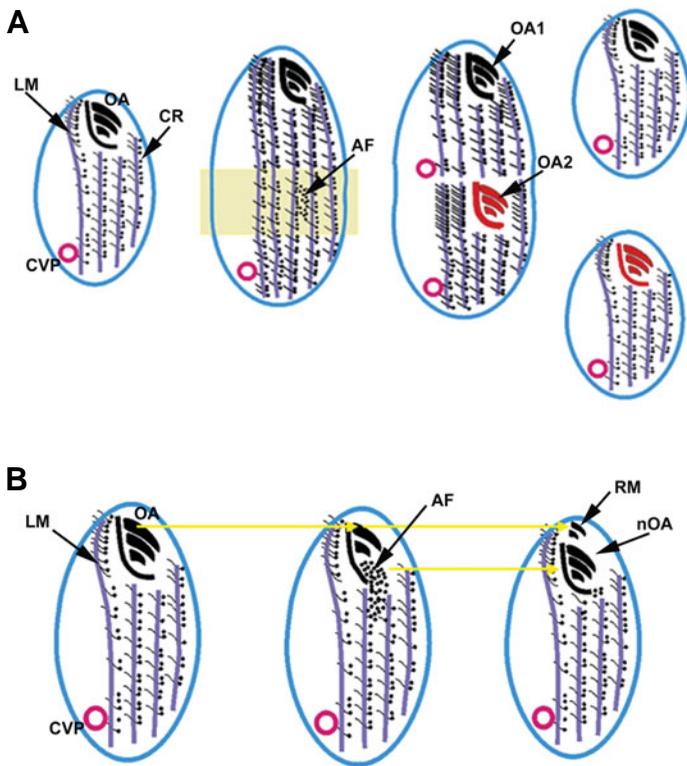


Fig. 1. Diagrams of the two types of morphogenesis in *Tetrahymena*. (A) Cortical patterns during segmental divisional morphogenesis. (B) Cortical patterns during oral replacement (OR) morphogenesis.

Nelsen *et al.*, 1994) (Fig. 1A). Prior to cytokinesis, the LM-bands maintain their continuity until the fission line separates them. Eventually, after the cytokinesis, the apical band reforms (Jerka-Dziadosz *et al.*, 2001; Thazhath *et al.*, 2002; 2004). Thus the parental cell undergoes continuous unidirectional elongation in the middle region of the parental cell with the local intercalation of basal bodies and the *in situ* addition of microtubular subunits into the LM-bands; the process which is consistent with Frankel's (1989) model of "the continuum growth cylinder". During early divisional morphogenesis the new unciliated basal bodies are added into ciliary rows. There are two "gradients" of this addition process: the anterior-posterior (Nanney 1975; Kaczanowski, 1978; Frankel *et al.*, 1981) and the circumferential gradient (Kaczanowski, 1978). In the nascent posterior daughter cell, the new oral primordium appears to the left of the postoral ciliary row #1 as an "anarchic field of basal bodies" (AF) (Fig. 1A). Its position corresponds to the site of the intersection of both gradients of the proliferation of the basal bodies (Kaczanowski, 1978). This anarchic field develops into the oral apparatus, which, at its anterior, contains fission line that runs perpendicularly across all ciliary rows (Frankel *et al.*, 1981; Nelsen *et al.*, 1994; Kaczanowska *et al.*, 1999; Strzyzewska-Jowko *et al.*, 2003) (Fig. 1A). This segmental pattern of cortex development is synchronized with a bipolar micronuclear mitosis in *T. thermophila* and with a bipolar macronuclear "amitosis" in both *T. thermophila* and *T. pyriformis* (reviewed by Frankel, 1999).

In *Tetrahymena*, the submembrane skeleton containing at least three epiplasmic proteins EpiA, EpiB and EpiC (Williams *et*

al., 1995) and at least two Ca^{2+} binding proteins, (Hanyu *et al.*, 1995; 1996) forms the continuous (with the exception of areas occupied by the microtubule composed structures) cortical shell (Williams *et al.*, 1990). Full knockout of the *EPC1* gene influences both cell shape and the fidelity of cortical development (Williams, 2004). Epi B protein is absent in the areas occupied by the oral apparatus, the LM-s, the all basal bodies and the contractile vacuole pores (Williams *et al.*, 1990; Williams *et al.*, 1995) (Fig. 2A). Thus in cells immunostained with the anti-EpiB antibody the spatial deployment of cortical microtubules is visible as the unstained "negative" pattern. Prior to cytokinesis, the decrease in the level of Epi B protein follows two segmental gradients in the shell of the apical regions of the parental cell and in the corresponding region of the opisthe (Fig. 2B; Kaczanowska *et al.*, 1993). In contrast, the "fenestrin protein", the other member of the membrane skeleton transiently appears in apical areas of both nascent offsprings (Nelsen *et al.*, 1994; Kaczanowska *et al.*, 2003).

Another type of morphogenesis, called the oral replacement (OR) occurs without cell division within a single *Tetrahymena* cell. In starved *Tetrahymena* the oral replacement occurs through a gradual regression and engulfment of the pre-existing oral apparatus at the apical area with the simultaneous morphogenesis of a new oral apparatus just posterior to the old one (Frankel, 1967; 1969; 1970; Williams and Frankel, 1973; Williams and Nelsen, 1973; Kaczanowski, 1976) (Fig. 1B, nOA). This new pole resumes the immunoreactivity with the apical pole marker (fenestrin), which coincides with the patterned polar withdrawal of Epi B immunoreactivity (Kaczanowska *et al.*, 2003) (Fig. 2C). During oral replacement of the starved *Tetrahymena* there is exchange and flux of stable subunits of microtubules, which is controlled by newly synthesized MAPs (Williams and Nelsen, 1973). The two types of *Tetrahymena* morphogenesis are summarized in Fig. 1 A, B and Fig. 2 A-C.

In this study, we asked whether the re-formation of an anterior-posterior axis and the ventral positioning of the oral apparatus occurring during *Tetrahymena* morphogenesis are controlled by CDC42- GSK3 β -MARK pathway regulated by a nutritional signal. It is known that this pathway is involved in the generation of cell polarity in metazoan neural cells (Doble and Woodgett, 2003) and non-neural cells (Etienne-Manneville and Hall, 2003). In differentiating neural cells, the effector mechanism for the establishment of cell polarity involves the cascade of activities of specific kinases. The GSK3 β (glycogen synthase kinase 3), and MARK kinase (Microtubule Affinity Regulating Kinase) regulate the lengthening and linear assembly/ disassembly of the bundles of microtubular subunits during the neuritogenesis through specific phosphorylation of a set of microtubule-associated proteins (MAPs) (Drewes *et al.*, 1997; Johnson and Stoothoff, 2004; Wiggan *et al.*, 2005). The GSK3 β kinase activates MARK/ PAR1 effector kinase by a structurally directed phosphorylation (Kosuga *et al.*, 2005) and then MARK/PAR1 destabilizes MAPs. This pathway is controlled by CDC42 complex (Cau and Hall, 2005) involved in posterior localization of MARK/PAR1 kinase (Schonegg and Hyman, 2006). Hence, the primary task in *Tetrahymena* genome research is the search for the putative genes encoding the CDC42- GSK3 β -MARK- MAP pathway. If these genes do really exist in *Tetrahymena* genome, the putative CDC42, GSK3 β (ser9) and MARK/PAR1 proteins should be identifiable on Western blots

of homogenized *Tetrahymena* cells with the available commercial antibodies against cognate metazoan proteins.

The next question concerns the character of the upstream components of this signaling pathway. During the neural differentiation of metazoan cells the anterior-posterior gradient of activity of GSK3 β renders the stabilized posterior pole and a highly active and structurally unstable, growing anterior cone of the neurite (Biernat *et al.*, 2002). This linear gradient of growth and structural stability of the neurite is induced by the upstream Wnt signaling transmitted to GSK3 β , and by the posterior location in cell of MARK/PAR1 protein (Doble and Woodgett, 2003). Therefore, we were also searching for the presence of possible orthologs of a Wnt signaling pathway in the genome of *Tetrahymena*.

The presence or absence of cell polarity during oral replacement (OR) morphogenesis in *Tetrahymena* can be tested by the use of the MPM-2 antibody against mitotic phosphoproteins. The MPM-2 monoclonal antibody binds to a plethora of phosphorylated proteins present in mammalian basal bodies and the mitotic spindle (Westendorf *et al.*, 1993). Previously we described the cortical patterns and polarity of MPM-2 immunostaining during cell cycle of *Tetrahymena* (Kaczanowska *et al.*, 1999). Our present studies were focused on the pattern of MPM-2 immunoreactivity in *Tetrahymena* cells during OR morphogenesis.

In non-neural metazoan cells, the signal upstream of PI-3-kinase drives the activation of Akt kinase regulating the CDC42-GSK3 β -MARK-pathway, which controls the polarity and asymmetry of the cytoskeleton (Ahringer 2003; Doble and Woodgett, 2003). In metazoan cells the specific inhibitor, wortmannin, inhibits activity of the PI-3K pathway and Akt kinase, hence induces abnormal activity of the GSK3 β (Kozma and Thomas, 2002). Wortmannin acts on the PI-3K (class I) pathway in *T. thermophila* and *T. pyriformis* (Leonaritis *et al.*, 2005) affecting, tentatively *via* insulin-like signaling (Christensen *et al.*, 2003), the food-vacuole formation (Zackroff and Hufnagel, 2001) and various components of the cytoskeleton (Kovacs and Pallinger, 2003). Since wortmannin arrests degradation of proteins at the initial sequestration step (Petiot *et al.*, 2000), we tested the effects of wortmannin on the divisional morphogenesis of *Tetrahymena*

pyriformis. Under conditions that increase a propensity for the induction of oral replacements, (Ryals *et al.*, 1997) we studied the effect of wortmannin on the stability of new oral structures for prospective posterior cell and the ability of the post-dividing cells to enter the oral replacement pathway.

We also focused on testing whether the structural presence of the oral apparatus is required for the establishment of anterior-posterior polarity and whether the oral apparatus is a marker for the establishment of the ventral side of cell. In these studies we have used conditional mutant I18G of *Tetrahymena thermophila* to generate an array of phenotypes with different degree of reduction of oral apparatuses and tested them for the presence of apical markers (i.e. fenestrin, and anti B epiplasm) (Nelsen *et al.*, 1994; Kaczanowska *et al.*, 2003).

We also discussed whether the results of our study are consistent with the hypothesis of the CDC42-GSK3 β -MARK pathway involvement in the generation of the cell polarity during the morphogenesis of *Tetrahymena*.

Results

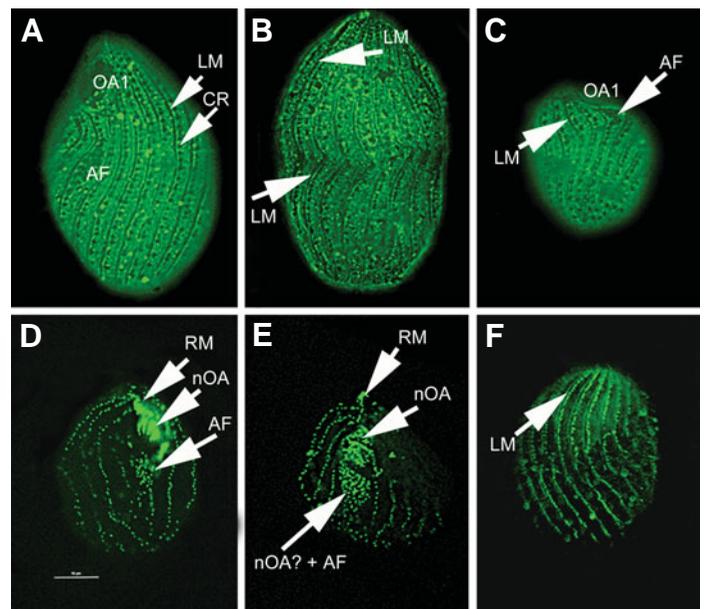
Search of *Tetrahymena* genome for the components of Cdc42-GSK3 β -MARK pathway

GSK3 β

We searched *Tetrahymena* genome for the possible homologs of the mammalian gene encoding GSK3 β protein. We used the query sequence of human GSK3 β (GSK3 β -human uniprot ID) and the tblastn computer program, provided by the www interface <http://tigrblast.tigr.org/er-blast/index.cgi?project=ttg>. We found DNA sequence Q248B3-TETH in the *Tetrahymena* genome encoding a protein homologous to a GSK3 β kinase with an extremely low expected value: 3.8e-63. Another blast search showed that all other proteins most closely related to the candidate *Tetrahymena* gene belong to the family of GSK3 β kinases present in various species of animals.

The results of blast analysis prompted us to test the antibody directed against metazoan inactivated GSK3 β kinase i.e. the

Fig. 2. *Tetrahymena* in two types of morphogenesis. (A-C) Immunostaining with the anti-B epiplasm antibody; "negative" staining is seen, i.e. the cortex remains unstained in the regions occupied by microtubular organelles. **(D-F)** Anti-MPM2 antibody immunoreactivity; positive staining of phosphoproteins associated with cortical structures of *Tetrahymena* in OR morphogenesis can be seen. **(A)** The early dividing cell with a "negative" pattern of cortical microtubules: in the OA1, AF region, basal body ciliary rows and the LM-bands. **(B)** The dorsal side of dividing cells with "negative" pattern of basal body ciliary rows and LM-bands, and with diminished immunostaining of apical area in parental cell, and in the area posterior to the fission line. **(C)** The cell in OR morphogenesis, Region of OA1 and the early anarchic field for new OA2 (AF) remain unstained; note the distinct "negative" pattern of the LM-bands. **(D)** Cell with extremely strong MPM-2 staining of the engulfed oral apparatus (RM) and with strong immunostaining of the newly formed oral apparatus (nOA) with the moderately immunostained dispersed anarchic field (AF). **(E)** Cell in OR at an advanced stage; the remnant (RM) of the engulfed oral apparatus is reduced to a strongly shining RM-pocket and some traces of the closed slit; the posterior shining field is subdivided into two entities (nOA and nOA?) along the ventral side. **(F)** Dorsal side of the cell in OR; note very intense and polarized immunostaining of LM-bands.



Therefore in agreement with previous report (Eisen *et al.*, 2006) it is unlikely that *Tetrahymena* genome is encoding MAP1B, MAP2, *tau* and MAP4 proteins.

MAPs of *Tetrahymena*

So far there are two identified MAPs that are able to bundle subunits of microtubules in experimental assay (Matui *et al.*, 2004). These are: the GAPDH protein and another protein probably corresponding to the fragment of the XMAP 215 microtubule-associated protein participating in the formation of the mitotic spindle in *Xenopus* cells. Eisen *et al.* (2006) reported in *Tetrahymena* two sequences of XMAP215: THERM_00439010 and THERM_00488190. Matui *et al.* (2004) also found another MAP in *Tetrahymena*, which has not been characterized yet.

In summary the *in silico* search of sequenced *Tetrahymena thermophila* genome (TIGR <http://www.tigr.org/tdb/e2k1/ttg/>) identified the orthologs of the GSK3 β - MARK2/PAR1/kin1 pathway. The corresponding bands were also detected in fractionated *Tetrahymena* by Western blotting with metazoan antibodies (Figs. 3,5,6).

Search of *Tetrahymena* genome for upstream components of Wnt signaling. Dishevelled and axin mediate upstream signaling cascades to regulate cell polarity in metazoa (Wallingford and Habas, 2005; Jiang *et al.*, 2005). We used as a query, the sequences for human dishevelled (uniprot ID dvl1_human) and human axin (uniprot ID axn1_human). These proteins contain smart domain dax. This is exclusively animal domain according to smart server, and therefore, as expected, we were unable to detect any homologous sequences in *Tetrahymena* genome.

β -catenin

First, we searched for the gene encoding β catenin in *Tetrahymena* genome using the tblastn computer program provided by [www.interface http://tigrblast.tigr.org/erblast/index.cgi?project=ttg](http://tigrblast.tigr.org/erblast/index.cgi?project=ttg) using the sequence of *Drosophila* β -catenin (ARM_DROME uniprot ID). We were unable to detect any homologous sequences in the *Tetrahymena* genome.

The catenin protein contains many *armadillo* repeats. Using a

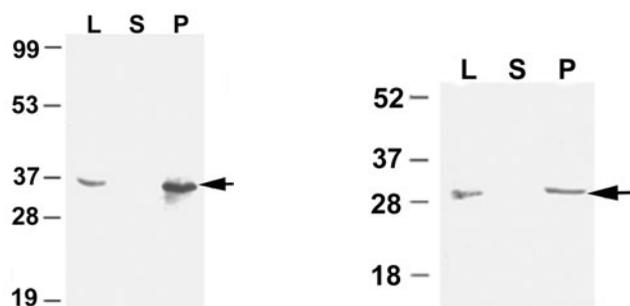


Fig. 5 (Left). Western blot of *Tetrahymena pyriformis* with polyclonal c-tak1 antibody against MARK/78kD (Upstate, Lake Placid, NY, USA) and developed by ECL. Total homogenate (L) and cytosolic (S) or particulate (mirosomal) (P) fractions; arrow points to the specific 36 kD band.

Fig. 6 (Right). Western blot of *Tetrahymena pyriformis* fractions with monoclonal antibody against cdc-42 (Santa Cruz BT, Santa Cruz, California, USA) recognizes the protein band of about 29 kD.

smart server <http://smart.embl-heidelberg.de/> we identified a putative protein in *Paramecium* containing many *armadillo* repeats (Q6BGJ0_PARTE). When we used this sequence as a query and we carried out blast search against the uniprot database we detected homology to the different importins including human α 6, (IMA5-HUMAN uniprot ID), plant MOS6 (Palma *et al.*, 2005) and 8 different importins in *Tetrahymena*.

Search for PAR6 with PAR6 α as a query. PAR6 protein is a fusion of widely distributed smart domains (PB1 and PDZ). According to smart server <http://smart.embl-heidelberg.de/> this type of fusion is present only in metazoan genes and is absent in *Tetrahymena*.

In summary, we concluded that the upstream components of a Wnt signaling pathway are not present in *T. thermophila* genome. In addition, it is likely that in *Tetrahymena*, the proteins with *armadillo* repeats may have totally different functions than β -catenin.

Morphological observations on re-polarization events in dividing cells and in cells in oral replacement

In mammalian cells the GSK3- β controls the mitotic spindle dynamics that is suppressed by such inhibitors like the lithium chloride (Wakefield *et al.*, 2003). We tested the spatial distribution of mitotic phosphoproteins (detected by MPM-2 antibody) in cells in OR. Kiersnowska and Golinska (1996) found a very weak phosphorylation of the LM-bands in the morphostatic cells in contrast to the strong staining of LM-bands in cells in OR (Fig. 2F). The area of the regressing and engulfed anterior region (Fig. 2D,E; RM) and the area of newly produced parts of the oral apparatus (Fig. 2 D, E; nOA) immunostained very strongly with the MPM-2 antibody. The posterior anarchic field of basal bodies (Fig. 2D; AF) showed lower intensity of immunostaining but stained more intensely than the basal bodies in ciliary rows at the posterior part of the cells. At the more advanced stage of oral morphogenesis (Fig. 2E) a strong fluorescence visible at the apical area corresponded to the remnant (RM) of the engulfed OA1 and the new OA (Fig. 2E; nOA). In addition, an anterior-posterior gradient of fluorescence of the abnormally organizing additional oral apparatus and fluorescence of AF were also visible (Fig. 2E; nOA?+AF). In some cells in OR, their dorsal side showed an anterior-posterior gradient of the immunostaining of the LM-bands (Fig. 2F; LM).

All these results are consistent with the hypothesis that the LM-bands and the oral structures in *Tetrahymena* undergo certain spatial and cell cycle determined changes which correlate with the anterior-posterior intensity of fluorescence of the antibody against MPM-2 phosphoproteins.

Studies on the effects of wortmannin on synchronized *Tetrahymena pyriformis*

Frankel (1967) and Frankel and Williams (1973) found that in *Tetrahymena*, the progress of the cell cycle and the acquisition of cell polarity depend on the external signals of nutrition and that the starved cells are mitotically arrested. The effects of wortmannin, the inhibitor of PI-3 kinase pathway was tested in synchronized *T. pyriformis* in constant presence of 1% PPY, the chelated Fe chloride and salts. The data are presented in Table 1. These data indicate that wortmannin added 30 min or later after the end of synchronizing treatment (EHT) does not affect the morphology,

with the exception of the enlargement of the forming OA2 (Fig. 7A,B) or the timing of synchronous cytokineses (approximately 90 min after the EHT the 25% of cells were in cytokinesis). After the cytokinesis the post-divided and rounded cells still lacked of internal part of cytopharynx, i.e. the deep fiber structures, which usually are produced 20-30 min after cytokinesis. In two types of treated samples (30-90 minutes, and 50-90 minutes), fixed at the time of synchronous cytokinesis, about 50% of cells were in the stages corresponding to the OR morphogenesis. Thus, it seems that the post-dividing cells and possibly the undivided cells (cells in synchronous rounding stage; Frankel, 1967) without internal part of cytopharynx start the oral replacement synchronously. Usually most of these cells form a single new oral replacement primordium (Fig. 7D), but some cells form a series of primordia in an anterior-posterior order with a tendency to curl the ciliary row #2 located posterior to OA1 that is reduced to a remnant engulfed pocket (Fig. 7C). Such behavior of ciliary rows #2 and rows n-1 and n-2 strongly suggest that the position of the newly formed apex become established at the posterior end of the disappearing OA1. Another type of cells was also present in wortmannin treated samples; these were mouth-less cells with an enlarged anarchic field (AF) of basal bodies marking the ventral side of the cell (Fig. 7E,F).

Analysis of the phenotypes of I18G mutant of *Tetrahymena thermophila*

We used anti-Epi B and anti-fenestrin antibodies to test polarity of I18G conditional mutant cells in different stages of morphogenesis. Overall, the I18G mutant cells kept in conditions yielding mutated phenotype were much smaller than control cells, but a subpopulation of mutant cells had normal size (Fig. 8 A,B). The mouth-less cells were descendants of dividing cells with underdeveloped OA2 (not shown) or cells with more or less advanced engulfment of the anterior oral apparatus. In I18G cells the process

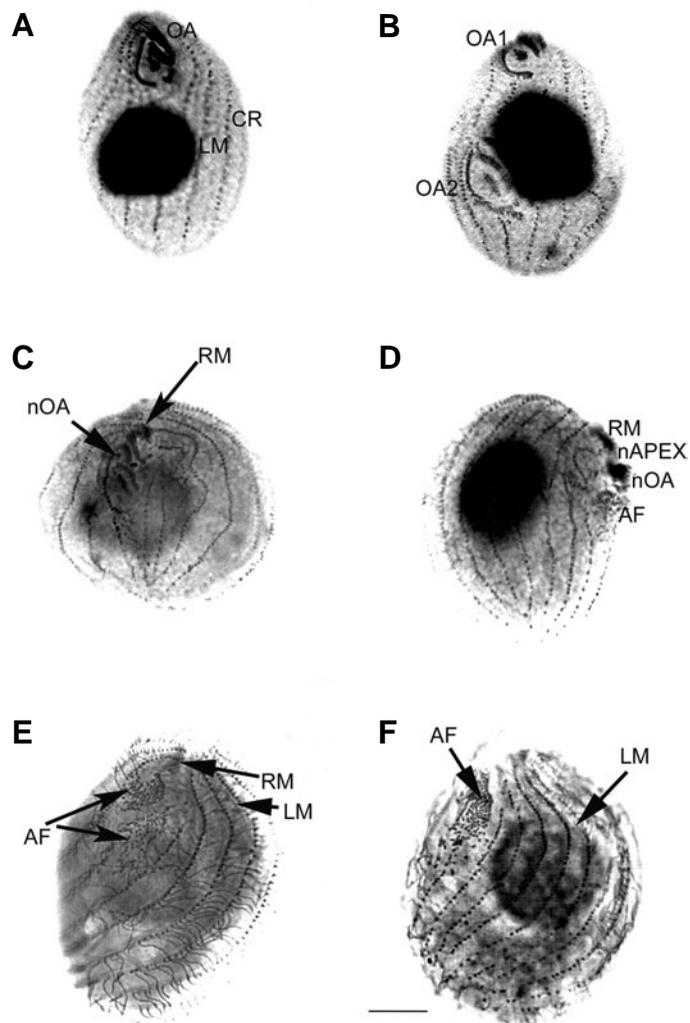


Fig. 7. Morphogenesis of synchronized *T. pyriformis* prone to enter into oral replacement morphogenesis treated with wortmannin 30 min after the end of heat treatment (EHT). Protargol stained specimens. (A) The interphase cell showing normal morphology. (B) The dividing cell fixed about 50 min after EHT showing normal morphology, except that OA2 is enlarged;(C-F) cells were fixed 120 min after EHT. (C) Cell in oral replacement morphogenesis with the engulfed regressed oral apparatus reduced to the remnant pocket (RM) and with developing new oral apparatus (nOA). (D) Cell at the same stage as shown in (C) but in side view; note: the rim of the remnant pocket, the membranellar cilia of the new oral apparatus (nOA) and the newly produced apex (nAPEX) with posterior position of the anarchic field (AF). (E) The mouth-less cell with its longitudinal microtubule (LM) bands, with the anterior residual remnant pocket (RM) and with two parts of the ventral anarchic field (AF). (F) Mouth-less cell with a single anarchic field (AF), and with two post-oral shortened ciliary rows marking ventral side of this cell.

of regression of apex and accompanying LMs and oral structures results in the formation of temporary pocket-like structure. We found that the border (slit) of this pocket is positive for the presence of EpiB (Figs. 8 C,D). After the completion of the resorption of old apex and the disappearance of the pocket-like structure, EpiB staining disappear and a new apex becomes temporarily positive for the presence of fenestrin (see below, Fig. 8H)

TABLE 1

EFFECTS OF WORTMANNIN TREATMENT ON ORAL DEVELOPMENT IN SYNCHRONIZED *T. PYRIFORMIS*

Time of treatment	Stages of development						OR	astomatous and others
	st. 0	st. 1-2	st. 3-4	st. 5	st. 6			
Control: Time after EHT								
50'	18	60	10.5	8	1	2.5	-	
90'	38	6	10	1	45	-	-	
120'	96.5		0.5	0.5	2.5	-	-	
Wortmannin added and fixed								
30-50'	45	12.5	35.5	0.5	0.5	3	3	
30-70'	53	2	2	1	16	26	-	
30-90'	16.5	0.5	2,75	1,75	21,15	56	-	
30-120'	75	0.5	2,15	-	-	22	-	
50-70'	26	11	22	1	15	25	-	
50-90'	29		18	1	-	50	2	
50-120'	43.5	7	10	-	-	38	1.5	

Effects of wortmannin treatment (2 µg/ml) on the progress of divisional morphogenesis and on the appearance of the oral replacement phenotypes in synchronized *Tetrahymena pyriformis*.

Times of addition of wortmannin and sample fixation indicate time in minutes that elapsed after EHT. Percentages of morphological stages counted in sample of about 200 well stained specimens

The results of fenestrin immunostaining of I18G cells (Fig. 8 E-H) were also instructive. Some of the I18G cells showing the apical fenestrin staining (Fig. 8E; APEX) are either in the particular stage of the resorption of anterior oral apparatus or in the early stages of the development of the posterior oral structures (Fig. 8E; OA?, nOA? and AF?). One of the cells in cytokinesis shows the anterior cell with the slightly engulfed oral apparatus with apical fluorescence signal indicating presence of fenestrin, and the posterior mouth-less cell also with a very strong apical signal for fenestrin (Fig. 8F; APEX). Fig. 8 (G,H) shows two small cells without oral apparatuses. These cells have strong signal of apical fenestrin (Fig. 8 G,H; APEX), and a very weak immunostaining of some ciliary rows at more distant positions.

All these observations indicate that the presence of the anterior oral apparatus is not required for the appearance of the fenestrin at the anterior pole, and that the engulfed slit is a marker of the ventral side of mouth-less cells.

Discussion

The first part of this study described the identification of the components of CDC 42-GSK3 β -MARK pathway in a ciliate *T. thermophila* genome and tested their relationship to the upstream Wnt signaling pathway (as observed in neural metazoan cells, reviewed by Doble and Woodgett, 2003). From the search *in silico* of the sequenced genome of *Tetrahymena thermophila* (TIGR <http://www.tigr.org/tdb/e2k1/ttg/>) we identified the orthologs of the GSK3 β -MARK2/PAR1 pathway. Some of the components of this pathway were also identified by Western blotting of fractionated *Tetrahymena*, using commercial antibodies against cdc42, mammalian GSK3 β (ser9), and mammalian MARK78 kD. The upstream components of a Wnt signaling pathway were not found in *T. thermophila* genome. On the other hand we found in *Tetrahymena* a subset of proteins with *armadillo* repeats, which may play totally different role than β -catenins.

In non-neural metazoan cells, upstream of GSK3 β , the PI-3-kinase-related signaling drives the activation of Akt kinase that controls polarity through the stabilization of structural bundling between MAPs and microtubules (Ahringer 2003; Doble and Woodgett, 2003). Accordingly, in synchronized *T. pyriformis* the addition of the inhibitor (wortmannin) to this pathway yielded the dividing cells with enlarged new oral apparatus, which were prone to enter into an oral replacement morphogenesis (Fig. 7 A-F).

We also wanted to determine whether the appearance of cell polarity during morphogenesis of *Tetrahymena* correlates with the gradient of structural stability of cortical microtubules, which might be controlled by the GSK3 β -MARK pathway. There are

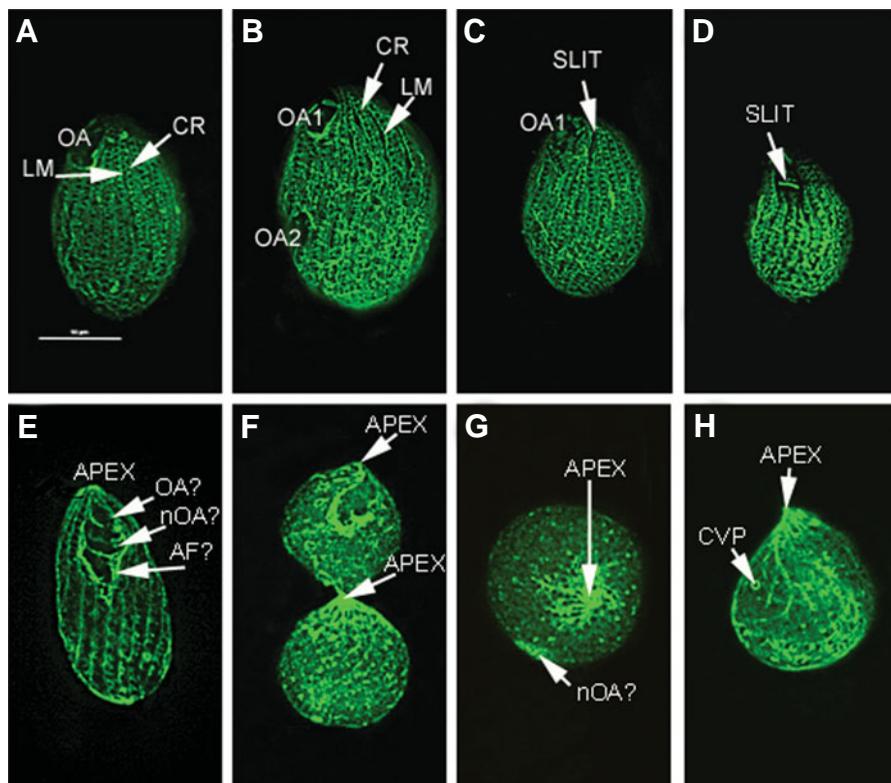


Fig. 8. The I18G phenotypes of *Tetrahymena thermophila*. (A-D) Specimens immunostained with the anti-Epi B antibody showing “negative” staining of microtubular structures. (A) The normal cortical pattern is negative at the subapical OA area, along the LM-bands, and in the basal bodies of ciliary rows (CR). (B) Cell of a normal phenotype at an early stage of divisional morphogenesis. (C) Cell at an early stage of engulfment of the oral apparatus along the slit (SLIT). The anterior pole is negative for anti-Epi B antibody. (D) Mouth-less cell at an advanced stage of engulfment of the anterior oral apparatus (shining SLIT) and with the apical region totally unstained. (E-H) I18G specimens immunostained with the anti-fenestrin antibody. (E) Cell with a shining apical pole (APEX) with a series of engulfing OA (OA?), a newly processed oral apparatus (nOA?) and with a posterior anarchic field (AF?). (F) Cell in advanced cytokinesis with the strongly shining apical areas in both daughter cells (APEX). (G) Small rounded mouth-less cell viewed from the apical pole (APEX) with putative new oral primordium (nOA?). (H) Mouth-less cell showing strong apical staining (APEX), residual diffused ventral staining and the single contractile vacuole pore (CVP).

some indirect evidences that support the hypothesis of the involvement of GSK3 β -MARK pathway in cortex stability. Jerka-Dziadosz and Frankel (1995) showed that in *Tetrahymena*, during divisional morphogenesis, the lithium chloride, the inhibitor of GSK3 β , affects the organization of opistor oral structures.

Williams and Nelsen (1973) correlated turnover of microtubule proteins with oral replacement. It is possible that deficiency of MAPs brings about decreased stability of cytoskeletal structures and increased propensity of starved cells for OR morphogenesis.

In this study we showed that the anterior-posterior difference in intensity of MPM-2 immunostaining correlates with the changes in the structural stability of microtubular organelles. At the anterior pole, both LM-bands and parts of the “old” oral apparatus were extremely unstable and underwent regression, the neighboring posterior area was also unstable, undergoing futile attempts to organize oral structures, whereas the posterior pole seemed to be normal (Fig. 2D-F). This was reminiscent of *T. thermophila* phenotypes observed in cells with the *EPC1* gene (epiplasm C

protein) gene knockout (Williams, 2004). This suggests (but does not prove) that during starvation of a wild type cells, or in mutants (mouth-less I18G and EPC1 knockout cells) the gradient of structural stability is present along the anterior-posterior axis of cells. At the anterior apical cortex, the regression and instability of microtubules and microtubule-associated structures and epiplasm are very high. The moderate structural stability is sufficient for attempts to form the new oral apparatus posterior to the apical zone. The posterior pole appears to be the most stable cortical zone. Studies with apical markers (Kaczanowska *et al.*, 2003) were consistent with the hypothesis that in the mouth-less cells (both after wortmannin treatment and in I18G phenotypes) the apical fenestrin immunostaining was independent of the presence of an oral apparatus. Nevertheless, some I18G phenotypes showed a spiraling of the LM- bands and associated ciliary rows. This indicates that the structural stability of the oral apparatus in *Tetrahymena* enforces a cylindrical pattern of cell growth.

The question arises whether this anterior-posterior spatial arrangement of regions of increasing instability of cortical structures represents the polar gradient of activation of some proteolytic activity in *Tetrahymena*? In this context MARK kinases with UBA domain (Fig. 4A) and c-TAK1 kinase which function depends on the ubiquitinating factor (Peng *et al.*, 1998; Wang *et al.*, 2001) are the first indications of a putative involvement of these kinases, or their upstream regulators in the mechanism of setting up cell polarity in *Tetrahymena* during OR. In polarized metazoan cells the MARK/ PAR1 kinase is active at early step of embryonic axis formation (La Carbona *et al.*, 2004) and is inactive at the posterior pole that is more resistant to proteolysis (Benton and St. Johnston, 2002). The GSK3 β -MARK-MAPs pathway also controls changes during mitotic cycle (Drewes and Nurse, 2003). It seems that the OR morphogenesis in starved *Tetrahymena* with anterior retraction of LMs and associated apical structure may parallel the events occurring during polar retraction of neurites in axon pruning events in neural cells (Hoopfer *et al.*, 2006).

In conclusion our data support hypothesis that GSK3 β -MARK-MAPs pathway may be involved in setting up a global cell polarity in a ciliate *Tetrahymena*.

Materials and Methods

Strain culture and synchronization

Tetrahymena thermophila CU428.1 (MPR1, ms, mt VII) were kindly provided by Dr J.Gaertig (originally derived from Cornell University, U.S.A.), and *Tetrahymena pyriformis* (GL-C) was kindly provided by Dr J.Nilsson (University of Copenhagen, Denmark). These strains were routinely cultured in 1% proteoso-peptone (Difco) and 0.1% yeast extract (1% PPY) in salt solution with FeCl₃ at 28°C (Nelsen *et al.*, 1981). Heat shock was applied to *T. pyriformis*, according to the routine procedure of Zeuthen (Williams and Scherbaum, 1959, Nelsen *et al.*, 1981). The end of the seventh shock was designated EHT (end of heat treatment) and all timing thereafter is given in minutes (Table 1).

The mutant I18G homozygous for single mutation in *Tetrahymena thermophila* (Tiedtke *et al.*, 1988) was a gift from Dr Leif Rasmussen (University of Odense, Denmark). Stock cultures were grown in proteoso-peptone-yeast extracts supplemented with salts at the permissive temperature of 28°C. They exhibited wild type phenotype. Experimental cultures were grown at 37°C (non permissive temperature) in the same medium supplemented with 1 μ g/ml folic acid and salts of Fe³⁺ and Cu²⁺. In this condition cells are able to proliferate yielding small cells with very variable phenotypes including some cells without oral apparatuses and

some cells in OR morphogenesis.

Study of the effects of wortmannin on the morphogenesis of *T. pyriformis*

These were performed according to the protocol of "synchronous rounding" (Frankel 1967, 1970; Williams and Frankel, 1973) except that *T. pyriformis* were permanently kept in 1% PPY, FeCl₃ and salt culture medium, and were given a standard seven shock heat synchronizing treatment. Control samples served to test the occurrence of the OR morphogenesis without the drug, whereas the experimental samples contained wortmannin (dissolved in DMSO) in final concentration of 2 μ g/ml. Wortmannin was added at 30min, 50min and 70 min after EHT i.e. when cells entered into divisional morphogenesis. The results of two different experiments containing cells with a high propensity for oral replacement after wortmannin treatment are shown in Table 1.

Permanent preparations

Protargol staining was performed according to routine protocol (Kaczanowska *et al.*, 1993). The preparations were used for analysis of the fractional distributions in different morphogenetic stages in accordance with accepted morphological criteria for divisional morphogenesis and for the oral replacement (Frankel, 1967; Williams and Frankel, 1973).

Immunostaining

The following antibodies were used: the MPM-2 antibody against a set of the mammalian mitotic phosphoproteins (1:200 dilution; gift from Dr P.N.Rao, Boulder, Colorado, U.S.A.), the anti B epiplasm serum (1:100 dilution; gift from Dr. N.E. Williams, Iowa University, U.S.A.) and the anti-fenestrin antibodies (9a7 against *T. thermophila* and 3A7 against *T. pyriformis*, both in dilution 1:40; (gifts from Drs J. Frankel and N. Williams, Iowa University, U.S.A.). Immunostainings was performed exactly as described earlier (Kaczanowska *et al.*, 1993; 1999; 2003).

Four commercial antibodies were also used: anti- tau5 (1:1000 dilution; Chemicon, Terneucula, California, USA), anti-GSK3 β (ser9) (1:500 dilution; Santa Cruz BT, Santa Cruz California, USA), anti-C-TAK1 (1:500 dilution; Upstate, Lake Placid, NY, USA) and anti-cdc42 (1:1000 dilution; Santa Cruz BT, Santa Cruz, California, USA). Storage and handling of antibodies were done according to donators' or manufacturers' protocols.

Cell fractionation

Cells were fractionated according to the method of Bollag *et al.* (1996). In short, cells were harvested by centrifugation at 1 000 rpm for 2 min at room temperature. Pellets were washed twice in a Tris medium containing 10 mM Tris (pH 7,3), 150 mM NaCl and 250 mM sucrose supplemented with the mixture of protease inhibitors (10 μ g/ml leupeptin, 5 μ g/ml aprotinin, 1 mM PMSF). Final pellets were resuspended in two volumes of cold Tris medium and transferred to chilled Potter homogenizer. Cells were broken by 40 hand strokes. The extent of cell breakage was monitored regularly with microscope inspection. The cell homogenate was centrifuged at 500g for 5 min to remove nuclei and debris. The supernatant was next centrifuged at 100 000g for 1h at 4 °C to obtain cytosolic and particulate fractions. Protein concentration was determined using Bradford methods (Bradford, 1976). After boiling for 5 min in Laemmli sample buffer (Laemmli, 1970) the cell fractions were analyzed by SDS-PAGE and Western blotting.

Electrophoresis and Western blot analysis

Equal amounts of proteins (30 μ g) from each fraction were separated by 10% SDS-PAGE with a Hoefer System (Amersham, USA) at pH 8.8 in the presence of SDS (Laemmli 1970). The resolved proteins were transferred to nitrocellulose membranes (Bio-Rad) at 100 V for 1h in transfer buffer containing 25 mM Tris (pH 8.3), 192 mM glycine and 20% methanol (Towbin *et al.*, 1979) by the Hoefer transfer system (Amersham, USA). Filters were blocked in blocking buffer of 2% BSA in TBS-Tween for 2 h and subsequently were incubated with primary antibody overnight

at 4°C, and with the HRP conjugated secondary antibody for 1h at room temperature. Finally, the immunoreactive protein bands were visualized using the Enhanced Chemiluminescence (ECL) detection system (Amersham, USA).

Research in silico

Search for putative components of CDC42- GSK3 β -MARK/PAR1-MAPs effector pathway. Macronuclear genome of *T. thermophila* had been sequenced and is available at <http://www.tigr.org/tdb/e2k1/tgt/>. We performed BLASTp search (Altschul *et al.*, 1997) for proteins that may control the patterned growth and oral replacement in *Tetrahymena* with reference to the polar differentiation of neural cells. The details of another searches are described in the Result section.

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References

- AHRINGER, J. (2003) Control of cell polarity and mitotic spindle positioning in animal cells. *Curr Opin Cell Biol* 15: 73-81.
- ALLEN, R.D. (1967) Fine structure, reconstruction and possible functions of components of the cortex of *Tetrahymena pyriformis*. *J Protozool* 14: 553-565.
- ALTSCHUL, S.F., MADDEN, T.L., SCHAFFER, A.A., ZHANG, J., ZHANG, Z., MILLER, W., LIPMAN, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25: 3389-3402.
- BENTON, R., ST JOHNSTON, D. (2002) Cell polarity: Posterior Par1 prevents proteolysis. *Curr Biol* 12: R479-R481.
- BIERNAT, J., WU, Y.Z., TIMM, T., ZHENG-FISCHHOFER, Q., MANDELKOW, E., MEIJER, L., MANDELKOW, E.M. (2002) Protein kinase MARK/PAR1 is required for neurite outgrowth and establishment of neuronal polarity. *Mol Biol Cell* 13: 4013-4028.
- BOLLAG, D.M., ROZYCKI, M.D., EDELSTEIN, S.J. (1996) Concentrating protein solution. In: «Protein Methods», Ed. Wiley Publishers pp. 71-93.
- BRADFORD, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
- CAU, J., HALL, A. (2005) Cdc42 controls the polarity of the actin and microtubule cytoskeletons through two distinct signal transduction pathways. *J Cell Sci* 118: 2579-2587.
- CHRISTENSEN, S.T., GUERRA, C.F., AWAN, A., WHEATLEY, D.N., SATIR, P. (2003) Insulin receptor-like proteins in *Tetrahymena thermophila* ciliary membranes. *Curr. Biol.* 13: R50-R52.
- DOBLE, B.W., WOODGETT, J.R. (2003) GSK-3: tricks of the trade for a multitasking kinase. *J. Cell Sci* 116: 1175-1186.
- DREWES, G., NURSE, P. (2003) The protein kinase kin1, the fission yeast orthologue of mammalian MARK/PAR-1, localises to new cell ends, after mitosis and is important for bipolar growth. *FEBS Letters* 554: 45-49.
- DREWES, G., EBNETH, A., PREUSS, U., MANDELKOW, E.M., MANDELKOW, E. (1997) MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* 89: 297-308.
- EISEN J.A., COYONE R.S, WU M., WU D., THIAGARAYJON M., *et al.* (2006) Macronuclear genome sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. *PLoS Biol* 4: 286-369.
- ETIENNE-MANNEVILLE, S., HALL, A. (2003) Cdc42 regulates GSK3 β and adenomatous polyposis coli to control cell polarity. *Nature* 421: 753-756.
- FRANKEL, J. (1967) Oral morphogenesis and synchronization: a comparison of "synchronous rounding" and synchronous division in *Tetrahymena*. *J Cell Biol* 35:165 A.
- FRANKEL, J. (1969) Participation of the undulating membrane in the formation of oral replacement primordium in *Tetrahymena pyriformis*. *J Protozool* 16: 26-35.
- FRANKEL, J. (1970) The synchronization of oral development without cell division in *Tetrahymena pyriformis* GL-C. *J exp Zool* 173: 79-100.
- FRANKEL, J. (1989) Pattern formation: Ciliate Studies and Models. Oxford Univ.Press.N.Y.
- FRANKEL, J. (1999) Cell Biology of *Tetrahymena thermophila*. In: *Methods in Cell Biology* 62 (ed DJ Asai and Forney JD): 27-125.
- FRANKEL, J., NELSEN, E.M., MARTEL, E. (1981) Development of the ciliature of *Tetrahymena thermophila* II Spatial subdivision prior to cytokinesis. *Dev Biol* 88: 39-54.
- HANYU, K., TAKEMASA, T., NUMATA, O., TAKAHASHI, M., and WATANABE, Y. (1995) Immunofluorescence localization of a 25-kDa Tetrahymena EF-hand Ca⁺⁺-binding protein, TCBP-25, in the cell cortex and possible involvement in conjugation. *Exp. Cell Res.* 219: 487-493.
- HANYU, K., NUMATA, O., TAKAHASHI, M., WATANABE, Y. (1996) Immunofluorescence localization of a 23-kDa Tetrahymena calcium binding protein, TCBP-23, in the cell cortex. *J.Biochem.* 119:914-919.
- HOOPFER, E.D., MCLAUGHLIN, T., WATTS, R.J., SCHULDINER, O., O'LEARY, D.D.M., LUO, L. (2006) Wld^o protection distinguishes axon degeneration following injury from naturally occurring developmental pruning. *Neuron* 50: 883-895.
- JERKA-DZIADOSZ, M., FRANKEL, J. (1995) The effects of lithium chloride on pattern formation in *Tetrahymena thermophila*. *Dev Biol* 171: 497-506.
- JERKA-DZIADOSZ, M., JENKINS, L.M., NELSEN, E.M., WILLIAMS, N.E., JAECKEL-WILLIAMS, R., FRANKEL, J. (1995) Cellular polarity in Ciliates: persistence of global polarity in a disorganized mutant of *Tetrahymena thermophila* that disrupts cytoskeletal organization. *Dev Biol* 169: 644- 661.
- JERKA-DZIADOSZ, M., STRZYZEWSKA-JOWKO, WOJSA-LUGOWSKA, U., KRAWCZYNSKA, W., KRZYWICKA, A. (2001) The dynamics of filamentous structures in the apical band, oral crescent, fission line and the postoral meridional filament in *Tetrahymena thermophila* revealed by monoclonal antibody 12G9. *Protist* 152: 53-67.
- JIANG, H., LUO, W., LIANG, X., RAO, Y. (2005) Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3 β and its upstream regulators. *Cell* 130: 120-123.
- JOHNSON, G.V.W., STOOHOFF, W.H. (2004) Tau phosphorylation in neural cell function and dysfunction. *J. Cell Sci.* 117: 5721-5729.
- KACZANOWSKA, J., BUZANSKA, L., OSTROWSKI, M. (1993) Relationship between spatial pattern of basal bodies and membrane skeleton (epiplasm) during the cell cycle of *Tetrahymena*: cda A mutant and anti-membrane skeleton 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. *Dev 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. (1975) A single-gene-dependent abnormality of adoral membranelles in *Tetrahymena pyriformis*, species 1. *Genetics* 81: 631-639.
- KACZANOWSKI, A (1976) An analysis of MP gene affected morphogenesis in *Tetrahymena pyriformis*, syngen 1 (species1) Ciliates. *J Exp Zool* 196:215-230.
- KACZANOWSKI, A. (1978) Gradients of proliferation of ciliary basal bodies and the determination of the position 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*: MPM2 immunogold labeling. *Acta Protozool* 35: 297- 308.
- KOSUGA, S., TASHIRO, E., KAJIJOKA, T., UEKI, M., SHIMIZU, Y., IMOTO, M. (2005) GSK3 β directly phosphorylates and activates MARK2/PAR-1. *J Biol.Chem.* 280: 42715-42722.

- KOZMA, S.C., THOMAS, G. (2002) Regulation of cell size in growth, development and human disease: PI3K, PKB and S6K. *BioEssays* 24:65-71.
- KOVACS, P., PALLINGER, E. (2003) Phosphatidylinositol 3-kinase-like activity in *Tetrahymena*. Effects of wortmannin and LY 294002. *Acta Protozool* 42: 277-285.
- LA CARBONA, S., ALLIX, C., PHILLIPE, M., LE GOFF, X. (2004) The protein kinase kin 1 is required for cellular symmetry in fission yeast. *Biol Cell* 96: 169-179.
- LAEMMLI, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- LEONDARITIS, G., TIEDTKE, A., GALANOPOULOU, D. (2005) D-3 phosphonositides of the Ciliate *Tetrahymena*: Characterization and study of their regulatory role in lysosomal enzyme secretion. *Bioch Biophys Acta* 1745: 330-341.
- MATUI, T., TOKURAKU, K., SHIMAZAKI, Y., KOTANI, S. (2004) Identification of an XMAP215-related 60kDa polypeptide as a major microtubule-associated protein in *Tetrahymena pyriformis* Japanese. *J Protozool* 37:11-18.
- NANNEY, D.L. (1975) Pattern of basal bodies addition in ciliary rows in *Tetrahymena*. *J Cell Biol* 65: 503-512.
- NELSEN, E.M., FRANKEL, J., MARTEL (1981) Development of the ciliature of *Tetrahymena thermophila* I Temporal coordination with oral development. *Dev Biol* 88: 27-38.
- NELSEN, E.M., WILLIAMS, N.E., Yi H, KNAAK, J., FRANKEL, J. (1994) "Fenestrin" and conjugation in *Tetrahymena thermophila*. *J Eukaryot Microbiol*. 41: 483-495.
- NG, S.F. (1978) Directionality of microtubule assembly: An in vivo study with the Ciliate *Tetrahymena*. *J. Cell Sci* 33: 227-234.
- NG, S.F. (1979) Unidirectional regeneration is an intrinsic property of longitudinal microtubules in *Tetrahymena*-an in vivo study. *J Cell Sci* 36:109-119.
- 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.
- PALMA, K., ZHANG, Y., LI, X. (2005) An importin alpha homolog MOS6 plays an important role in plant innate immunity. *Curr Biol* 15: 1129-1135.
- PENG, C.Y., GRAVES, P.R., OGG, S., THOMA, R.S., BYRNES, M.J., WU, Z., STEPHENSON, M.T., PIWNICA-WORMS, H. (1998) C-TAK1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. *Cell Growth & Differ*. 9:197-208.
- PETIOT, A., OGIER-DENIS, E., BLOMMAART, E.F., MEIJER, A.J., CODOGNO, P. (2000) Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J. Biol. Chem.* 275: 992-998.
- RYALS, P.E., SMITH-SOMERVILLE, H.E., BUHSE, H.E. jr. (2002) Phenotype switching in polymorphic *Tetrahymena*: a single-cell Jekyll and Hyde. *Int Rev Cytol*. 212: 209-238.
- SCHONEGG, S., HYMAN, A.A. (2006) CDC42 and RHO-1 coordinate actomyosin contractility and PAR protein localization during polarity establishment in *C. elegans* embryos. *Development* 133: 3507-3516.
- STRZYZEWSKA-JOWKO I., JERKA-DZIADOSZ, M., FRANKEL, J. (2003) Effect of alteration in the global body plan on the deployment of morphogenesis-related protein epitopes labeled by the monoclonal antibody 12G9 in *Tetrahymena thermophila*. *Protist* 154:71-90.
- TASSAN, J.-P., LE GOFF, X. (2004) Review: An overview of the kin1/Par1/mark family. *Biol of Cell* 96: 193-199.
- THAZHATH, R., LIU, C., GAERTIG, J. (2002) Polyglycylation domain of β tubulin maintains axonemal architecture and affects cytokinesis in *Tetrahymena*. *Nat. Cell Biol* 4:256-259.
- THAZHATH, R., JERKA-DZIADOSZ, M., DUAN, J., WLOGA, D., GOROVSKY, M.A., FRANKEL, J., GAERTIG, J. (2004) Cell context-specific effects of the beta-tubulin glycylation domain on assembly and size of microtubular organelles. *Mol Biol Cell* 15: 4136-4147.
- TIEDTKE, A., HUNSELER, P., RASMUSSEN, L. (1988) Growth requirements of a new food-vacuole-less mutant of *Tetrahymena*. *Eur J Protistol*. 23: 350-353.
- TOWBIN, H., STAHELIN, T., GORDON, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheet: Procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350-4354.
- WANG, C., DENG, L., HONG, M., GR, AKKARAJU., INOUE, J.-I., CHEN, Z.J. (2001) TAK1 is an ubiquitin- dependent kinase of MKK and IKK. *Nature* 412: 346-351.
- WAKEFIELD, J.G., STEPHENS, D.J., TAVARE, J.M. (2003) A role for glycogen synthase kinase-3 in mitotic spindle dynamics and chromosome alignment. *J Cell Sci* 116: 637-646.
- WALLINGFORD, J.B, HABAS, R. (2005) The developmental biology of *Dishevelled*: an enigmatic protein governing cell fate and cell polarity. *Development* 132: 4421-4436.
- WESTENDORF, J.M, RAO, P.N., GERACE, L. (1993) Cloning of cDNAs for M-phase phosphoproteins recognized by the MPM-2 monoclonal antibody and determination of the phosphorylated epitope. *Proc Natl Acad. Sci USA* 91: 714-718.
- WIGGIN, G.R, FAWCETT, J.P., PAWSON, T. (2005) Polarity proteins in axon specification and synaptogenesis. *Dev. Cell* 8:803-816.
- WILLIAMS, N.E, SCHERBAUM, O.H. (1959) Morphogenetic events in normal and synchronously dividing *Tetrahymena pyriformis* GL. *J Embryol Exp Morph* 7:241-256.
- WILLIAMS, N.E., FRANKEL, J. (1973) Regulation of microtubules in *Tetrahymena* I. Electron microscopy of oral replacement. *J Cell Biol* 56:441-457.
- WILLIAMS, N.E., NELSEN, E.M. (1973) Regulation of microtubules in *Tetrahymena*. II Relation between turnover of microtubule proteins and microtubule dissociation and assembly during oral replacement. *J Cell Biol* 56: 458-465.
- WILLIAMS, N.E., HONTS, J.E., JAECKEL-WILLIAMS, R.F. (1987) Regional differentiation of the membrane skeleton 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.
- WILLIAMS, N.E. (2004) The epiplasm gene EPC1 influences cell shape and cortical pattern in *Tetrahymena thermophila*. *J.Eukaryot.Microbiol.* 51: 201-206.
- ZACKROFF, R.V., HUFNAGEL, L.A. (2001) Wortmannin, phagocytosis and anti-actin drug resistance in *Tetrahymena thermophila*. *J Eukaryot Microbiol* Abstract 33.

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