Forward Genetics and Cortical Patterns in *Tetrahymena*

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WHY NOW?

1. It is now technically possible to clone genes of interest in *Tetrahymena* efficiently by functional complementation of mutations obtained by classical forward genetics.

2. Stocks bearing mutations selected at Iowa will shortly be deposited at the *Tetrahymena* stock center at Cornell University, and will be available to all interested researchers.

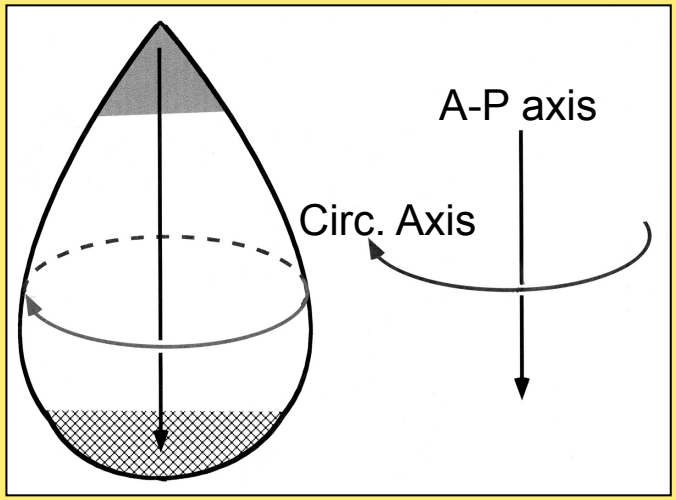
Original Goal: Find genes that influence cortical patterns in *Tetrahymena*

Methods used:

1. Induce mutations using nitrosoguanidine
2. Bring recessive mutations to expression by assortment or cytogamy
3. Screen for alterations of cell shape at 40^{o} C.
4. Carry out-
   - -conventional genetic analysis
   - -complementation crosses
   - -chromosome-arm assignment (for some)
   - -diagnosis of phenotype (via silver staining & SEM)

⟶ 80 alleles of 49 genes (complementation groups)
(20 published)

Mutations affecting:

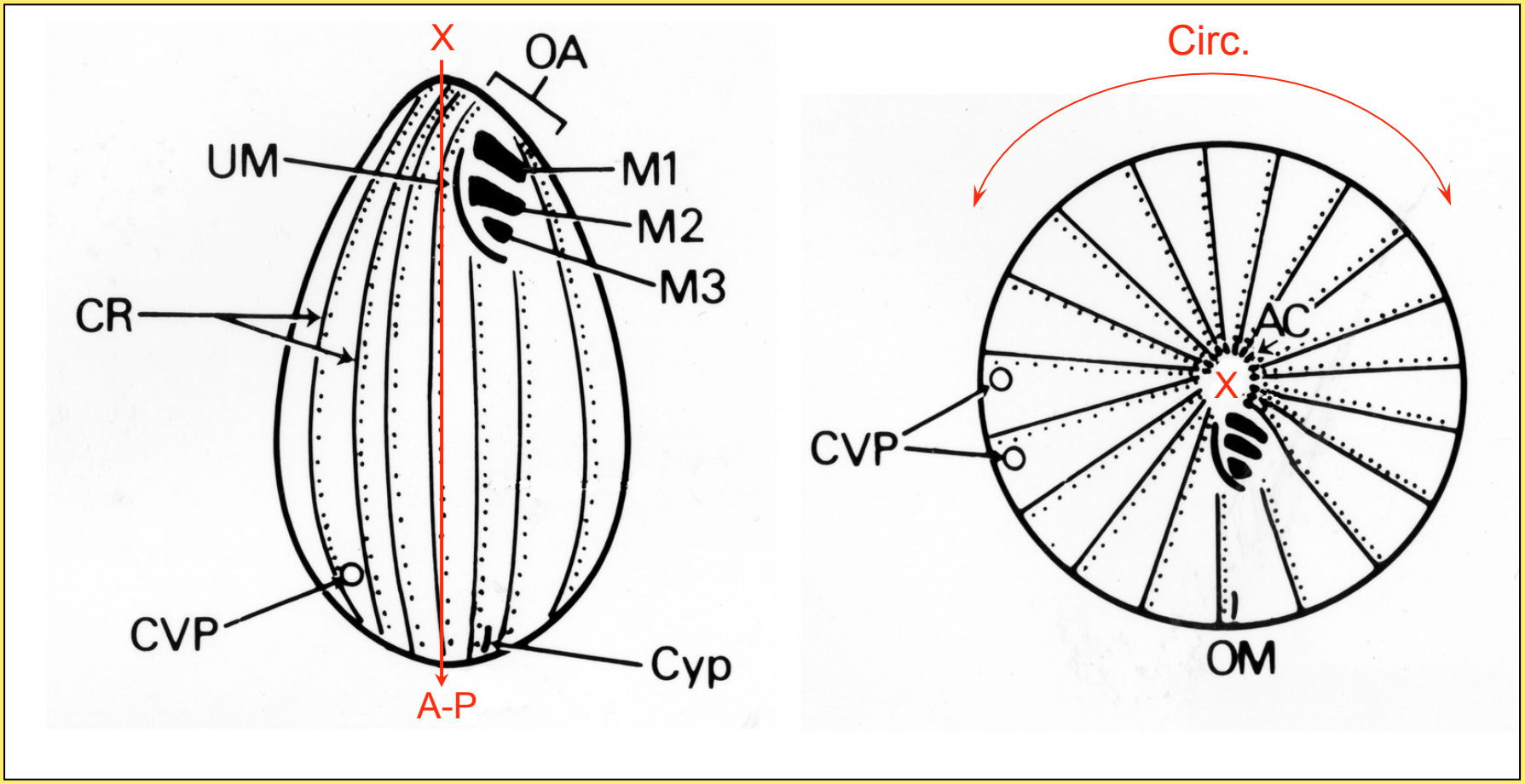


1. Positioning of cortical landmarks along the circumferential axis
2. Positioning….along the anteroposterior axis
3. Number and regularity of ciliary rows
4. Structure of the oral apparatus

⟶ First: Two introductory slides

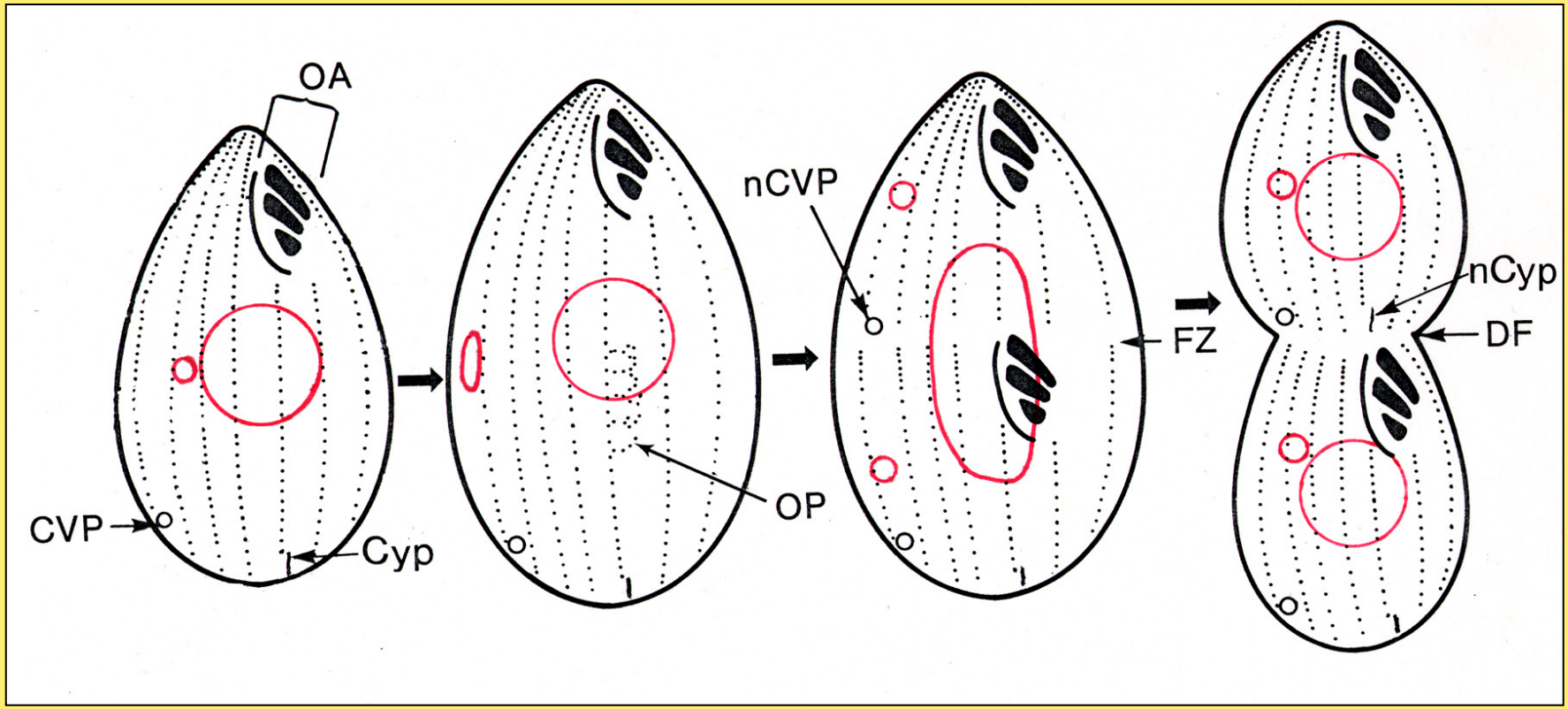
Introductory slide #1

Cortical anatomy of *Tetrahymena*



Introductory slide #2

Cortical subdivision (segmentation) of *Tetrahymena*



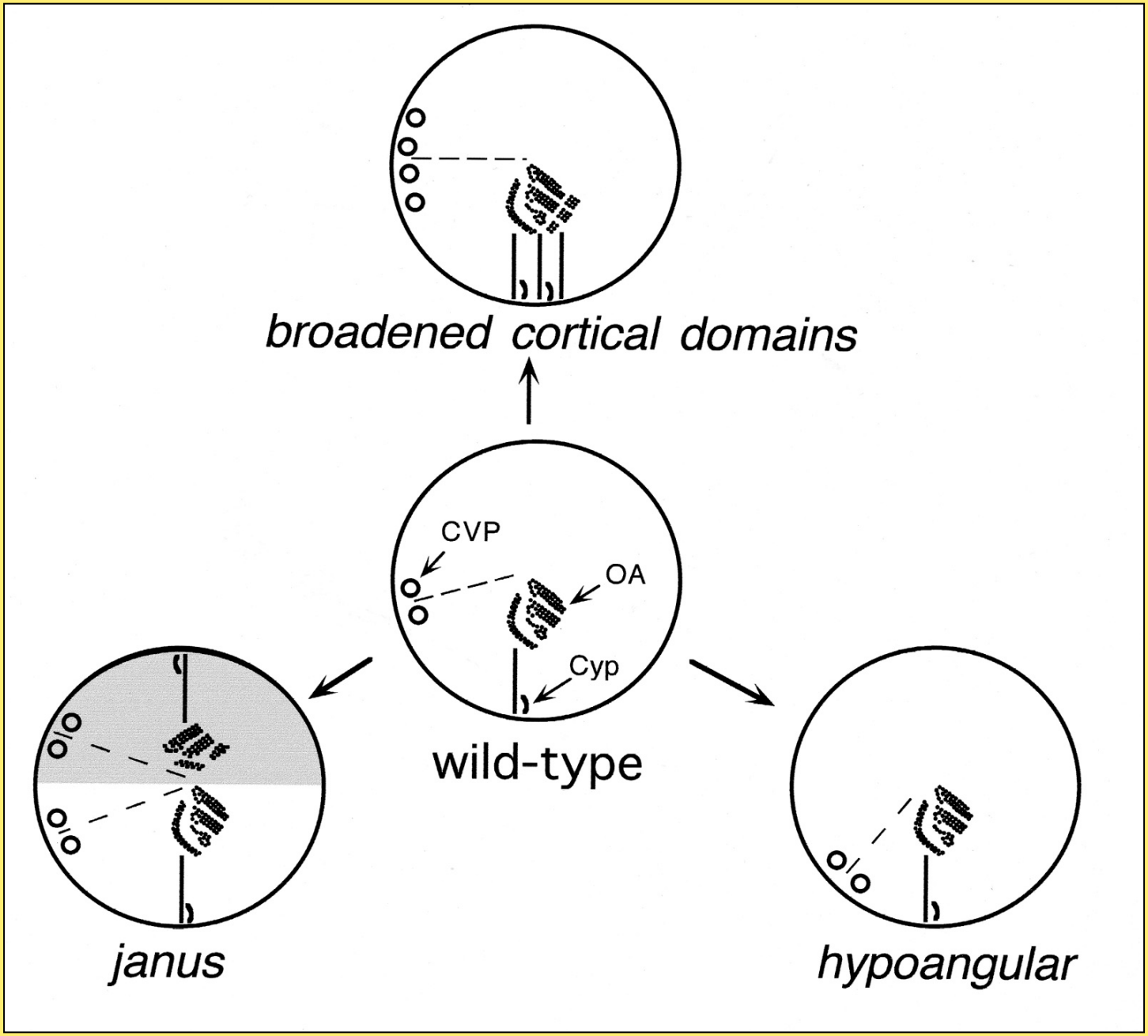
Mutations affecting:

1. Positioning of cortical landmarks along the circumferential axis
2. Positioning….along the anteroposterior axis
3. Number and regularity of ciliary rows
4. Structure of the oral apparatus

janus (jan)A, janB, janC*
hypoangular (hpo)1
broadened-cortical domains (bcd)1

*Temperature-sensitive mutations are indicated in red

Three old friends…..



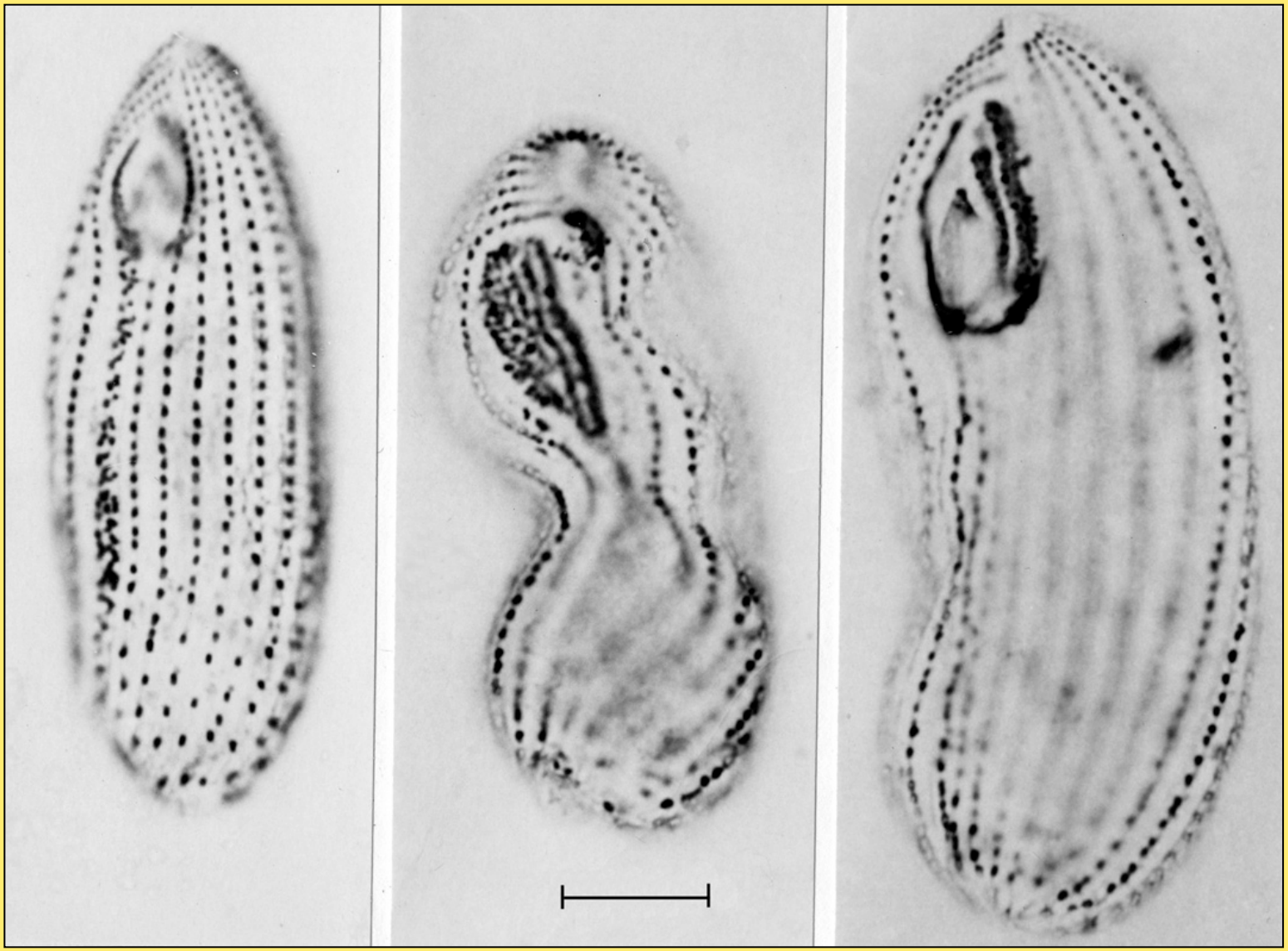
Mutations affecting:

1. Positioning…along the circumferential axis
2. Positioning of cortical landmarks along the anteroposterior axis
3. Number and regularity of ciliary rows
4. Structure of the oral apparatus

Subdivision fails: *pseudomacrostome (psm)A,B,C,D*
Subdivision aborts: *cell division arrest (cda)A, H*
Site of subdivision displaced-
-posteriorly: *conical (con)1;* ***elongated (elo)1*;*** *(psmA, B, C)*
-anteriorly: ***cdaI,*** *cdaK, (psmD)*

***Unpublished mutations are indicated in boldface**

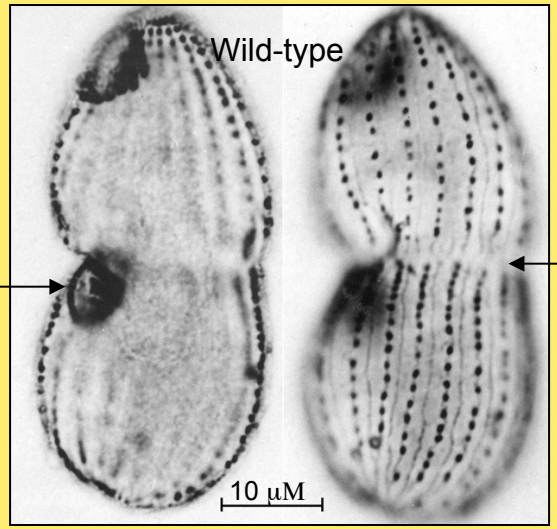
Subdivision fails



(J. Embryol. Exp, Morph. *82*: 41-66)

pseudomacrostomeA-1 at 28^{o} C.

Subdivision aborted:



Dev. Biol. 58: 255-275 (Figs. 1 & 2)

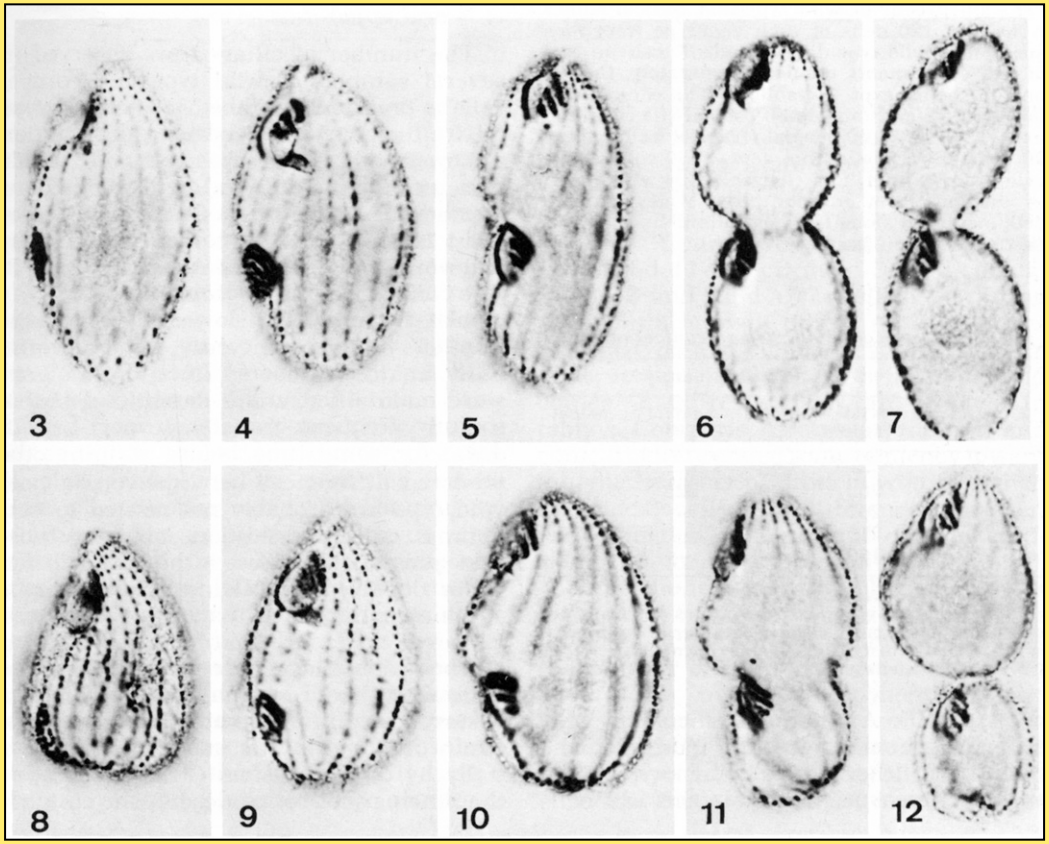
Dev. Biol. 58: 255-275 (Figs. 3 & 4)

40° C.

(unpublished)

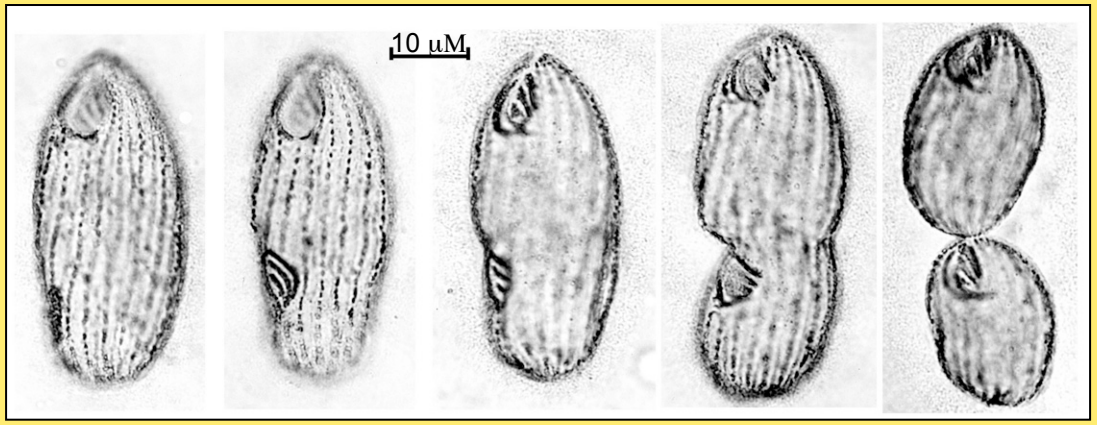
Subdivision displaced posteriorly

Wild-type (D)
(29° C)



Doerder, F.P. *et al.*
J. Exp. Zool.
192: 237-258

conical1-1
(29° C)

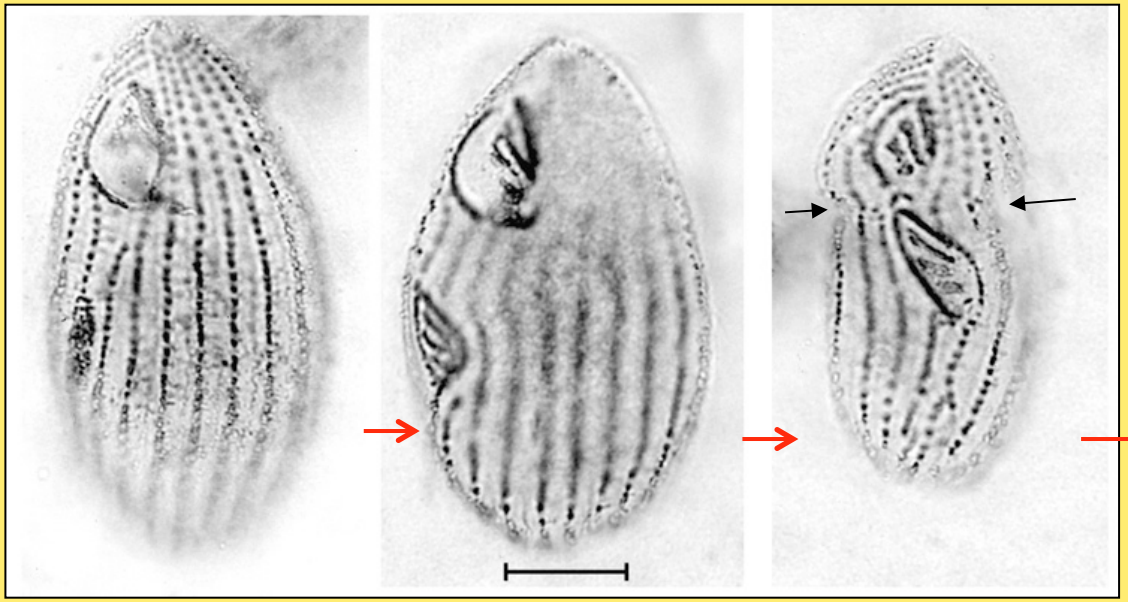


elongated 1-1
(29° C)

(unpublished)

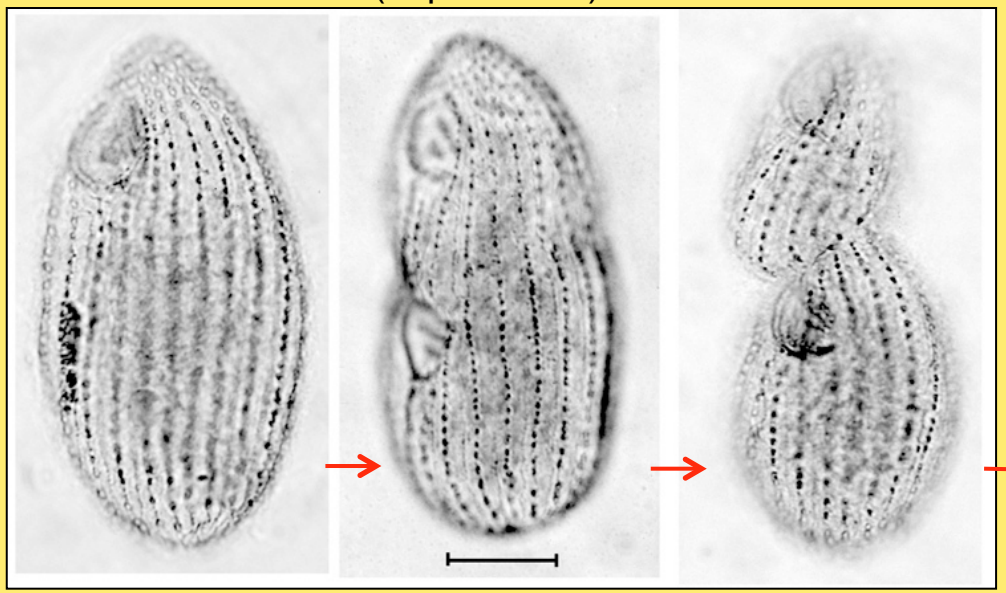
Subdivision displaced anteriorly

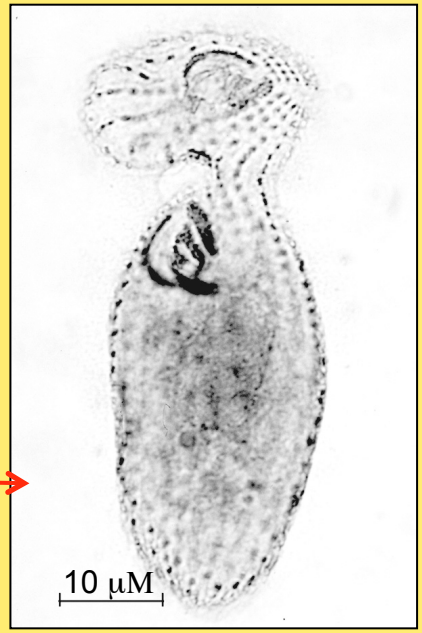
"hammerhead" mutants



cdaI-3

(unpublished)





cdaK-1

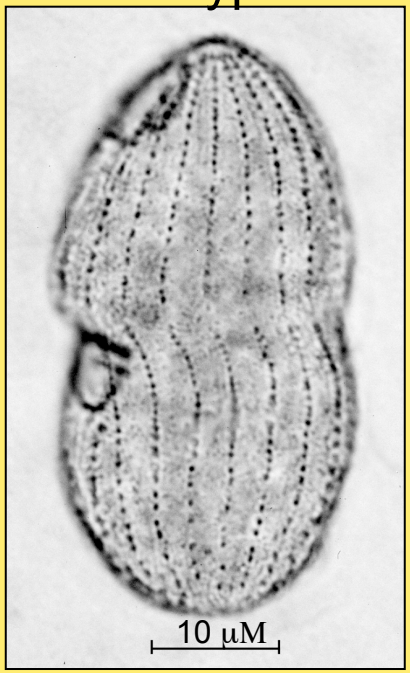
(A. Krzywicka *et al.* Europ. J. Protistol. *35*: 342-352)

Mutations affecting:

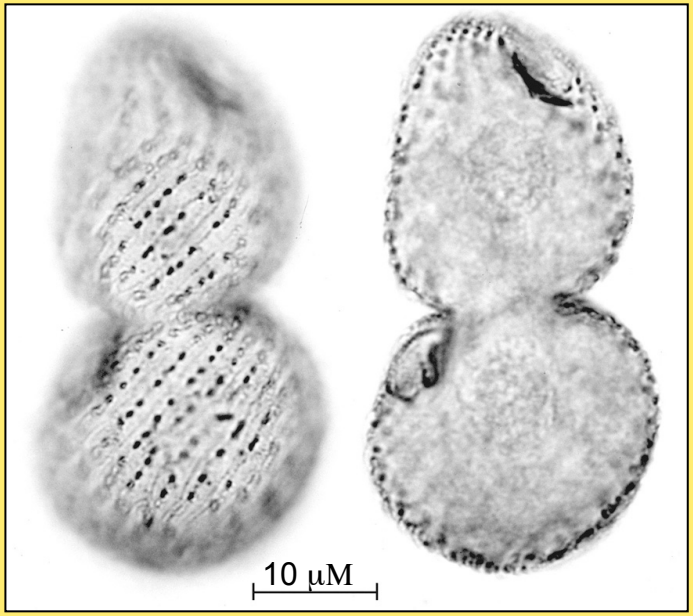
1. Positioning …along the circumferential axis
2. Positioning….along the anteroposterior axis
3. Number and regularity of ciliary rows
4. Structure of the oral apparatus

Too few rows: ***low kinety number (lkn)1***
Rows twisted around the cell: ***twi1, 2***
Rows disorganized*:* *disA*, ***B, C, D, E***; *big1*

wild-type

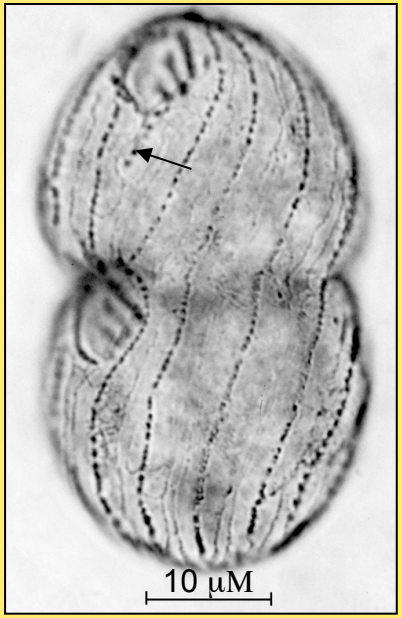


twisty1-1

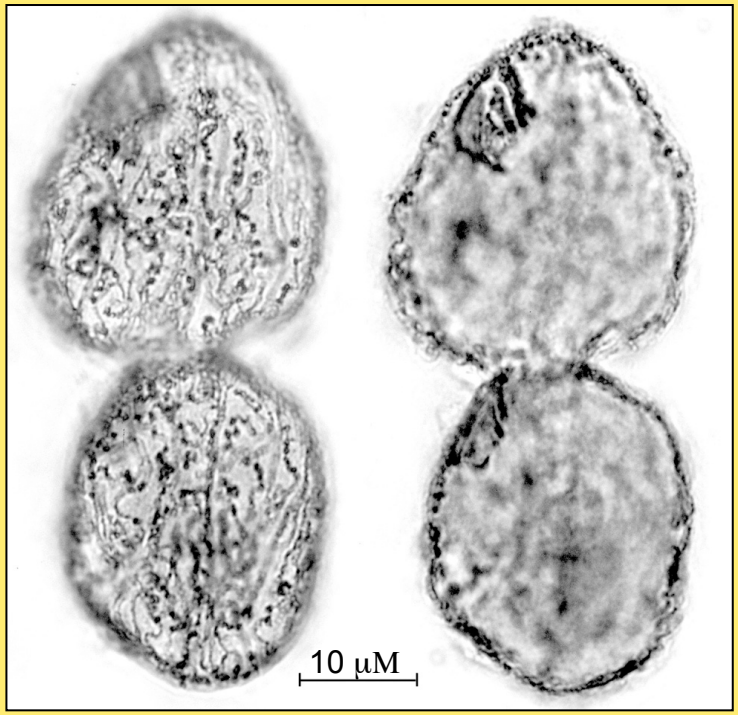


(unpublished)

lkn1-1



disorganizedA-1



(Jerka-Dziadosz *et al. Dev. Biol.* ***169****, 644-661)*

Ciliary rows abnormal…

…Cortical subdivision normal

(unpublished)

Mutations affecting:

1. Positioning… along the circumferential axis
2. Positioning….along the anteroposterior axis
3. Number and regularity of ciliary rows
4. Structure of the oral apparatus

Undulating-membrane organization:

misaligned undulating membrane (mum)1

Membranellar number and organization:

membranellar pattern (mpA), mpC, mpD,* ***mpG, mpH;*** *(****cdaI****)*

General oral organization:

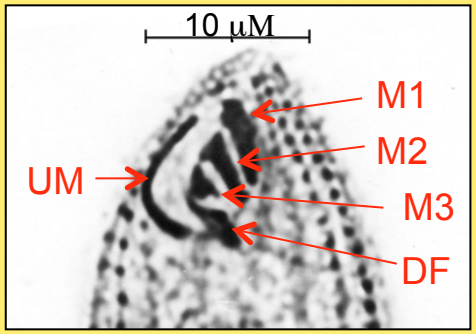
mpF; defective oral apparatus (doa)1, 2; *NP1**; phg#*

*Kaczanowski, A. 1975 Genetics 81: 631-639 (D-strain segregant)

**Orias, E. and Pollock, N.A. 1975 Exp. Cell Res. 90: 345-356 (macronuclear mutant)

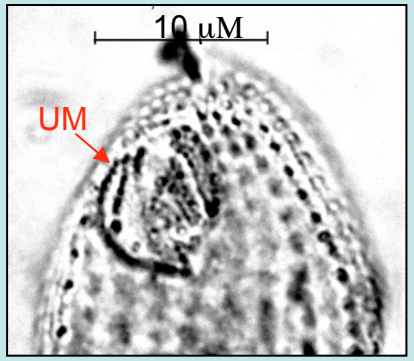
#Tiedtke, A. *et al.* 1988. Europ. J. Protistol. *23: 350-353*

Oral-apparatus mutants



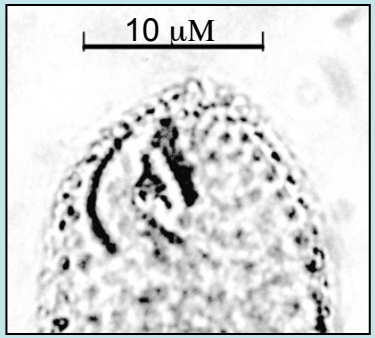
Wild-type

(UM)



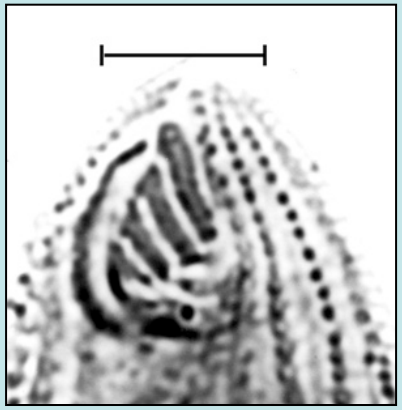
mum1-1

(membranelles)



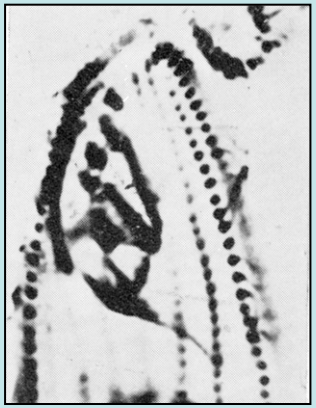
mpG-1

mpC-2



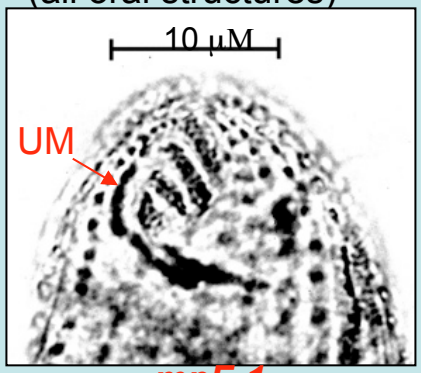
mpD-1

(Genetics *81* 631-9, Fig. 2c)

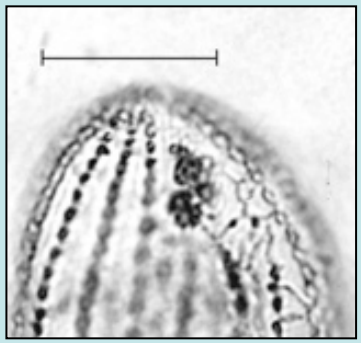


mpA-1 (Kaczanowski)

(all oral structures)



mpF-1

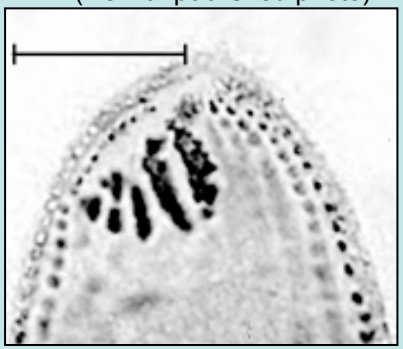


doa1-1

(Exp. Cell Res. *90* 345-56, Fig. 5A)

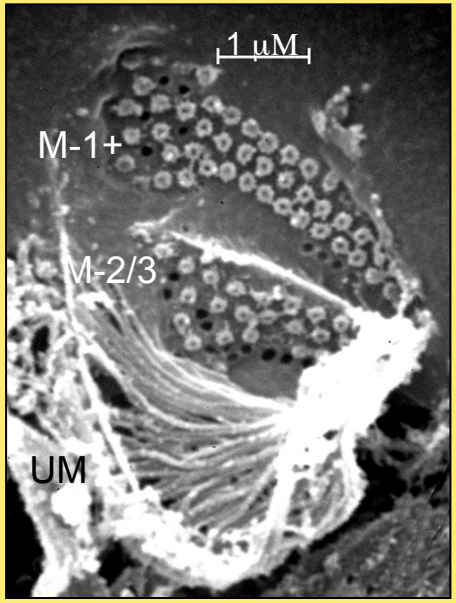
NP1 (Orias and Pollock)

(New unpublished photo)

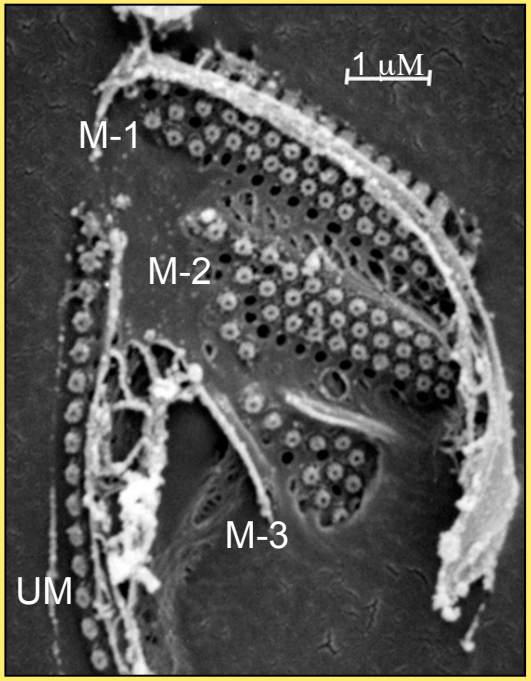


phg [Tiedtke *et al.*]

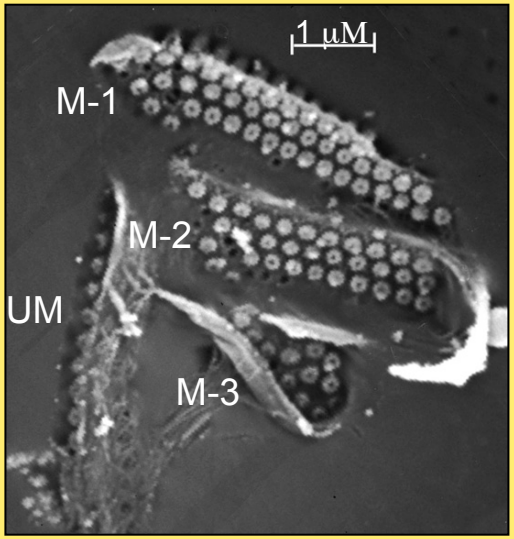
A gradient of membranellar patterns



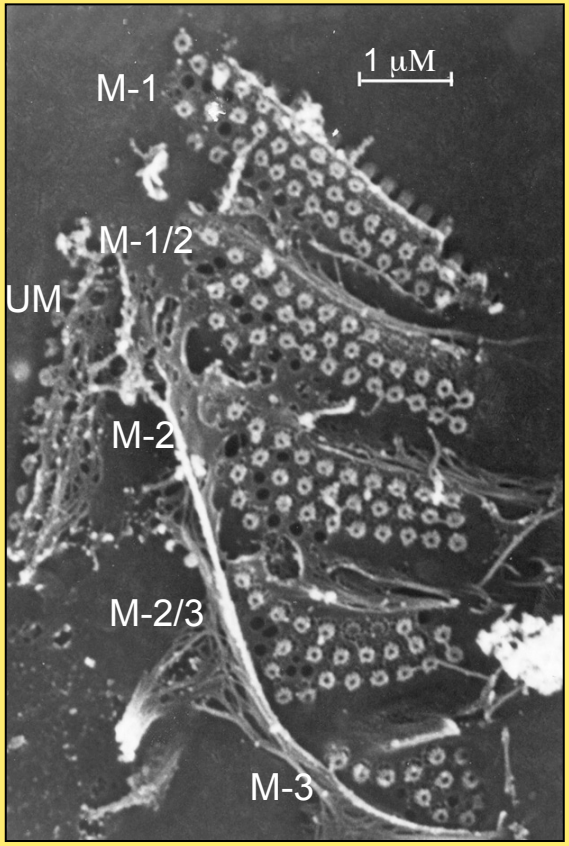
mpG-1 - 30° C. (2 membranelles)
(unpublished)



Wild-type - 37° C



mpG-1 - 30° C (3 membranelles)



mpD-1 - 37° C.

J.E.E.M. *82*: 67-95 (Fig. 12)

Legacy-mutant contributors

Isolation and genetic analysis

- *conical 1-1, cdaA-1:* F.P. Doerder
- *mp(A-1):* A. Kaczanowski
- *NP-1*: E. Orias and N.A. Pollock
- *phg:* A. Tiedtke, P. Hunseler, and L. Rasmussen
- All others: L.M. Jenkins

Analysis of phenotypes

- *janus:* J. Bakowska, E. Cole, M. Jerka-Dziadosz, L.M. Nelsen, I. Strzyzewska-Jowko
- *broadened cortical domains:* E. Cole, K. Stuart
- *hypoangular:* E.M. Nelsen, C.A. Stoltzman
- *pseudomacrostome:* J. Bakowska, L.M. Jenkins, E.M. Nelsen,
- *conical*: G. Cleffmann, L.E. DeBault, F.P. Doerder, D.H. Lynn, G.E. Schafer
- *cell-division arrest:* L. Buzanska, G. Cleffmann, L.E. DeBault, A. Frankel, M. Frontczak, K. Gonda. D.W. Gregory, K. Hanyu, M. Hirono, L.M. Jenkins, E. Joachimiak, J. Kaczanowska, M. Katoh, M. Kiersnowska, W. Krawczynska, A. Krzywicka, E. Martel, J. Mohler, E.M. Nelsen, K. Nishibori, O. Numata, H. Ohba, I. Ohmori, M. Ostrowski, H. Suzuki, R. Tamura, M. Takahashi, Y. Watanabe, D.N. Wheatley, D. Wloga. T. Yasuda
- *disorganized:* K. Aufderheide, R.J. Jaeckel-Williams, M. Jerka-Dziadosz, E.M. Nelsen, N.E. Williams
- *misaligned-undulating membrane:* T. J. Lansing
- *membranellar-pattern:* J. Bakowska, A. Kaczanowski, E.M. Nelsen,
- *non-phagocytosis (NP1):* J.E. Honts, E. Orias, N.A. Pollock, G.B. Silberstein, N.E. Williams,
- *phagocytosis (defective) (phg):* P. Hünseler, E.M. Nelsen, L. Rasmussen, A. Tiedtke

Tetrahymena **Cortical pattern mutant bibliography**

(Organized by mutant, following the sequence of the Powerpoint presentation; chronological within each class of mutants; chromosome-arm localization indicated where known)

Review

Frankel, J. (2008). What do genic mutations tell us about the structural patterning of a complex single-celled organism? Euk. Cell **7**, 1617-1639

Mutants affecting positioning of cortical landmarks along the circumfeential axis:

janus (jan) (janA – 3R; janB – 2; janC – 1R)

Jerka-Dziadosz, M. and Frankel, J. (1979). A mutant of *Tetrahymena thermophila* with a partial mirror-image duplication of cell surface pattern. I. Analysis of the phenotype. *J. Embryol. Exp. Morph.* **49**, 167-202.

Frankel, J. and Jenkins, L.M. (1979). A mutant of *Tetrahymena thermophila* with a partial mirror-image duplication of cell surface pattern. II. Nature of genic control. *J. Embryol. Exp. Morph.* **49**, 203-227.

Jerka-Dziadosz, M. (1981). Patterning of ciliary structures in *janus* mutant of *Tetrahymena* with mirror-image cortical duplications. An ultrastructural study. *Acta Protozool.* **20**, 337-356.

Frankel, J. and Nelsen, E.M. (1981). Discontinuities and overlaps in patterning within single cells. *Phil. Trans. Roy. Soc. Lond. B* **295**, 525-538.

Frankel, J., Jenkins, L.M., and Bakowska, J. (1984). Selective mirror-image reversal of ciliary patterns in *Tetrahymena thermophila* homozygous for a *janus* mutation. *Roux's Arch. Dev. Biol.* **194**, 107-120.

Frankel, J. and Nelsen, E.M. (1986). How the mirror-image pattern specified by a *janus* mutation of *Tetrahymena* comes to expression. *Dev. Genet.* **6**, 213-238.

Frankel, J. and Nelsen, E.M. (1987). Positional reorganization in compound *janus* cells of *Tetrahymena thermophila*. *Development* **99**, 51-68.

Frankel, J., Nelsen, E.M., and Jenkins, L.M. (1987). Intracellular pattern reversal in *Tetrahymena thermophila: janus* mutants and their geometrical phenocopies. In W.F. Loomis (ed.), *Genetic Regulation of Development (Society for Developmental Biology Symposium* **45**), Alan R. Liss, New York, pp. 219-244.

Cole, E.S., Frankel, J., and Jenkins, L.M. (1988) [see under *broadened cortical domains*]

Cole, E.S. and Frankel, J. (1991). Conjugal blocks in *Tetrahymena* pattern mutants and their cytoplasmic rescue. II. *janus A*. *Dev*. *Biol*., **148**, 420-428.

Strzyzewska-Jówko, I., Jerka-Dziadosz, M., and Frankel, J. (2003). Effect of alteration of the global body plan on the deployment of morphogenesis-related epitopes labeled by the monoclonal antibody 12G9 in *Tetrahymena thermophila*. *Protist* **154,** 71-90

broadened cortical domains (bcd) – 3R

Cole, E.S, Frankel, J., and Jenkins, L.M. (1987). *bcd*: A mutation affecting the width of organelle domains in the cortex of *Tetrahymena thermophila*. *Roux's Arch*. *Dev*. *Biol*. **196**, 421-433.

Cole, E.S., Frankel, J., and Jenkins, L.M. (1988). Interactions between the *janus* and *bcd* cortical pattern mutants in *Tetrahymena thermophila*: An investigation into global intracellular patterning mechanisms using double-mutant analysis. *Roux's Arch*. *Dev*. *Biol*. **197**, 476-489.

Cole, E.S. and Frankel, J. (1991). Conjugal blocks in *Tetrahymena* pattern mutants and their cytoplasmic rescue. II. *janusA*. *Dev*. *Biol*. **148**, 420-428.

Cole, E.S. and Stuart, K. (1991). Biochemical and cytological evidence for an overabundance of mucocysts in the *bcd* pattern mutant of *Tetrahymena thermophila*. *J*. *Protozool*. **38**, 536-547.

hypoangular (hpo) - 3R

Frankel, J., Jenkins, L.M., Nelsen, E.M., and Stoltzman, C.A. (1993). *hypoangular:* a gene potentially involved in specifying positional information in a ciliate, *Tetrahymena thermophila*. *Dev*. *Biol*., **160**, 333-354.

<u>Mutants affecting positioning of cortical landmarks along the anteroposterior axis:</u>

pseudomacrostome (psm) (psmA – 5; psmB – 4, psmC-5; psmD -3R)

Frankel, J. (1979). An analysis of cell-surface patterning in *Tetrahymena*. In S.Subtelny and I.R. Konigsberg (eds.), *Determinants of Spatial Organization (Society for Developmental Biology Symposium* **37**). Academic Press, New York, pp. 215-246.

Frankel, J. (1983). What are the developmental underpinnings of evolutionary changes in protozoan morphology? In B.C. Goodwin, N. Holder, and C.G. Wylie (eds.), *Development and Evolution, British Society for Developmental Biology Symposium* **6**. Cambridge University Press, Cambridge, pp. 279-314.

Frankel, J., Jenkins, L.M., Bakowska, J., and Nelsen, E.M. (1984a). Mutational analysis of patterning of oral structures of *Tetrahymena*. I. Effects of increased size on organization. *J*. *Embryol*. *Exp*. *Morph*. **82**, 41-66.

conical (con)

Doerder, F.P., Frankel, J., Jenkins, L.M., and DeBault, L.E. (1975). Form and pattern in ciliated protozoa: Analysis of a genic mutant with altered cell shape in *Tetrahymena pyriformis*, syngen 1. *J. Exp. Zool.* **192**, 237-258.

Lynn, D.H. (1977). Proportional control of organelle position by a mechanism which simultaneously monitors cell size of wild-type and conical form-mutant *Tetrahymena*. *J. Embryol. Exp. Morph.* **42**, 261-274.

Schäfer, E. and Cleffmann, G. (1982). Division and growth kinetics of the division mutant conical of *Tetrahymena*. A contribution to the regulation of generation time. *Exp. Cell Res.* **137**, 277-284.

cell-division-arrest (cda) (cdaA-1R – E. Hamilton))

Frankel, J., Jenkins, L.M., Nelsen, E.M., and Doerder, F.P (1976). Mutations affecting cell division in *Tetrahymena pyriformis*, syngen 1. I. Selection and genetic analysis. *Genetics* **83**, 489-506.

Frankel, J., Jenkins, L.M., and 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., and Jenkins, L.M. (1977). Mutations affecting cell division in *Tetrahymena pyriformis*, syngen 1. II. Phenotypes of single and double homozygotes. *Dev. Biol.* **58**, 255-275.

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

Frankel, J., Mohler, J., and Frankel, A.W.K. (1980). Temperature-sensitive periods of mutations affecting cell division in *Tetrahymena thermophila*. *J. Cell Sci.* **43**, 59-74.

Frankel, J., Nelsen, E.M., and Martel, E. (1981). Development of the ciliature of *Tetrahymena thermophila*. II. Spatial subdivision prior to cytokinesis. *Dev. Biol.* **88**, 39-54.

Joachimiak, E., J. Kaczanowska, M. Kiersnowska, M., and A, Kaczanowski (2004). Syndrome of the failure to turn off mitotic activity in *Tetrahymena* thermophila: in *cdaA1* phenotypes. Acta Protozool. **43**: 291-301.

Tamura, R., Takahashi, M., and Watanabe, Y. (1984). Molecular mechanism of cell division in *Tetrahymena thermophila*. I. Analysis of execution period of a division-arrest ts-mutant. *Zool. Sci. (Tokyo)* **1**, 50-61.

Ohba, H., Ohmori, I., Numata, O., and Watanabe, Y. (1986). Purification and immunofluorescence localization of the mutant gene product of a *Tetrahymena cdaA1* mutant affecting cell division. *J. Biochem. (Tokyo)* **100**, 797-808.

Buzanska, L. (1990). Integration of oral structures in the cdaA1 mutant of *Tetrahymena thermophila*. Acta Protozool. **29**: 37-46.

Buzanska, L., Gregory, D.W., and 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.

Watanabe, Y., Ohba, H., Hirono, M., and Yasuda, T. (1990). Analysis of furrow formation and furrowing during cell division in *Tetrahymena* using cell-division-arrest mutants. *Ann. N.Y. Acad. Sci.* **582**, 166-177.

Kaczanowska, J. (1990). Integration of cortical structures in cdaA1 mutant of *Tetrahymena thermrophila*. *Acta Protozool.* **29**, 275-290.

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

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

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**,149-58

Numata, O, Suzuki, H., Ohba, H., and Watanabe, Y. (1995). The mutant gene product of a *Tetrahymena* cell-division-arrest mutant *cdaA* is localized in the accessory structure of specialized basal body close the division furrow. *Zool. Sci. (Tokyo)* **12**, 133-135.

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

Krzywicka, A., Kiersnowska, M., Wloga., and Kaczanowska, J. (1999). Morphogenesis of the fission line in a ciliate *Tetrahymena:* Analysis of *cdaK1* phenotypes and rearrangements of membrane skeletal proteins during cell division. *Europ. J. Protistol.* **35**, 342-352.

Gonda, K., Nishibori, K., Ohba, H., Watanabe, A., and Numata, O. (1999) Molecular cloning of the gene for p85 that regulates the initiation of cytokinesis in *Tetrahymena*. *Biochem. Biophys. Res. Comm.* **264**, 112-118.

Gonda, K., Katoh, M., Hanyu, K., Watanabe, Y., and Numata, O. (1999). Ca^{2+} and p85 cooperatively regulate an initiation of cytokinesis in *Tetrahymena*. *J. Cell Sci.* **112**, 3619-3626.

Numata, O. and Gonda, K. (2001). Determination of division plane and organization of the contractile ring in *Tetrahymena*. *Cell Structure and Function* **26**, 593-601.

Mutants affecting the number and regularity of ciliary rows:

twisty (twi)

No publications

low kinety number (lkn)

No publications

disorganized (dis)

Frankel, J. (1979) *[see under pseudomacrostome*]

Aufderheide, K.J. (1980). Mitochondrial associations with specific microtubular components of the cortex of *Tetrahymena thermophila*. II. Response of the mitochondrial pattern to changes in the microtubule pattern. *J. Cell Sci*. **42**, 247-260.

Jerka-Dziadosz, M., Jenkins, L.M., Nelsen, E.M., Williams, N.E., Jaeckel-Williams, R.J., and 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.

Mutants affecting the structure of the oral apparatus

misaligned undulating membrane (mum)

Lansing, T.J., Frankel, J., and Jenkins, L.M. (1985). Oral ultrastructure and oral development in the misaligned undulating membrane mutant of *Tetrahymena thermophila*. *J. Protozool*. **32**, 126-139.

membranellar-pattern (mp) (mpC – 3[L?]; mpD – 3R); mpG – 4)

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. *J. Exp. Zool*. **196**, 215-230.

Frankel, J. (1979) [*see under pseudomacrostome*]

Frankel, J., Jenkins, L.M., Bakowska, J., and Nelsen, E.M. (1984a) [*see under pseudomacrostome*]

Frankel, J., Nelsen, E.M., Bakowska, J., and Jenkins, L.M. (1984b). Mutational analysis of patterning of oral structures in *Tetrahymena*. II. A graded basis for the individuality of intracellular structural arrays. *J. Embryol. Exp. Morph.* **82**, 67-95**.**

defective oral apparatus (doa) No publications
non-phagocytosis (NP1)

Orias, E. and Pollock, N. A. (1975). Heat-sensitive development of the oral organelle in a *Tetrahymena* mutant. *Exp. Cell Res.* **90**, 345-356.

Silberstein, G.B. (1979). Acid hydrolases and their release in food vacuole-less mutants of *Tetrahymena thermophila*. *J. Protozool.* **26**, 519-524.

Silberstein, G.B., Orias, E., and Pollock, N. (1975). Mutant with heat-sensitive capacity for phagocytosis in *Tetrahymena*. Isolation and genetic characterization. *Genet. Res.* **26,** 11-19.

Williams, N.E. and Honts, J.E. (1987). The assembly and positioning of cytoskeletal elements in *Tetrahymena*. *Development* **100**, 23-30.

phagocytosis (phg$^-$* = **II8G))

Kaczanowska, J., S. Kaczanowski, M. Kiersnowska, H. Fabczak, K. Tulodziecka, and A. Kaczanowski, 2008. Patterning and acquisition of cell polarity during cell cycle and oral replacement in a ciliate *Tetrahymena*. Int. J. Dev. Biol. **52:** 249-258

Tiedtke, A., Hünseler, P., and Rasmussen, L. (1988). Growth requirements of a new food-vacuole-less mutant of *Tetrahymena Europ. J. Protistol.* **23**, 350-353.

vacA *(oral defect undetectable by light microscopy—not covered in Powerpoint presentation)*

Suhr-Jessen, P.B. and Orias, E. (1979). Mutants of *Tetrahymena thermophila* with temperature-sensitive food vacuole formation. I. Isolation and genetic characterization. *Genetics* **92**, 1061-1077.

Suhr-Jessen, P.B. and Orias, E. (1979). Mutants of *Tetrahymena thermophila* with temperature-sensitive food vacuole formation. II. Physiological and morphological studies. *Exp. Cell Res.* **124**, 317-327.

Reviews

Jerka-Dziadosz, M. and Beisson, J. (1990) Genetic approaches to ciliate pattern formation: from self-assembly to morphogenesis. *Trends Genet*. **6**, 41-45.

Frankel, J. (2000) Cell biology of *Tetrahymena thermophila. Methods in Cell Biology* **62**, 27-125.

Frankel, J. (2000). Cell polarity in ciliates. In: *Cell Polarity*, ed. D.G. Drubin. Oxford University Press, Oxford, pp. 78-105.

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