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Review

Genetic and Epigenetic Factors Affecting Meiosis Induction in Eukaryotes Revealed in *Paramecium* Research

Małgorzata PRAJER

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This review presents studies of the induction of meiosis undertaken on the ciliate *Paramecium,* a unicellular model eukaryotic organism. Meiosis in *Paramecium*, preceding the process of fertilization, appears in starved cells after passing a defined number of divisions (cell generations), starting from the last fertilization. Investigations were performed on clones of cells entering autogamy, a self-fertilization process. Genetic as well as epigenetic factors, i.e. endo- and exogenous factors, affecting the induction of meiosis and changing the duration of the interautogamous interval (IAI), were analyzed. The results show that: (1) Meiosis induction is controlled genetically by the somatic macronucleus. However, besides the nuclear factors, the cytoplasmic protein immaturin also affects this process (HAGA & HIWATASHI 1981); (2) Epigenetic factors, such as non-genetically disturbed cytoskeleton structures and changes in the cell architecture observed in doublet *Paramecium* cells, exert internal mechanical stress (INGBER 2003), which constitutes the endogenous impulse accelerating meiosis; (3) Mild osmotic stress, acting as an exogenous factor, can initiate the specific MAP kinases signaling pathway resulting in earlier meiosis induction, as in other unicellular eukaryotes (SEET & PAWSON 2004).

Key words: Autogamy, interautogamous interval, doublet cells, glucose, sorbitol, insulin, cellular stress, *Paramecium aurelia* species complex.

Małgorzata PRAJER, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland. E-mail: prajer@isez.pan.krakow.pl

The unicellular ciliate *Paramecium*, the genome of which was recently sequenced (AURY *et al.* 2006), exemplifies a complete and independent eukaryotic organism. It constitutes the perfect model system for the physiological and genetic studies of mechanisms regulating the sexual processes, development and morphogenesis on a cellular and molecular level. Hence, research concerning meiosis induction and clonal cycle regulation have been undertaken on this excellent model organism. In this paper the results of these studies are reviewed and summarized.

Meiosis is a basic process propagating the germ line information into successive generations in sexually reproducing eukaryotes. Eukaryote cells of both unicellular and multicellular organisms have a limited potential to divide even in nutrient rich conditions. Human cells cultured *in vitro* age with progressing divisions and die after a defined number of cell divisions, called the "Hayflick limit" (HAYFLICK & MOORHEAD 1961). Similarly, *Paramecium* cells die after reaching the "Sonneborn limit" of divisions, if they do not undergo the sexual process. Under standard conditions of daily reisolation culture, clones of the *Paramecium aurelia* species complex die after about 250-350 cell generations (SONNEBORN 1954). Thus, the role of sexual reproduction involving meiosis and fertilization is fundamental in preventing ageing and death (SMITH-SONNEBORN 1981; TAKAGI 1988, 2000).

The sexual processes of *Paramecium,* conjugation and autogamy, are unique and characteristic only for the ciliates (SONNEBORN 1974; MIYAKE 1996). They both are induced by starvation and begin with meiosis followed by fertilization. Conjugation is a sexual process in which two reactive cells of complementary mating type participate, while autogamy is a self-fertilization process that occurs in unpaired cells, in the absence of complementary mating type partners. This process is equivalent, in terms of nuclear phenomena, to conjugation of complementary mating type cells, with the exception that the zygotic nucleus is formed

from two haploid nuclei of the same cell. Therefore, autogamy results in homozygous cell progeny and can be considered as a means of preventing the progression of ageing and restoring the vigour of a clone (SONNEBORN 1954). This self-fertilization process is considered to be one of the most striking features in all 15 species of the *P. aurelia* complex (SONNEBORN 1975).

According to SONNEBORN (1957, 1974) the clonal cycle of *Paramecium* begins with meiosis and fertilization in a sexual process. It consists of the following, successive periods:

1. Immaturity, when the cells are not able to undergo sexual processes;

2. Maturity, when cells can conjugate or slightly later undergo autogamy;

3. Senescence, the longest period characterized by phenotype instability and gradual loss of reproductive abilities (TAKAGI 1988).

4. Death ends the clonal cycle, unless the cells earlier enter the sexual process that begins a new clonal cycle.

During the run of clonal life, sexual processes are induced by starvation after a specific number of divisions, starting from the last fertilization. The period between two successive conjugations is termed "mating immaturity" and that between two successive autogamies "the interautogamous interval" (SONNEBORN 1957, 1974) or "autogamous immaturity" (TAKAGI 1988). The duration of both periods is measured by the number of cell divisions (i.e. the cell cycle or cell generations) (SONNEBORN 1970; SMITH-SONNEBORN & REED 1976). Therefore, the interautogamous interval (IAI) can be defined as the number of vegetative divisions following autogamy, which is required for cells of a clone to have a high probability of undergoing the next self-fertilization process, if they are starved.

In the *P. aurelia* species complex, as in other ciliates, the germ and somatic lines differentiate at the nuclear level. The nuclear apparatus is composed of two types of nuclei: one large, highly polyploid somatic macronucleus (~1000n in *P. tetraurelia)* and two small, diploid, germ line micronuclei (RAIKOV 1982; PRESCOTT 1994). The macronucleus, transcriptionally very active, is responsible for the phenotype of the cell, while the micronuclei are silent during vegetative growth but their role in the sexual process is very important. They undergo meiosis to transmit the germ line genome to the next generation.

During the sexual process micronuclei undergo meiosis to produce 8 genetically identical haploid nuclei, one of which enters the paroral region. Only this nucleus survives and undergoes mitosis yielding two haploid pronuclei; reciprocal exchange of pronuclei between two mates takes place in conjugation, while in the cell engaged in autogamy, two identical pronuclei fuse together during karyogamy. In both cases the fusion of two pronuclei results in the formation of a diploid, zygotic nucleus which next divides mitotically twice yielding four nuclei. The different kinds of nuclei originate from these identical diploid nuclei. Two of these, situated anteriorly in the cell, become the new micronuclei, while two posterior ones will differentiate into the macronuclear anlagen (MIKAMI 1980; GRANDCHAMP & BEISSON 1981). The latter nuclei begin to enlarge. Then, the germ line genome is extensively rearranged during the development of a new somatic macronucleus. These rearrangements involve the amplification of the diploid micronuclear genome and the programmed and reproducible elimination of a large fraction of germline DNA (GRATIAS & BÉTERMIER 2001; YAO *et al.* 2002; BÉTERMIER 2004; LE MOUËL *et al.* 2003; NOWACKI *et al.* 2005). This process begins after a few DNA replication cycles and is completed by the first cell division, when micronuclei divide mitotically, while the macronuclear anlagen are distributed without fission into two daughter cells initiating two new clones. During successive vegetative divisions the micronuclei run mitotic fissions, while the macronucleus divides by a non-mitotic process. After micronuclear meiosis, the parental somatic macronucleus is progressively fragmented into small pieces which segregate randomly to daughter cells and are then degraded.

Prokaryotes are potentially immortal; they stop dividing only when food resources become unavailable. The phenomenon of mortality was acquired by eukaryotes as a result of evolution. According to TAKAGI (2000), both the differentiation into "germ" and "soma", and sexual reproduction represented by meiosis and fertilization may be involved in mortality. The "germ" inherits the immortal nature of prokaryotes, while the "soma" can utilize costly energy for processes other than division. The "germ" came to be associated with sexual reproduction. Both the "germ" and the "soma" proliferate, however, the proliferation of "soma" is limited. In ciliates, during asexual reproduction the germ line is produced from the germ one, and the somatic line from the somatic one. In sexual reproduction, both the germ and somatic lines are produced from the germ line, while the old somatic line is destroyed (Fig. 1). Therefore, the sexual process not only fulfils its usual function, such as recombination of the gene pool, but also plays another important role yielding the replacement of the old macronucleus by a new one. In ciliates the mechanism for initiation of meiosis seems to be coupled to the mechanism of destroying the soma during apoptotic degradation of the old macronucleus.

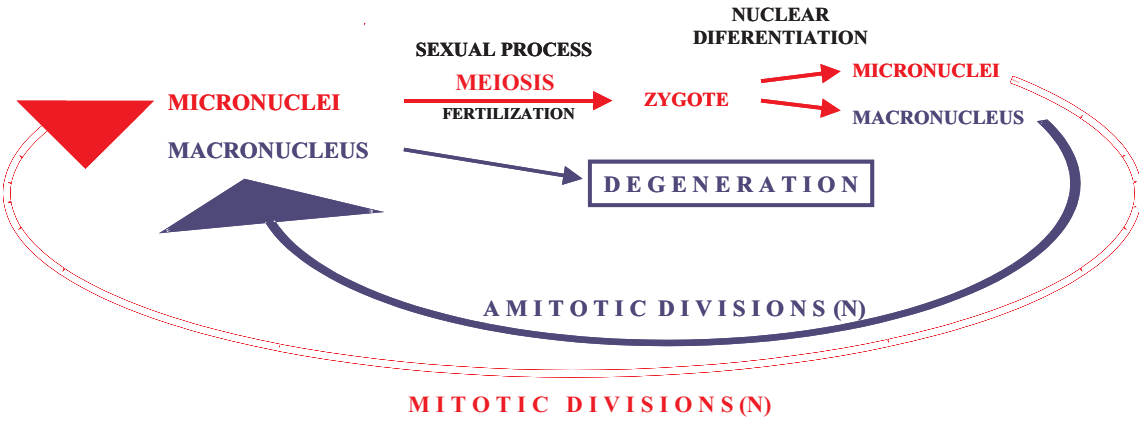

Fig. 1. Immortal germ line and mortal somatic line during the clonal cycle of *Paramecium*. Germ line is drawn in red, somatic line in blue. N – number of vegetative divisions during sexual immaturity.

The mechanism of clonal cycle regulation and of sexual process induction were studied in representative *Paramecium aurelia* species. The investigations were performed in clones of cells entering autogamy. Genetic as well as epigenetic, i.e. endo- and exogenous factors, affecting the induction of meiosis and changing the duration of the interautogamous interval, expressed as the number of generations between two successive autogamies, were analyzed.

Genetic factors control meiosis induction

Studies of the interautogamous interval undertaken in the representatives of all species belonging to the *P. aurelia* complex revealed that the duration of this period is stable and characteristic for a given species and even for a stock (SONNEBORN 1957). Moreover, a correlation between cell size and the length of the IAI exist. This interval in species with small sized cells was found to be shorter than in species having larger cells. From nearly 10 up to about 60 fissions were needed in different species belonging to the *P. aurelia* complex for mass autogamy to occur (KOŚCIUSZKO & PRAJER 1988). Studies concerning the duration of IAI in the mutants of *P. tetraurelia* revealed that this period is in general shorter than in wild stock of this species (PRAJER & KOŚCIUSZKO 1999). The isolation of mutants with long autogamous immaturity (KOMORI *et al.* 2004, 2005) confirmed that the potential ability to induce meiosis is genetically controlled.

The nuclear dualism in *Paramecium aurelia* species provides a unique opportunity for the study of nuclear factors affecting the run of the clonal cycle and meiosis induction at autogamy. The role of the macronucleus in this process has been investigated by microtransplantation of macronuclear karyoplasm. As it was shown by KOŚCIUSZKO and KOIZUMI (1983), autogamy can be induced in *P. tetraurelia* cells of young clonal age when their macronuclei are injected with macronucleoplasm from cells of older clonal age. Investigations performed by MIKAMI and KOIZUMI (1983) demonstrated that the transplantation of a whole macronucleus derived from aged cells as well as partial elimination of the macronucleus accelerate autogamy. Therefore, they concluded that clonal age should be measured in rounds of chromatin replication or DNA synthesis, rather than in the number of cell divisions. They suggested that the length of the period during which autogamy cannot be induced by food deprivation is encoded in the macronucleus. The macronuclear karyoplasm from cells of *P. tetraurelia* stock with a short interautogamous interval transplanted into cells of a stock with a longer IAI caused, likewise, a distinct shortening of the IAI in the recipient clones (KOŚCIUSZKO & PRAJER 1989). Similar experiments concerning the mating reactivity in *Paramecium caudatum* confirmed the above hypothesis (MIKAMI & ITOH 1995). The possibility of inducing autogamy immediately after macronuclear regeneration (PREER 1968) was also in accordance with this assumption. Thus, the "counting" of the fission number appears to reside within the macronucleus itself (HAGA 1995). These results indicated the direct role of the macronucleus in the control of the interautogamous interval in *P. tetraurelia.*

On the other hand, the duration of sexual immaturity is also regulated by immaturin, a cytoplasmic protein inhibiting the expression of mating reactivity as revealed by cytoplasm transplantation (MIWA *et al.* 1975; MIWA 1979a, 1979b, 1984, HAGA & HIWATASHI 1981). This protein exhibits strong DNAse activity (HAGA & SAKAZUME 1996). Similarly, cytoplasm immature for autogamy contains a factor(s) retarding the self-fertilization process (KOŚCIUSZKO & PRAJER 1992; PRAJER 1994; PRAJER & KOŚCIUSZKO 1994, 1998). HAGA (1995) suggested that immaturin synthesis at various stages

in the immaturity period depends on the clonal age of the macronucleus which, as the somatic nucleus, represents a developmental clock controlling the intrinsic temporal programming of ageing.

Disturbed cell architecture as an endogenous, epigenetic factor inducing meiosis

For a better understanding of the mechanisms affecting meiosis induction and regulating IAI duration, subsequent approaches were focused on their association with morphogenesis and developmental processes in abnormal forms of *Paramecium,* such as artificially obtained doublet cells. In these types of cells autogamy was observed at a substantially increased rate than in normal, wild cells (IFTODE & ADOUTTE 1993; PRAJER *et al.* 1999).

Doublets are semi-stable cells resulting from the fusion of normal cells, which occasionally appear during conjugation (SONNEBORN 1963). They can be obtained more systematically through electrofusion of sister cells (GAERTIG *et al.* 1988) or by heat shock applied to conjugating cell pairs (PRAJER *et al*. 1999). Homopolar, symmetrical doublets have two almost complete sets of cortical structures, with two oral apparatuses placed at the same latitude, at an angle of about 180°. They are semistable, in the sense that although they can yield doublet cells during vegetative division, they tend to evolve towards normal, singlet cells in the course of divisions through a regulation process (Fig. 2) (FAURÉ-FREMIET 1948; SONNEBORN 1963; KACZANOWSKA & DUBIELECKA 1983). The correlations between cortex and organelles in doublet cells during the regulation process were extensively examined by PRAJER *et al.* (1999) and IFTODE *et al*. (2001). The changes of the main cortical and nuclear structures which can be observed in regulating populations are schematically presented in Fig. 3. At the beginning of clonal life, almost perfect doublets must have double nuclear structures, comprising two macronuclei and two

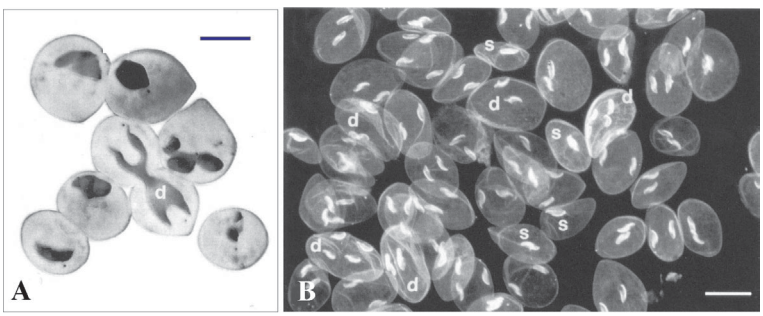

Fig. 2. Doublet populations during regulation to singlet state. A: Nearly perfect doublets (180°-160°) of *P. undecaurelia.* Different macronuclear structures of various size are visible. d – dividing doublet. Fernandez-Galiano's staining. B: *P. tetraurelia* doublets and singlets during an advanced stage of the regulation process. Many cells with two oral regions situated closely on the same side. CTR 210 antibody. d – doublets during division, s – singlet cells. Bars = 10 μm. Modified from PRAJER *et al.* (1999).

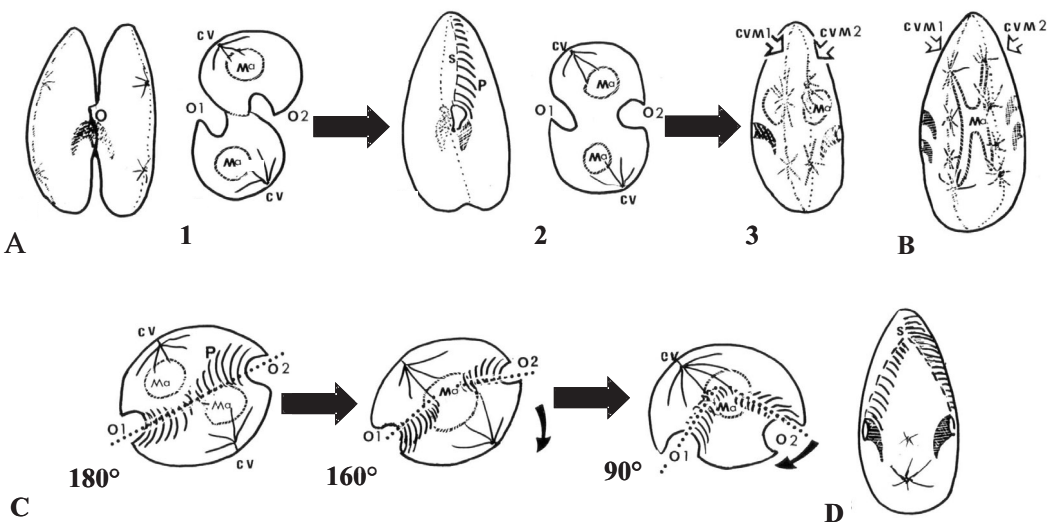

Fig. 3. Cortical and nuclear organization of symmetrical doublets in schematic presentation. A1: Cell architecture shortly after fusion: frontal view and cross-section. A2: The fusion zone progressively enlarges between two oral regions (O1, O2). The paratene area (P) bounds each oral region to the pole, along the anterior suture (s). A3: Doublet before regulation with two macronuclei (Ma) and two contractile vacuoles on two meridians (CVM1, CVM2), oppositely located. B: Division of symmetrical doublet with one macronucleus being "quartered" between two CVMs. C: Changes of the angle between two oral areas during progressing regulation to singlet state; cross-sections. D: Ventral surface of regulating doublet. Modified from PRAJER *et al.* (1999).

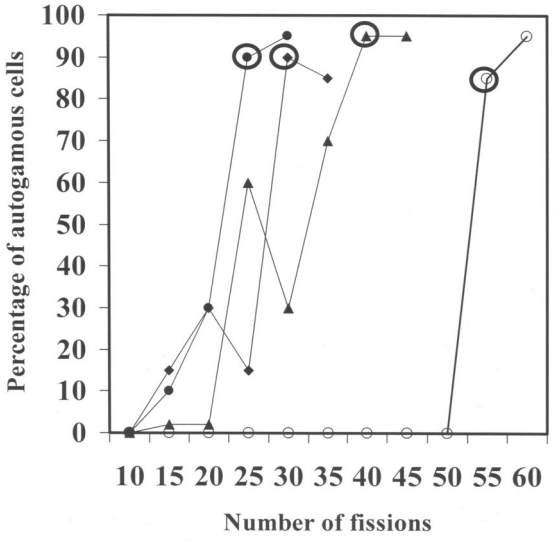

Fig. 4. Age dependent increase of autogamous cells in three sublines of *P. undecaurelia* doublets. Lines with black squares, circles and triangles – particular doublet sublines. Line with empty circles – control singlet line. Large circles – first mass autogamy in each cell line. From PRAJER *et al.* (1999).

sets of micronuclei. However, dimacronucleate doublets give rise rapidly to unimacronucleate progeny with one macronucleus substantially larger and having about twice the DNA content (Fig. 2A) as that of a normal cell (MORTON & BERGER 1978); such changes were considered by FAURÉ-FREMIET (1948) as the first step in the process of regulation towards the singlet state.

The process of autogamy was thoroughly studied in *P. tetraurelia* characterized by a short interautogamous interval, and *P. undecaurelia* which has a long IAI, more convenient for precise observation (PRAJER *et al.* 1999). An increase in the frequency of autogamy and a highly significant decrease in duration of the interautogamous interval, in comparison with singlet lines, in regulating doublet lines of both species was observed (Fig. 4). During reversion to the singlet state, doublets displayed abnormalities in nuclear processes during both vegetative and sexu al stages (PRAJER *et al.* 1999) such as: (1) a systematic DNA unbalance (Fig. 5), and in consequence: (2) an unequal distri-

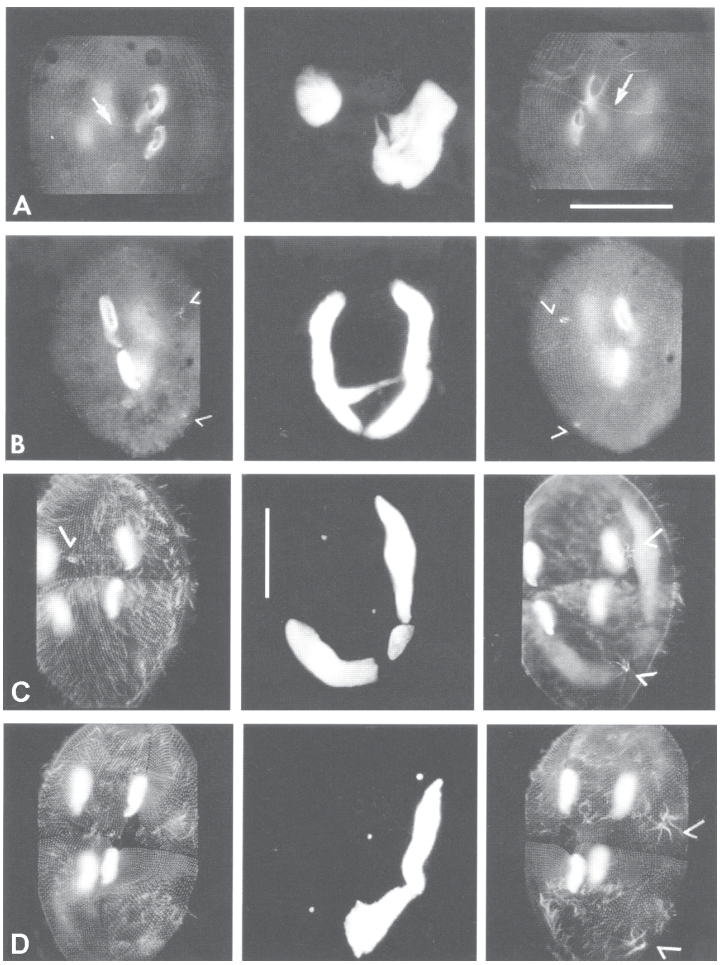

Fig. 5. Relation between contractile vacuole number, shape and position of the macronuclear structures during division in almost perfect doublets of *P. tetraurelia* (A, B), and *P. undecaurelia* (C, D). Each image of the nuclear apparatus is placed between the two cortical views of the same cell. Two micronuclei are visible in C and three in D. In A the fission furrow is decorated on both side of doublet (arrows). New CVs are not well decorated. A: Early division stage with different size macronuclei. B: Two macronuclei elongating along the two complete CV meridians (external half arrows). C: Shape of three elongating nuclear structures in a cell with normal CVM1 and only one contractile vacuole in the middle of CVM2 (half arrows). D: Shape of a single macronucleus in doublet with only one CVM with two CVs. Antibodies: CTR 210 (A, B), TEU 348 (C, D). Hoechst staining of nuclear apparatus. Bars = 60 μm. From PRAJER *et al.* (1999).

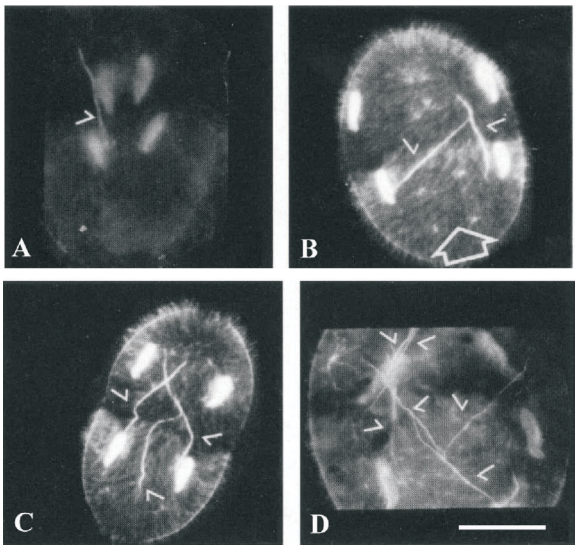

Fig. 6. Mispositioning of a different number of micronuclei during the division of a *P. tetraurelia* doublet. A-D: From one to six micronuclear separation spindles (half arrows). Two CV meridians are present in B. Antibody 6-IIB-1. Bar = 60 μm. Modified from PRAJER *et al.* (1999).

bution of macronuclear material between daughter cells during vegetative fission; and (3) an abnormal number of micronuclei (Fig. 6) and macronuclear anlagen at autogamy. According to the observations PRAJER *et al.* (1999), cortical and nuclear events were correlated during division by means of the contractile vacuole system and the cell volume seemed to be the determinant in this process. In regulating doublets, the enlarged cell volume leads to an increase in the number of contractile vacuoles (CVs) and to changes in their positioning (Fig. 7). It was suggested that abnormal number, size and position of macronuclei in doublets are related to the number, spread and positioning of the CVs. During division the position of the macronucleus seemed to be controlled by the number and position of the contractile vacuoles (Fig. 5). Therefore, the hypothesis of a general link between cortex and nuclei was reinforced. This may well account for the abnormalities in macronuclear partition observed in unbalanced doublets since macronuclei appear to be "quartered" between both dorsal surfaces bearing the CV meridians (Figs 2 A; 3 B). In consequence, this leads to the gross asymmetries in the distribution of the macronuclear DNA to the two daughter doublets (BERGER & MORTON 1980). The homopolar doublets underwent a complex cortical reorganization over the time of regulation (for details see IFTODE *et al.* 2001). The important event in this process is the increasing dominance of one oral region and final invagination and resorption of the other one (Figs 8 & 9), preceding the reversion to the singlet state. Therefore, the increased global imbalance of regulating doublets have consequences for cell architecture. Changes in cell form and the concomitant reduction in cell volume may cause internal modifications in the positioning of organelles and nuclei which maintain their linkage to the cortex (PRAJER *et al*. 1999).

The tendency of abnormal doublet cells to take the form of single cells through the regulation process can be explained by INGBER's (1993, 1998,

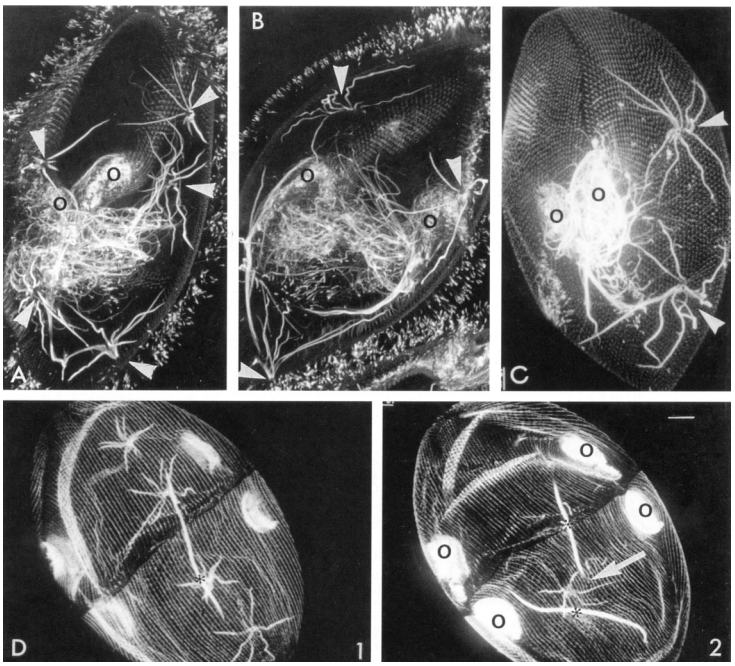

Fig. 7. Different steps of contractile vacuole meridian (CVMs) regulation. A: Five CVs (arrow heads) on two meridians. Some channels are shorter on the CV near one oral apparatus (O). B: Three CVs. One of them positioned in the posterior end of the cell. C: Two large CVs on one CV meridian remain in a 90° doublet. D: Dividing 140° doublet. One oral meridian is normal (1), while the another one bears only one posterior CV (arrows in 2); two micronuclear spindles are present (asterisk). One of them is situated completely in the posterior daughter cell. The microtubular spindle invades the entire cortex. TEU 348 antibody. Bar = 10 μm. From PRAJER *et al.* (1999).

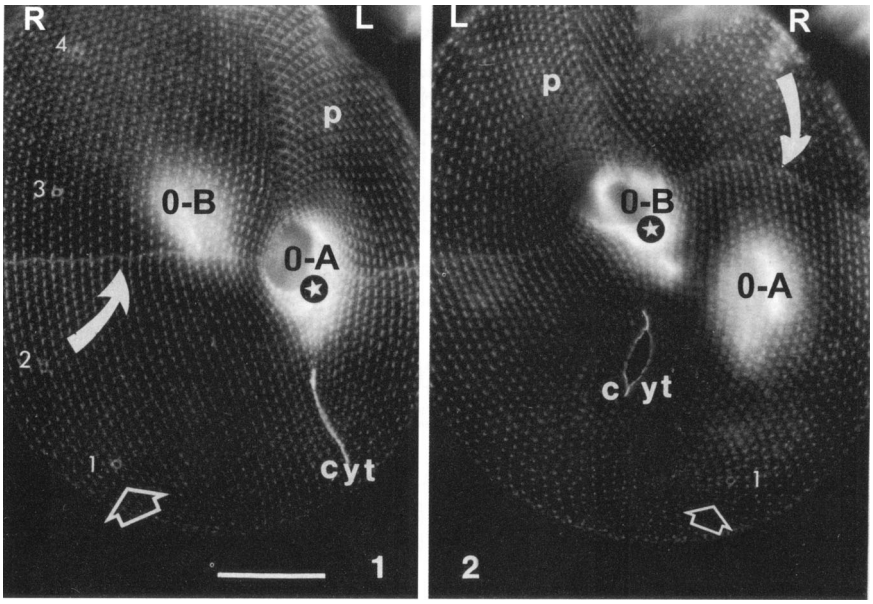

Fig. 8. Two surfaces of an almost perfect doublet (160°) of *P. tetraurelia* at the beginning of division. 1: View from the outside of the cell, with the oral apparatus O-A in focus, and the oral apparatus O-B on the opposite side of the cell. 2: In view through the cell the oral apparatus O-B is in focus. cyt – cytoproct, ✪ – oral anlage, open arrows – CV meridians, 1-4 - CV pores (1, 3 – parental, 2, 4 newly formed), curved arrows – fission line. R – right and L – left side of each semicell. CTR210 antibody. Scale bar = 15 μm. From IFTODE *et al.* (2001), with permission.

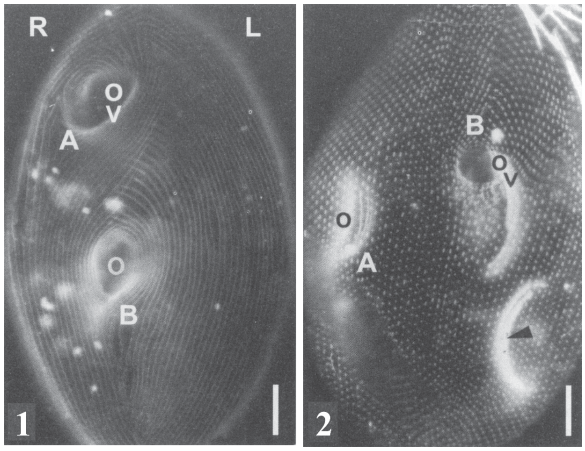

Fig. 9. Displacement and resorption of oral structures in regulating doublets of *P. tetraurelia*. 1: The oral region of oral meridian A is disappearing into an oral vacuole OV. The cytoproct and postoral suture are no longer visible. Oral meridian B is normal. R – right and L – left side of cell. Antibody S-8. 2: The oral apparatus of oral meridian B is invaginating into an oral vacuole (OV). The oral apparatus (o) of the oral meridian A, situated on the other side of the cell, is out of focus. A third set of oral structures undergoing digestion in the endoplasm is visible (arrowhead). Antibody IA2. Scale bar: 10 μm. From IFTODE *et al.* (2001), with permission.

2003) tensegrity model. According to this model the cytoskeleton of eukaryotic cells is a complex of an interweaving meshwork of filamentous and tubular biopolymers. They constitute the mechanical basis of all cytoskeletal structure coordination. Cells use tensegrity (i.e. tensional integrity) architecture for their organization. The cell architecture based on mechanical forces is globally integrated and dynamic. Internal mechanical tension is a driving force that directs cytoskeleton pattern formation. Hence, all the alterations in the balance of cellular mechanical forces will drive a structural remodeling cascade at the molecular level. In accordance to the tensegrity model, KACZANOWSKA *et al.* (1995) demonstrated that during *Paramecium* cell division, the mechanical consequences of phosphorylation-dependent assembly or disassembly of certain proteins are involved in the mechanics of pattern formation that govern the equatorial positioning of the oral apparatus. Tensegrity is a plausible model explaining the mechanism of the cellular response to mechanical stress exerted on *Paramecium* doublets if initial mechanical cortical symmetry is broken at the moment of fusion of two macronuclei into one large structure, which is considered as a first step of the regulation process. The progressing reduction of kinety number, associated with an altered form of the macronucleus and closely related to the changes in size and number of the contractile vacuole system, gradually affect the structural equilibrium in doublets. Hence, the structural symmetry and tensegrity deteriorates. Interactions of tensegrity forces can constitute the mechanism driving the local regulation of cytoskeletal tubulin polimerization/depolimerization. Therefore, metabolic alteration during the cell cycle such as phosphorylation/dephosphorylation or cytoskeletal element contraction/decontraction, associated with the morphogenesis process, can modify the fate and positioning of the macronuclei. However, the regulating abnormal cells tend to recover their structural integrity during successive abnormal di-

visional morphogenesis and finally establish the single state structural balance. It may be that during the regulation process the disturbed internal mechanical forces drive the shape modification as well as the reorganization of the cytoskeleton pattern towards singlet formation. Therefore, it is likely that frequent meiosis at autogamy, resulting in shortening of the interautogamous interval in *Paramecium* doublets, are induced directly by mechanical stress, consistent with the tensegrity model. Thus, in doublet cells some epigenetic stimuli such as changes in the structures of the cortex, connected with enlargement of the cell surface induced by increased cell volume, have an impact on the induction of the sexual process.

Exogenous factors affecting the induction of meiosis

Besides the genetic and endogenous epigenetic factors, the induction of meiosis can also be controlled by environmental factors. The effects of such exogenous factors on the length of the interautogamous interval were investigated in *Paramecium primaurelia* (PRAJER 2005). The cells of this species, characterized by a relatively long autogamous immaturity period in comparison with the other representatives of the *P. aurelia* complex (KOŚCIUSZKO & PRAJER 1988), were separately submitted to the action of glucose, sorbitol and insulin. These substances were used as the supplementary component of culture medium, in the maximum concentration that did not affect the rate of daily divisions, the general outline of the cells nor the swimming behavior. All these agents always induced autogamy earlier in comparison with the control series of cells cultured in non-supplemented medium, and shortened the interautogamous interval (Fig. 10).

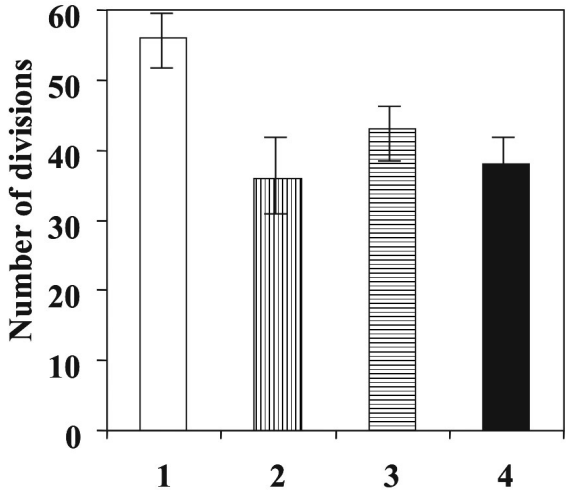

Fig. 10. Effects of glucose, sorbitol and insulin on IAI duration in *P. primaurelia* lines. 1 – control cells. Cells treated with: 2 – glucose, 3 – sorbitol, 4 – insulin. According to PRAJER (2005).

Glucose as an ubiquitous nutrient for eukaryotic cells is a source of carbon and energy. The mechanisms involved in glucose sensing are tightly connected with metabolism pathways in animals as well as in plants (ROLLAND *et al.* 200l). In mammalian cells and in yeasts external glucose in the culture medium induces the expression of specific glucose-regulated proteins that may lead to apoptosis (LEE 2001) or inhibit entry into meiosis (HONIGBERG & PURNAPATRE 2003). In *Paramecium,* shortening of the autogamous immaturity period was consistently induced by treatment of growing cells with low doses of glucose. Since glucose may affect the cells both as a nutritional and osmotic agent, a non-metabolized sugar, sorbitol, having a similar molecular size as glucose, was also used in concentrations identical to those of glucose (thus the osmolarity of the culture medium for both agents was equivalent). The effect of sorbitol on the IAI duration was similar to that of glucose. Such results suggest that a mild dose of glucose delivers an osmotic shock as does sorbitol, rather than a nutritional effect resulting in the inhibition of meiosis.

The mechanisms promoting adaptation to variable environmental conditions have been highly conserved during eukaryote evolution. Extracellular signals generate a number of different types of cellular responses which are implicated in the control of cell growth and developmental mechanisms. It has been established that mitogen activated protein kinases (MAPKs), a class of signaling intermediates, play an essential role in the development of many different types of organisms (PEARSON *et al.* 2001). A subclass of the stress activated protein kinases (SAPK) belonging to the group of MAP kinases, includes p38 kinase(s) which responds to osmotic stress stimuli such as shocks of hyperosmolarity (MARTIN-BLANCO 2000). This invertebrate p38 kinase has high sequence similarity to the Hog 1 kinase involved in hyperosmotic shock protection in *Saccharoromyces cerevisiae* (PEARCE & HUMPREY 2001). Thus, this kinase family has been evolutionary conserved from yeast to mammals. Indeed, WANG *et al.* (1999) found that mild hyperosmotic shock of sorbitol applied to *Tetrahymena* activated the expression of mRNA kinases including TpMAPK, which shows significant sequence similarity to the p38 kinases. A different TpNrk also revealed high homology to other NIMA-related protein kinases (WANG *et al.* 1998; NAKASHIMA *et al.* 1999) involved in the control of cell cycle regulation in response to osmotic shock. Thus, the evolutionary conservation of stress activated signaling pathways between mammals and yeast indicates the importance of this regulatory pathway in cellular physiology.

Osmotic stress may induce the activation of parallel pathways of the MAP-related kinases in *Paramecium* cells. It is well documented that the stress activated kinases can modulate the course of the cell cycle and affect the checkpoint activation. The mechanisms promoting meiosis in unfavorable conditions are evolutionarily conserved and may resemble the induction of sporulation in the mutant sho1 of yeast (SEET & PAWSON 2004). In these cells with disturbed homeostasis and characterized by diminished function of the mutated scaffold protein Pbs2, high osmolarity shock treatment activated the mating pathways instead of an osmoresponse. It may be speculated that *Paramecium,* as *Tetrahymena,* can modify the rate of protein synthesis of MAP kinase cascades as well as signal transduction pathways, and change gene expression in response to various environmental conditions. However, further experiments are needed to confirm this hypothesis.

On the other hand, an insulin hormone was used as a candidate for a sugar dependent factor complementary to glucose, and its effect on IAI duration was investigated. Treatment with the entire molecule of insulin applied in much lower concentrations than sugars also shortened the IAI duration. All these results taken together suggest that glucose affects the IAI duration both as an osmotic agent and as a metabolite inducing insulin dependent pathways. The potential homologs of genes encoding insulin receptors and insulin itself in the ciliate genome were not found (for details see PRAJER 2005). However, this hormone can affect the life functions of protozoans (CSABA & LANTOS 1975; KOHIDAI *et al.* 1992; CHRISTENSEN 1993; CSABA & HEGYESI 1994; CSABA & KOVACS 1995; MUGNAINI *et al.* 1995; SHEMAROVA *et al.* 2002; CHRISTENSEN *et al.* 2003), although these unicellular organisms can produce proteins immunologically similar to insulin. On the other hand, this hormone molecule may mimic the effect of unknown environmental or autocrine factors. It was suggested that insulin pathways may exert an effect on lifespan by regulating a variety of genes including the cellular stress response. Some exogenous proteins can nonspecifically stimulate the activation of phagocytosis. Excessive endocytosis can affect cell homeostasis and according to this hypothesis, may induce meiosis. Thus, the entire molecule of insulin in *Paramecium* cells may act as an unspecific inducer of pinocytosis or phagocytosis crucial for the induction of shortening of the IAI duration. Therefore, the non-receptor mechanism of insulin accumulation must be taken into account in this organism. It may be energy independent of the endocytotic pathway (HARADA *et al.* 1992) or the non-receptor trafficking of insulin through passive transcapillary diffusion (HAMILTON-WESSLER *et al.* 2002).

On the basis of the reviewed articles it can be concluded that:

i – The interautogamous interval (IAI) is controlled genetically by the macronucleus and its duration is characteristic for particular species and even for stocks. However, besides the nuclear factor, the cytoplasmic protein immaturin also plays a role in the regulation of the IAI.

ii – Epigenetic factors such as changes in the cell architecture observed in artificially produced doublets of *Paramecium* induce frequent autogamies during doublet regulation towards the normal singlet cells. Such non-genetically disturbed cytoskeletal structures, induced by increased cell volume and the modified ratio of cell surface to volume, exert internal mechanical stress. This constitutes the endogenous, epigenetic impulse accelerating meiosis and shortening the IAI duration.

iii – Mild osmotic stress, acting as an exogenous factor, can initiate the specific MAP kinases signaling pathway resulting in the induction of earlier meiosis, as in other unicellular eukaryotes (SEET & PAWSON 2004), and shortening the IAI duration.

It can be speculated that numerous factors can be engaged in the initiation of meiosis. However, the role of epigenetic stimuli may be significant, as revealed in *Paramecium* research and confirmed in numerous recent studies undertaken mostly on higher eukaryotes, in which meiosis is the specialized cell division that is essential for germ cells to develop into functional haploid gametes. It was suggested that this process can be cell-autonomously initiated by a putative meiosis-inducing substance such as retinoic acid, which functions as an inducer of meiosis in mouse embryonic gonads (BOWLES *et al*. 2006; KOUBOWA *et al.* 2006). Retinoic acid induces the expression of specific genes triggering meiosis by inducing molecular cascades cooperating in establishing the particular chromosome structure which initiates and promotes meiosis (MATSUI & HAYASHI 2007). Therefore, a specific epigenetic status of germ line chromatin structure is needed for the initiation of meiosis. Heterochromatin assembly and gene silencing probably regulate the process of meiosis. Changes in histone modifications may define the chromatin status required for meiosis specific gene expression (HAYASHI *et al.* 2005). The establishment of a specific chromatin structure before the initiation of meiosis is necessary for proper progression of meiotic prophase, as well as for exit of this process (YOSHIDA *et al.* 2007). On the other hand, it was revealed that cellular stress induces pairing of homologous heterochromatin as a genetically controlled response to genotoxic and

non-genotoxic stressors (ABDEL-HALIM *et al.* 2006). Stress events inducing the expression of a set of highly conserved "stress proteins" acting through the MAP kinases pathway may activate proteins engaged in meiosis (LAROSA & DOWNS 2007). All these results suggest that meiosis initiation is a complex process, in which an important role is played by epigenetic factors activating the cellular stress response. However, numerous mutually correlated events need to be extensively studied at the molecular level.

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