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The conjusome: a novel structure in *Tetrahymena* found only during sexual reorganization

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SUMMARY

A unique structure, the conjusome, has been identified and initially characterized in *Tetrahymena thermophila*. The conjusome appears only during a specific phase of conjugation. Immunofluorescence microscopy reveals that the conjusome is strongly labeled by antibodies to the protein Pdd1p. Pdd1p is a chromodomain protein and participates in the formation of chromatin-containing structures in developing macronuclear anlagen. Recent studies suggest that Pdd1p is physically associated with the elimination of specific germ-line sequences from developing macronuclei (anlagen) and may play a role in heterochromatin assembly. The conjusome contains Pdd1p, but it is devoid of any detectable DNA. The conjusome appears before DNA elimination begins in the developing anlagen and after Pdd1p is found in the parental macronucleus. Transmission electron microscopic observations reveal that the conjusome is not a membrane-bounded structure. The conjusome ranges in size from about 1 μm to sizes approaching 7 μm, depending on its maturity. It is composed of a coarse reticulum of a fibrous, electron dense material, interspersed with apparent background cytoplasm. Our initial characterization does suggest a number of possible functions for what may be a new, transient organelle.

Key words: Anlagen, Pdd1p, Macronucleus, Ciliate, Conjugation

INTRODUCTION

A diagnostic feature of the Ciliophora is the possession of two types of differentiated nuclei in the same cell: the germline micronucleus, and the somatic macronucleus. The polycopy macronucleus is transcriptionally active, and determines most of the phenotype of the cell, while the diploid micronucleus is transcriptionally silent and contributes little to cellular phenotype (Ng, 1986; Prescott, 1994). This distinction between micro- and macronuclei, sexual versus vegetative function, is analogous to the germ line/soma line differentiation found in metazoans. During conjugation, cells of complementary mating types pair and initiate the sexual process of nuclear reorganization (Bruns and Brussard, 1974). After micronuclear meiosis and subsequent reciprocal exchange and fusion of haploid gamete nuclei, mitotic progeny of the zygotic nucleus differentiate and give rise to the new macronuclei and micronuclei (Nanney, 1953; Orias, 1986). After the second post-zygotic mitosis in *Tetrahymena thermophila*, two undifferentiated nuclei migrate to the anterior pole and two to the posterior pole of the cell. The two nuclei that migrate anteriorly become determined to develop into macronuclei, while the two that migrate posteriorly will become micronuclei (Nanney, 1953). During this interval when new macronuclei (macronuclear anlagen) and new micronuclei are developing, the parental macronucleus begins its programmed nuclear death by a highly regulated process (Nanney, 1953). Thus, late in conjugation, three different nuclear fates occur in parallel within a common cytoplasm: apoptosis of the parental macronucleus (Davis et al., 1992; Mpoke and Wolfe, 1996; Madireddi et al., 1996), micronuclear differentiation, and macronuclear differentiation.

Large-scale rearrangements and changes of the genome take place within the macronuclear anlagen of ciliates (Coyne et al., 1996; Klobutcher and Jahn, 1991; Madireddi et al., 1995; Prescott, 1994). These events include transcriptional activation (Wenkert and Allis, 1984), DNA excision (Yao et al., 1984; Yao and Gorovsky, 1974), chromatin remodeling (Allis and Wiggins, 1984; Stargell et al., 1993), nucleolar biogenesis and gene amplification (Orias, 1986; Prescott, 1994; Yao, 1986; Yao and Gall, 1979), and chromosome breakage (Yao et al., 1990) followed by telomere addition (Greider and Blackburn, 1989). In *Tetrahymena* anlagen, specific micronuclear-limited DNA segments are removed (~15% of the genome; Yao and Gorovsky, 1974). Pdd1p, a conjugation-specific polypeptide, localizes to electron-dense heterochromatic-like structures at the periphery of developing anlagen (Smothers et al., 1997a) and co-localizes with micronuclear-specific DNA sequence deletion elements in the anlagen (Madireddi et al., 1996). These heterochromatic structures, resembling mature nucleoli,

have now been shown to be unique organelles in developing anlagen (Smothers et al., 1997a). The abundant nature of Pdd1p suggests that it plays a structural role, forming a complex with deletion-specific DNA-binding factors, thereby promoting a change in chromatin structure that brings appropriate boundaries of eliminated sequences into the correct conformation for excision (Coyne et al., 1996; Madireddi et al., 1994) and ligation. Recently, biochemical analyses have demonstrated a physical link between Pdd1p and germ-line restricted chromatin (Smothers et al., 1997b). At certain times in conjugation, transient levels of Pdd1p have also been found in the parental macronucleus (Madireddi et al., 1994). Programmed DNA degradation in the parental and in developing macronuclei occurs at approximately the same phase of conjugation (Austerberry et al., 1984; Davis et al., 1992; Mpoke and Wolfe, 1996; Yokoyama and Yao, 1984). No direct functional link has been found between Pdd1p and DNA degradation in the parental macronucleus. However, Pdd1p is present when the parental macronucleus begins to degenerate (Madireddi et al., 1994).

Observations, using phase contrast or Nomarski optics, of living, immobilized pairs of conjugating *T. thermophila* at specific phases of conjugation, revealed a hitherto unreported structure in the anterior of each conjugant partner. This structure did not label with DAPI or other DNA-specific stains. Staining with antibodies directed against Pdd1p revealed strong labeling. This paper is a description of this new structure, which we have named the conjusome.

MATERIALS AND METHODS

Cell culture conditions

Genetically marked strains of *Tetrahymena thermophila* Nanny & McCoy, 1976, CU 427 (*Chx/Chx*[cy-s]VI) and CU 438 (*Pmr/Pmr*[pm-s]IV) were grown independently to densities of ~250,000-500,000 cells/ml in proteose peptone and yeast extract medium (PPY) as described by Ng and Frankel (1977), and starved in Dryl's solution (Dryl, 1959) for 18-24 hours prior to mixing. The cell strains were provided by Peter Bruns (Cornell University, Ithaca, NY). In order to induce cells to be sexually reactive, equal numbers of cells of complementary mating type were mixed following starvation, according to established procedures (Martindale et al., 1982). Matings with greater than 80-90% pairing and kinetics similar to those previously described (Martindale et al., 1982) were observed in all experiments reported. All time points, reported in hours, refer to the time from mixing of complementary mating types.

Rotocompression and light microscopy

Living, conjugating cells were picked individually at appropriate times by micropippeting and were placed into a rotocompressor (Aufderheide, 1986). Immobilized, rotocompressed conjugants were examined using high resolution Nomarski DIC or Zernike phase contrast optics on an Olympus BH2 microscope. No deleterious effects were observed when cells were placed back into growth medium and cultured.

Immunofluorescence and acid phosphatase staining

Living cells were prepared for in vivo fluorescence by incubating conjugating cells in 0.3 mg/ml DAPI and 0.1 mg/ml Acridine Orange simultaneously for one hour (similar to the method of Mpoke and Wolfe, 1997). Cells were then resuspended and washed 3× in Dryl's before observation on a UV fluorescent microscope. Observations were also made using only Acridine Orange (0.3 mg/ml) in living cells. All observations were made while cells were under rotocompression. Kodak Royal Gold 400 was used to record color images.

All cells were prepared for immunostaining as previously described (Wenkert and Allis, 1984), with the following modifications: after fixation and resuspension in methanol, cells were washed twice in PBS and then stored overnight in PBS with 2% BSA. Cells were incubated for one hour the following day at room temperature with immune or preimmune serum (appropriately diluted in PBS according to the titer of the immune serum with 2% BSA). The cells were then washed twice in PBS followed by a one hour incubation in Cy3-conjugated goat anti-rabbit serum (Sigma). For DNA staining, 0.1 mg/ml DAPI in 70% EtOH was added to the cell suspension after labeling and then washed out with PBS before mounting. This concentration of DAPI allowed excellent detection of all nuclei with a mercury lamp without interfering with the red fluorescence imparted by the Cy3-immunolabeled structures. The specificity of the primary antibody to Pdd1p has been demonstrated in previous reports (Madireddi et al., 1994). Background basal body staining is a phenomenon common to rabbit polyclonal sera (Turksen et al., 1982). Labeled cells were resuspended in 150 μl of PBS, mounted on slides and examined on an Olympus BH2 microscope using an epifluorescence illumination system mounted with standard fluorescent clusters. To record black and white images, Kodak Technical Pan Film was exposed using an Olympus C-35AD-2 camera.

Cytochemical localization of acid phosphatase was done according to Anderson's adaptation of the Gomori technique (Anderson, 1982). Observations were made using brightfield optics at appropriate time points in conjugation.

Electron microscopy and immunocytochemistry

Appropriately staged mating cells were fixed in 0.5% glutaraldehyde, 2.0% paraformaldehyde in 0.1 M cacodylate buffer, dehydrated and embedded in LR white and polymerized at 65°C for 24 hours. Ultrathin sections were mounted on colloidon coated copper grids and were stained for 5 minutes in aqueous uranyl acetate and 30 seconds in Reynolds' lead citrate or subjected to immunodetection.

For immunodetection, grids were incubated for 1 hour in blocking solution (2% BSA and 0.1% sodium azide in PBS). Grids were then incubated for 1 hour in Pdd1p primary antiserum diluted 1:100 in PBS solution followed by 5× 5 minute washes in PBS. Samples were then incubated for 40 minutes with secondary antibody (goat anti-rabbit conjugated to 10 nm gold beads; Amersham). Secondary labeling was followed by 5× 5 minute washes in PBS and then two washes in dH_2O and then stained with uranyl acetate and Reynold's lead citrate as described above. All examinations were done on a Zeiss 10C transmission electron microscope operating at 60 kV.

RESULTS

The conjusome appears at a distinct time and place during conjugation

With the use of the rotocompressor (Aufderheide, 1986), the cells were held motionless, thus offering the observer a detailed view of living cells. Observations of conjugants showed that the time of appearance of the conjusome corresponded closely with the condensation of the parental macronucleus. The parental macronucleus became 'grainy' in appearance at 5.5 to 6.5 hours postmixing of complementary mating types. This conspicuous event correlated with the development of macronuclear anlagen and the appearance of the conjusome (Fig. 1A). Although the mass matings were fairly synchronous, the precise timing of mating events can differ some among conjugating pairs. Visual markers, such as the appearance or state of mitotic spindles, were necessary to identify the exact

stage of conjugation in a given pair. We were thus able to correlate the conjusome with specific nuclear events during conjugation.

The appearance of the conjusome at approximately 6 hours corresponded very closely to the time when anlagen first appeared following the last post-zygotic mitosis (arrows, Fig. 2B). The conjusome was usually in close proximity, and anterior, to the two anlagen at this stage in development (Figs 1A, 2A,B). The parental macronucleus also started to condense at about this time. The series of Nomarski images in Fig. 1 show the conjusome within two different living, conjugating pairs at early and late stages of parental macronucleus condensation. The conjusome was present throughout the time that the parental macronucleus condensed (condensation takes approximately 0.5-1.5 hour). The parental macronuclei linger in their condensed state for a number of hours before undergoing pycnosis and complete degeneration and resorption. The early conjusome was small, less than 1.0 μm in diameter (Fig. 2A). It subsequently (see Fig. 2B) was capable of reaching dimensions rivaling those of early, developing anlagen (5.0-7.0 μm in diameter). The conjusome is normally spherical in form, although it occasionally has assumed a somewhat crescent and/or elongated shape. Once the old macronucleus condensed, the conjusome began to shrink and eventually disappeared.

Fig. 3 shows a time series of anti-Pdd1p staining in the three structures that display this protein at selected times during sexual reorganization: the parental macronucleus, the conjusome, and the developing macronuclei. The parental macronucleus showed antibody labeling for Pdd1p at roughly 4.0 hours post mixing. This labeling persisted for about 1.0-3.0 hours, and ceased at approximately the same time the conjusome appeared. The conjusome appeared and also stained positive for Pdd1p at approximately 6 hours, which is 0.5-1.0 hour before the developing anlagen began to stain with the Pdd1p antibody. During the time that the conjusome was present, it labeled very intensely, often much more so than either the parental or developing macronuclei for Pdd1p (see Fig. 2). At the light microscopic level, Pdd1p was distributed evenly within the structure. Developing anlagen began to stain positive for Pdd1p at about 7.0 hours into conjugation. The parental macronuclei subsequently showed the presence of Pdd1p for a second time, at 10.0-12.0 hours. Pdd1p staining of the parental macronucleus, which is often asynchronous in the two cells of a conjugating pair, lasted approximately 0.5-1.0 hour. Conjugation ends with the separation of cells at about 12 hours. The anlagen continue to develop and remain Pdd1p positive until about 15 hours from the time of mixing (Madireddi et al., 1994). Occasionally, small structures other than the conjusome stained for Pdd1p in fixed specimens. These structures were often seen near the parental macronucleus or conjusome. It is important to note that occasionally, the DNA stain DAPI, revealed that these smaller structures in fact contained DNA. This is in distinct contrast to the conjusome, which was always DAPI negative and contained no detectable DNA.

Acridine orange staining for DNA, RNA and lysosomal activity in the conjusome was negative in living cells (Fig. 4B). At the time the conjusome was visible, the structure also did not stain for acid phosphatase by the Gomori technique, a classic indicator of digestive vacuoles and lysosomes. As

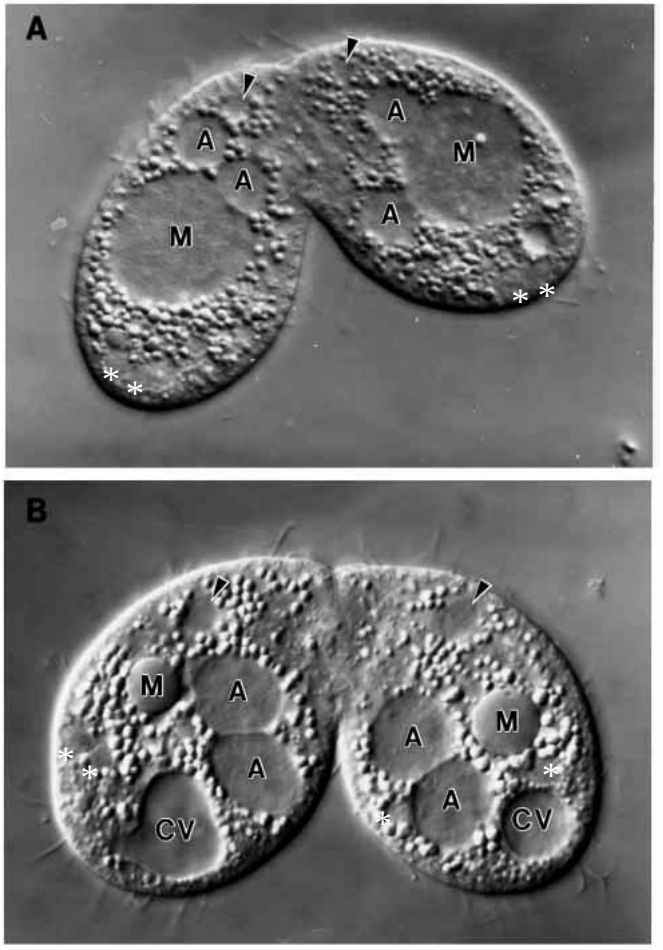

Fig. 1. Nomarski images of conjugating cells. Conjusome (arrowheads) were present at 6.0 hours (A). At this time, macronuclear anlagen (A) were just starting to enlarge and differentiate. The parental macronucleus (M) is beginning to condense. The micronuclei (asterisks) are still in the posterior of the cell. Conjugating pairs at 7-8 hours show a conjusome that has grown in size (B). The parental macronucleus has condensed and the macronuclear anlagen are much larger than in A. The micronuclei have migrated from the posterior of the cell. Note that A corresponds to the same time period as the fixed pairs in Fig. 2A and B. Cells in B are at approximately the same stage as the conjugant pairs in Fig. 2C and D. Contractile vacuoles (CV) were functioning in both pairs but were photographed before systole in B. ×1200.

expected, there were other acid phosphatase positive bodies in these cells (data not shown). Furthermore, immunocytochemistry using antibodies directed against ubiquitin showed no detectable ubiquitin in the conjusome (data not shown). The conjusome thus did not show characteristics typical of a lysosome or of the ubiquitin/proteasome pathway.

Electron microscopy

We examined conjugant cells using transmission electron microscopy (TEM). TEM revealed that the conjusomes were not membrane bounded (Fig. 5). The conjusome was composed of a coarse reticulum (see arrows in Fig. 6B). This reticulation forms a contiguous three dimensional meshwork that appears

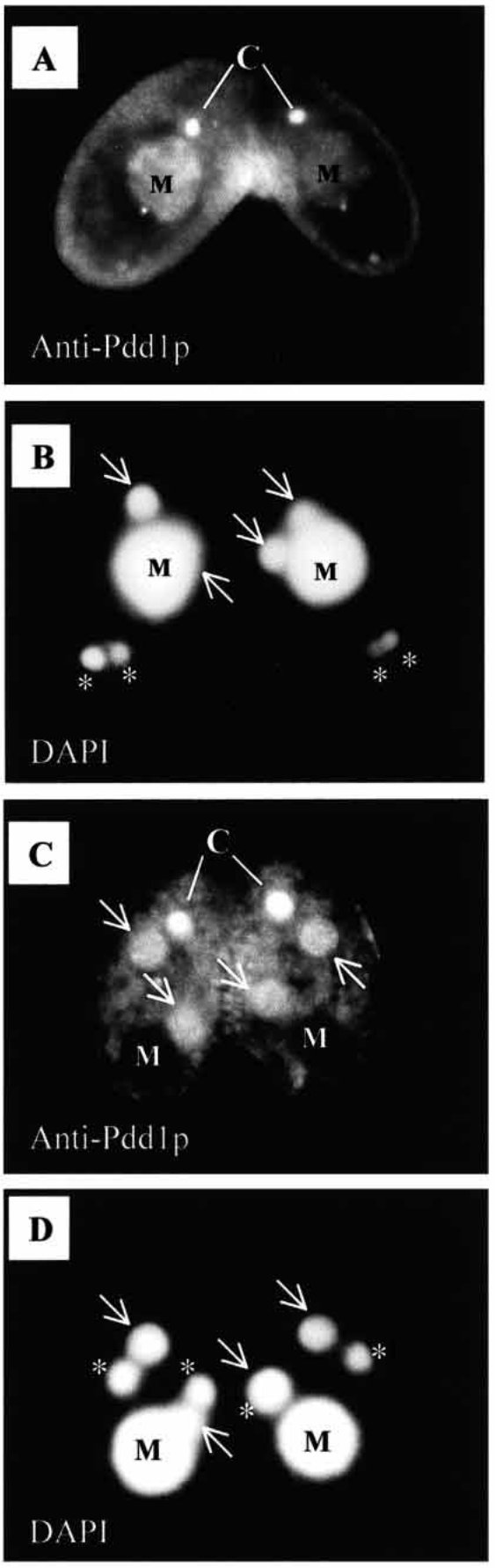

Fig. 2. Indirect immunofluorescence and DAPI staining in conjugating pairs. Fluorescent antibody staining in 6 hour (A) and 8 hour conjugant pairs (C) showed antibody directed against Pdd1p staining the conjusome (C) in each cell. The 6 hour pair also showed light staining of the parental macronucleus, while the 8 hour cells revealed the presence of Pdd1p in the developing anlagen (arrows, C). The same 6 hour (B) and 8 hour (D) pairs were also counterstained for DNA with DAPI. Anlagen (arrows) and micronuclei (asterisks) were visible. The parental macronucleus (M) also stained brightly with DAPI. The conjusomes did not stain with DAPI. The parental macronucleus had just started degenerating in the 6 hour pair and the micronuclei had not yet migrated anteriorly (B). The conjusomes were larger and the macronucleus no longer stained positive for Pdd1p antibodies in the 8 hour conjugants (C). The anlagen (arrows) showed their characteristic staining pattern at 8 hours while the micronuclei did not show Pdd1p immunolabeling in either pair. ×1000.

to be the framework of the conjusome. Intermingled within this reticulation were ribosomes. The concentration of ribosomes within the structure did not appear different from that of the cytoplasm. However, electron-lucent bodies found throughout the cytoplasm were excluded from the conjusome. The presence of Pdd1p was substantiated by immungold labeling using the Pdd1p antibody. Interestingly, the labeling (arrowheads) colocalized almost exclusively with the reticulum within the conjusome (Fig. 6B).

Immunolabeling of the *cnj9* mutant shows a distinct phenotype

To characterize the possible function(s) the conjusome has in the cell during conjugation, antibody staining was examined in a mutant line that does not develop new macronuclei. Nuclei in the *cnj9* mutant fail to complete the second post-zygotic division (Cole and Soelter, 1997). This inability to undergo the last mitosis left each cell with two nuclei, which both appeared to remain micronucleus-like. They are somewhat larger than normal micronuclei, but smaller than developing anlagen. In conjugating pairs of *cnj9* cells that show this characteristic phenotype, Pdd1p labeling was seen only in a very large conjusome at 6.0-11.0 hours postmixing (Fig. 7A). As in wild-type, the conjusome in this mutant lacked DNA (Fig. 7B). The conjusome in *cnj9* cells persisted longer than in wild-type cells, although the duration of the persistance varied from pair to pair. It is important to note that the conjusomes persisted longer in this mutant and conjugation events appear to be delayed to some degree in *cnj9* cells (Cole and Soelter, 1997). In the *cnj9* mutant, the parental macronucleus stained positive for Pdd1p early on, as in the wild-type. The timing of events in labeled *cnj9* cells and the conjusome was also substantiated by observations of living, rotocompressed, conjugants of this mutant type.

DISCUSSION

Documentation of conjugation and sexual reorganization in *Tetrahymena thermophila* has been done predominantly by examination of fixed specimens (Nanney, 1953; Ray, 1956; Martindale et al., 1982; Numata et al., 1985; Orias, 1986; Gaertig and Fleury, 1992; Madireddi et al., 1994; Nelson et al., 1994). With the use of the rotocompressor (Aufderheide,

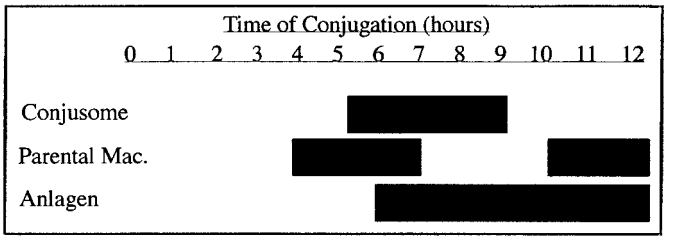

Fig. 3. Staining of the three different organelles was assessed by observing fixed and immunolabeled cells at the appropriate time points of conjugation. The blocks correspond to positive staining of that organelle to the antibody directed against Pdd1p during that time in conjugation.

1986), we have been able to immobilize and observe sexual reorganization in living cells. Rotocompression allows the experimenter to have an unprecedented view into the cell because it holds the specimen still and also gently flattens it. Observations of living, conjugant pairs with Nomarski and phase-contrast optics have allowed us to discover a number of important phenomena that occur during conjugation, most notably the appearance of a previously undocumented structure, the conjusome.

We have shown here that a transient structure appears during sexual reorganization in *Tetrahymena*. The conjusome appears in the anterior of conjugating cells during the time that early macronuclear anlagen development takes place. With the microscopic techniques we have used, the conjusome is visible for a period of 2-4 hours during conjugation. The ontogeny of the conjusome is not clear at this time. The conjusome ranges in diameter from approximately 1 μm to 7 μm. The conjusome is not membrane bounded and consists of a reticulum that forms its structural basis. This reticulum stains positive for the chromodomain protein Pdd1p. We hope to identify other proteins within the conjusome to see how they are temporally regulated.

Possible roles of the conjusome

We have a number of hypotheses suggesting what role the conjusome is playing during conjugation. The first is that the conjusome is acting as a 'dump' for Pdd1p and possibly other components from the parental macronucleus. In this fashion it may be degrading the components it collects. The second hypothesis is that the cells recycle Pdd1p, and possibly other components, from the parental macronucleus into the developing macronuclear anlagen. In this regard, the conjusome acts as a reservoir for components from the old macronucleus and stores or processes them for the newly developing macronuclei. Finally, the conjusome may be acting as a production or distribution center for Pdd1p. It is possible that combinations of any of the above mentioned three hypotheses may operate.

Thus far, we have been unable to document enzymatic activity in the conjusome that would indicate that the conjusome is acting as a site for degradation of any components from the parental macronucleus. Moreover, a site of degradation uncompartmentalized by a membrane is not consistent with usual eukaryotic lysosomal activity. Furthermore, degradation taking place by the usual ubiquitin/proteasome pathway seems unlikely since we do not

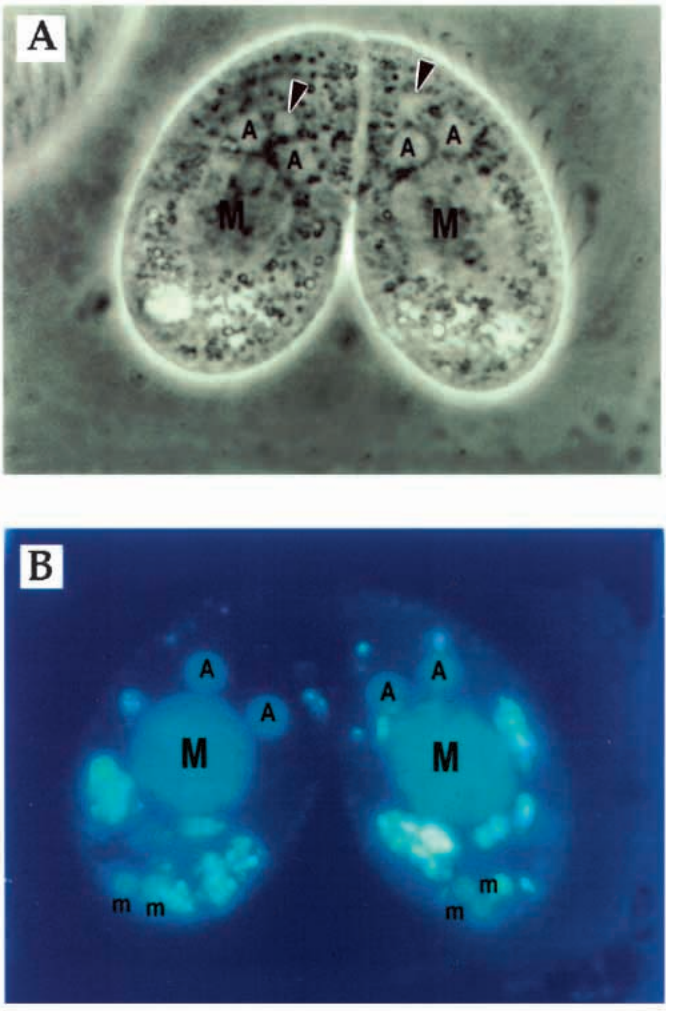

Fig. 4. Phase contrast and fluorescent microscopy of 6 hour living, conjugating pairs. Conjusomes (arrowheads) were in close proximity and anterior to early anlagen (A) in both cells (A). In vivo fluorescence microscopy of the same pair from A stained with Acridine Orange and DAPI revealed that the conjusome did not show a detectable presence of DNA, RNA or lysosomal activity (B).

find detectable amounts of ubiquitin in the conjusome. We do, however, have evidence that small Pdd1p-positive structures exist during the time the conjusome is present. Some small structures also contain DNA. It might be possible these smaller structures originated from the condensing parental macronucleus. These Pdd1p-positive structures could be precursors to the conjusome or it may be that these structures are part of some unknown transport mechanism that shuttles Pdd1p and possibly other components into and/or out of the conjusome.

If the Pdd1p protein found in the conjusome originates in the parental macronucleus, then it would seem plausible that *Tetrahymena* are using the conjusome as a reservoir to store or process this protein and possibly other components. The temporal coordination of the presence and absence of Pdd1p in the conjusome and the developing and parental macronuclei fits with this hypothesis. The close proximity of the conjusome to the developing macronuclei and the disappearance of the conjusome as Pdd1p staining in the developing macronuclei

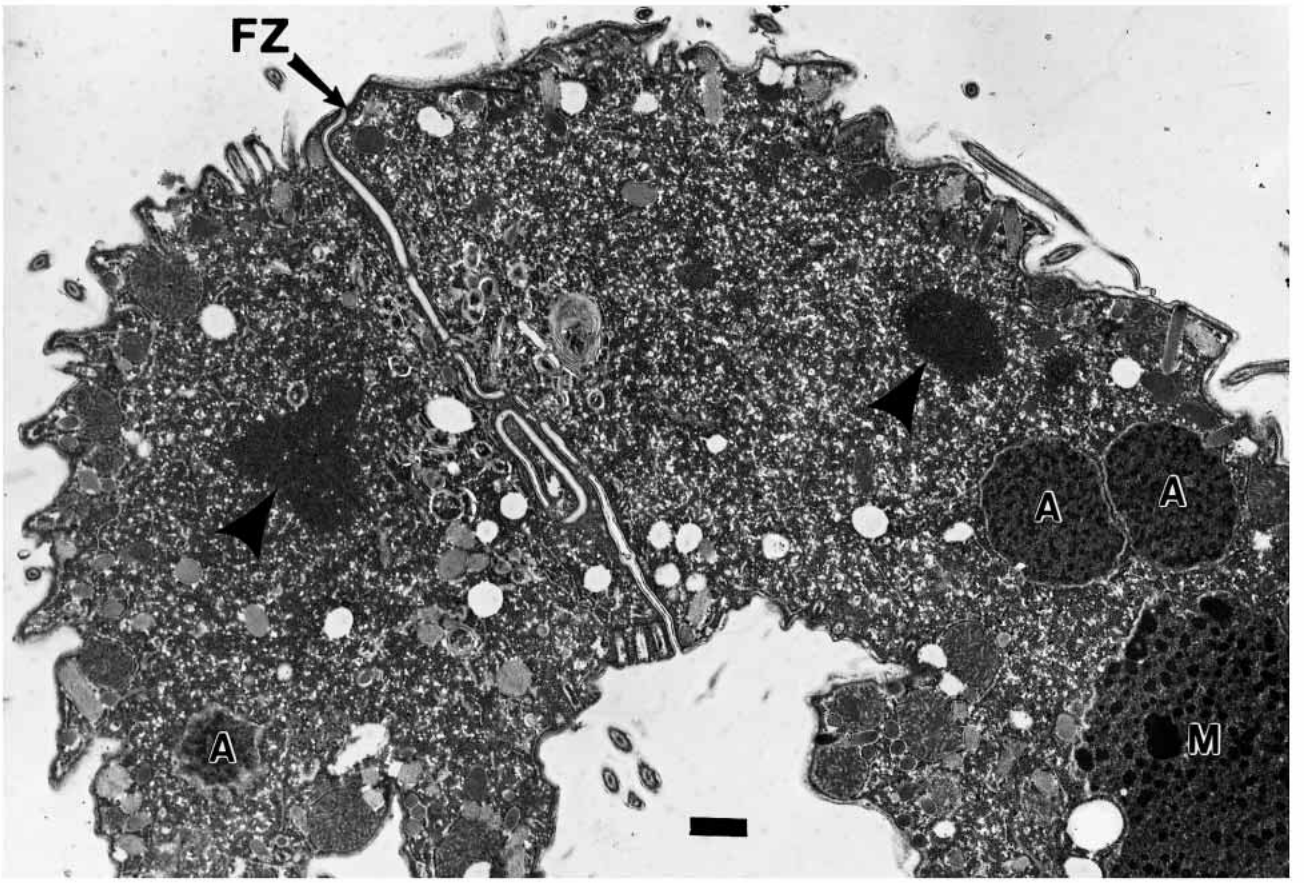

Fig. 5. Ultrastructural observation of conjusomes. Mating cells at 8 hours were fixed and prepared for transmission electron microscopy. Conjusomes (arrowheads) were visable in the anterior of each cell. The macronuclear anlagen (A) and the parental macronucleus (M) were visible in the cell on the right. The fusion zone (FZ) is the point of contact between the mating cells. Bar, 1 μm.

increases also is consistent with this view. In addition, the data obtained from the mutant *cnj9* lend credence to the idea that the components of the conjusome are destined to be transferred to the developing anlagen. When anlagen are not present, the conjusome has nowhere to deliver the Pdd1p, and thus the amount of Pdd1p remains high and the conjusome appears large. These observations suggest that the conjusome serves as a reservoir, possibly storing the Pdd1p and other components to support development in the anlagen. The *cnj9* data also do not lend support to the degradation hypothesis.

The presence of ribosomes within the conjusome could mean that Pdd1p is translated within the structure. The high concentration of Pdd1p in the structure and the significant translation rate at this period in development (Madireddi, 1994) makes it reasonable to believe that its production may take place within the conjusome as well. The translation of Pdd1p in the conjusome is very intriguing when considering that the fate of macronuclear anlagen is dependent on their localization in the anterior of the cell. It is possible, however, that little or no translation take place in the conjusome. The localization of Pdd1p may merely be the result of some unknown mechanism which shuttles cytoplasmically translated Pdd1p to the conjusome for further processing or distribution.

Other possible functions

If Pdd1p originates from the parental macronucleus and is stored or processed in the conjusome for subsequent use in the developing anlagen, the conjusome and its associated structures might be involved in some aspects of epigenetic phenomena that have been observed in ciliates (Meyer and Duharcourt, 1996; Chalker and Yao, 1996). It has clearly been shown that the parental macronucleus communicates with, and can have a maternal effect on, the developing macronuclei (Preer, 1997). The mechanism of this type of communication is unknown at this time, but experiments (Sonneborn and Schneller, 1979; Epstein and Forney, 1984) show that some somatic mutations are transmitted in a maternal fashion and not inherited in a typical Mendelian style.

The conjusome is spatially associated with the developing anlagen in the anterior of the cell during the time of nuclear differentiation. It has been shown that the developmental fates of nuclei are related to their location within the cell (Kaczanowski et al., 1991; Gaertig and Fleury, 1992) at critical times. The presence of the conjusome in the cell anterior at the time of macronuclear determination may be merely coincidental. The conjusome might be acting independently of these events and is merely coordinate with nuclear events in time and not causally linked to their development. This association warrants further investigation. Interestingly, the conjusome has some structural resemblance to the granulofibrillar material (GFM) found in the mitochondrial cloud in *Xenopus laevis* (Heasman et al., 1984) and the

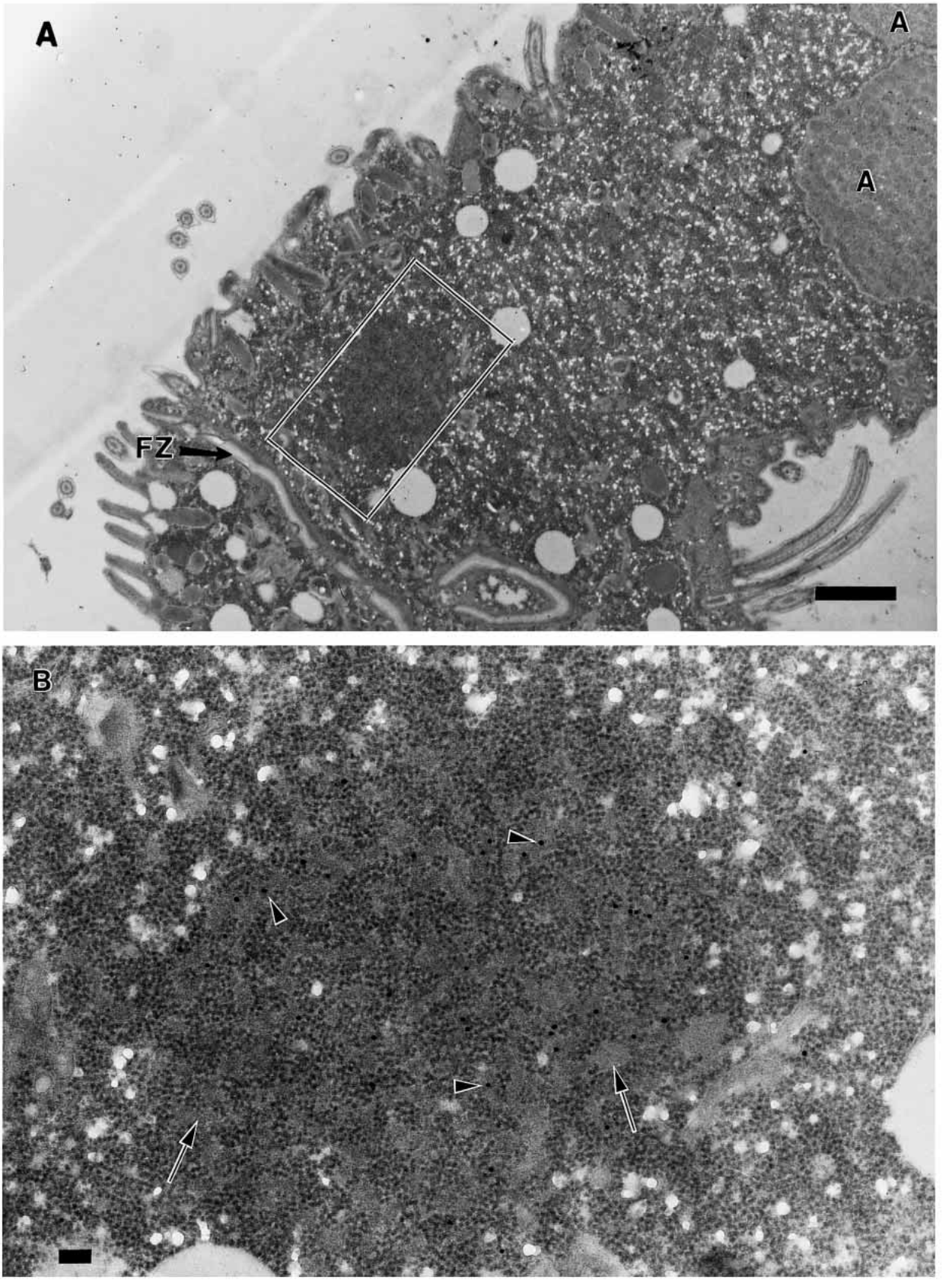

Fig. 6. Pdd1p immunogold cytochemistry of conjusomes. Mating cells at 8 hours were fixed and processed for immunogold cytochemistry using Pdd1p antibodies. The conjusome was present at lower magnification (A). Bar, 1 μm. The area inside the rectangle is shown at higher magnification (B). Note the electron dense reticulation (arrows) and the immunogold labeling (arrowheads) within the conjusome. Bar, 100 nm.

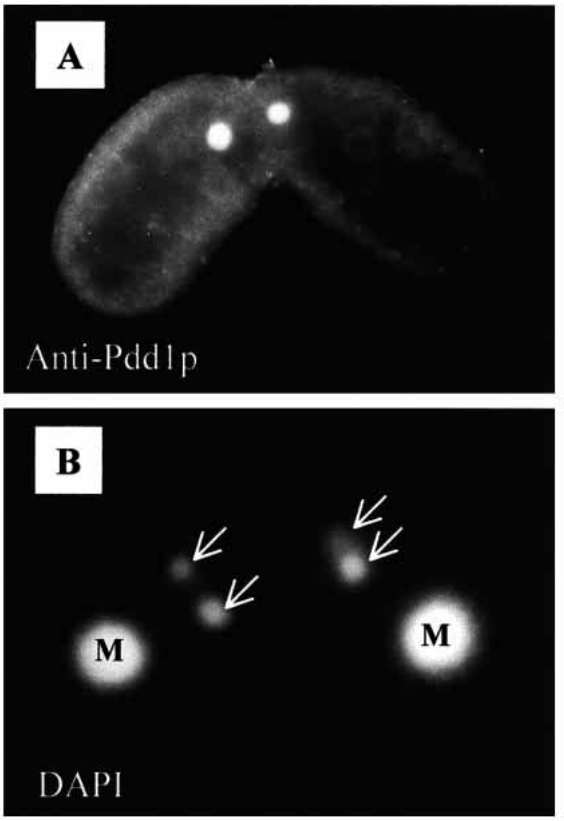

Fig. 7. Indirect immunofluorescence and DAPI staining in a mutant *cnj9* conjugating pair. Note the Pdd1p antibody labeling of the conjusome in each cell (A). The conjusomes were the only organelle positive for antibody labeling of Pdd1p in this pair at 10.0 hours. DAPI staining showed the parental macronucleus (M) and undifferentiated nuclei (arrows) of the same conjugant pair (B). Conjusomes, as in wild-type conjugant cells, did not stain positive for DAPI. Note that the parental macronucleus had already condensed. ×1000.

germline (P) granules of *Caenorhabditis elegans* (Wolf et al., 1983). Similar polar granules have been found in *Drosophila* (Mahowald, 1971; Illmense and Mahowald, 1974). It has been suggested that there is a relationship between the GFM and these germinal granules (Heasman et al., 1984). The significance of this structural similarity to the function of the conjusome is not known.

The microscopy data we have presented demonstrate that the conjusome has a unique structural appearance. Immunofluoresence data suggest that its protein constituents are uniquely expressed during conjugation and specifically localized to the conjusome. The appearance of the conjusome is time-limited, and highly coordinated to nuclear events during conjugation. All of these data suggest to us a specific function for this structure. For these reasons, we believe the conjusome is worthy of recognition as an organelle in *Tetrahymena*. We would also be interested to learn whether homologous structures are found in other ciliophorans. We are investigating further the structures and functions of the conjusome in development during the events of sexual reorganization.

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REFERENCES

Allis, C. D. and Wiggins, J. C. (1984). Histone rearrangements accompany nuclear differentiation and dedifferentiation in *Tetrahymena. Dev. Biol.* **101**, 282-294.

Anderson, R. O. (1982). Cytochemical localization of acid phosphatase (lysosomal and digestive activity marker enzyme). *Protocol. Protozool.* C22.1-22.7.

Aufderheide, K. J. (1986). Identification of the basal bodies and kinetodesmal fibers in living cells of *Paramecium tetraurelia* Sonneborn, 1975 and *Paramecium sonneborni* Aufderheide, Daggett & Nerad, 1983. *J. Protozool.* **33**, 77-80.

Austerberry, C. F., Allis, C. D. and Yao, M.-C. (1984). Specific DNA rearrangements in synchronously developing nuclei of *Tetrahymena. Proc. Nat. Acad. Sci. USA* **81**, 7383-7387.

Bruns, P. J. and Brussard, T. B. (1974). Pair formation in *Tetrahymena pyriformis:* An inducible developmental system. *J. Exp. Zool.* **183**, 337-343.

Chalker, D. L. and Yao, M. C. (1996). Non-Mendelian, heritable blocks to DNA rearrangement are induced by loading the somatic nucleus of *Tetrahymena thermophila* with germ line-limited DNA. *Mol. Cell Biol.* **16**, 3658-3667.

Coyne, R. S., Chalker, D. L. and Yao, M.-C. (1996). Genome downsizing during ciliate development: nuclear division of labor through chromosome restructuring. *Annu. Rev. Genet.* **30**, 557-578.

Cole, E. S. and Soelter, T. A. (1997). A mutational analysis of conjugation in *Tetrahymena thermophila.* 2. Phenotypes affecting middle and late development: Third prezygotic nuclear division, pronuclear exchange, pronuclear fusion, and postzygotic development. *Dev. Biol.* **189**, 233-245.

Davis, M. C., Ward, J. G., Herrick, G. and Allis, C. D. (1992). Programmed nuclear death; apoptotic-like degradation of specific nuclei in conjugating *Tetrahymena. Dev. Biol*. **154**, 419-432.

Dryl, S. (1959). Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during conjugation or autogamy. *J. Protozool.* **6** (suppl.), 25.

Epstein, L. M. and Forney, J. D, (1984). Mendelian and non-Mendelian mutations affecting surface antigen expression in *Paramecium tetraurelia. Mol. Cell. Biol.* **4**, 1583-1590.

Gaertig, J. and Fleury, A. (1992). Spatiotemporal reorganization of intracytoplasmic microtubules is associated with the nuclear selection and differentiation during developmental process in the ciliate *Tetrahymena thermophila. Protoplasma* **167**, 74-87.

Greider, C. W. and Blackburn, E. H. (1989). A telomeric sequence in the RNA of *Tetrahymena* telomerase is required for telomeric repeat synthesis. *Nature* **337**, 331-337.

Heasman, J., Quarmby, J. and Wylie, C. C. (1984). The mitochondrial cloud of *Xenopus* oocytes: The source of germinal granule material. *Dev. Biol.* **105**, 458-469.

Illmense, K. and Mahowald, A. P. (1974). Transplantation of posterior pole plasm in *Drosophila.* Induction of germ cells at the anterior of the egg. *Proc. Nat. Acad. Sci. USA* **71**, 1016-1020.

Kaczanowski, A., Ramel, M., Kaczanoska, J. and Wheatley, D. (1991). Macronuclear differentiation in conjugating pairs of *Tetrahymena* treated with the antitubuline drug nocodazole. *Exp. Cell Res.* **195**, 330-337.

Klobutcher, L. A. and Jahn, C. L. (1991). Developmentally controlled genomic rearrangements in ciliated protozoa. *Curr. Opin. Genet. Dev.* **1**, 397-403.

Madireddi, M. T., Davis, M. C. and Allis, C. D. (1994). Identification of a novel polypeptide involved in the formation of DNA-containing vesicles during macronuclear development in *Tetrahymena. Dev. Biol*. **165**, 418-431.

Madireddi, M. T., Smothers, J. F. and Allis, C. D. (1995). Waste not, want not: Does DNA elimination fuel gene amplification during development in ciliates? *Semin. Dev. Biol.* **6**, 305-315.

Madireddi, M. T., Coyne, R. C., Smothers, J. F., Mickey, K. M., Yao, M.-C. and Allis, C. D. (1996). Pdd1p, A novel chromodomain-containing protein, links heterochromatin assembly and DNA elimination in *Tetrahymena. Cell* **87**, 75-84.

Martindale, D. W., Allis, C. D. and Bruns, P. J. (1982). Conjugation in *Tetrahymena thermophila* (a temporal analysis of cytological stages). *Exp. Cell Res.* **140**, 227-236.

Mahowald, A. P. (1971). Origin and continuity of polar granules. In *Origin and Continuity of Cell Organelles* (ed. J. Reinert and H. Ursprung), pp. 159-169. Springer-Verlag, New York.

Meyer, E. and Duharcourt, S. (1996). Epigenetic programming of developmental genome rearrangements in ciliates. *Cell* **87**, 9-12.

Mpoke, S. and Wolfe, J. (1996). DNA digestion and chromatin condensation during nuclear death in *Tetrahymena. Exp. Cell Res*. **225**, 357-365.

Mpoke, S. and Wolfe, J. (1997). Differential staining of apoptotic nuclei in living cells: Application to macronuclear elimination in *Tetrahymena. J. Histochem. Cytochem.* **45**, 675-683.

Nanney, D. L. (1953). Nucleo-cytoplasmic interactions during conjugation in *Tetrahymena. Biol. Bull.* **105**, 133-148.

Nelson, E. M., Williams, N. E., Yi, H., Knaak, J. and Frankel, J. (1994). 'Fenestrin' and conjugation in *Tetrahymena thermophila. J. Euk. Microbiol.* **41**, 483-495.

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

Ng, S. F. (1986). The somatic function of the micronucleus of ciliated protozoa. *Prog. Protistol.* **1**, 215-286.

Numata, O., Sugai, T. and Watanabe, Y. (1985). Control of germ cell nuclear behavior at fertilization by *Tetrahymena* intermediate filament protein. *Nature* **314**, 192-194.

Orias, E. (1986). Ciliate conjugation. In *The Molecular Biology of Ciliated Protozoa* (ed. J. G. Gall), pp. 45-84. Academic Press, Orlando, FL.

Prescott, D. M. (1994). The DNA of ciliated protozoa. *Microbiol. Rev.* **58**, 233-267.

Preer, J. R., Jr (1997). Whatever happened to *Paramecium* genetics? *Genetics* **145**, 217-225.

Ray, C., Jr (1956). Meiosis and nuclear behavior in *Tetrahymena pyriformis. J. Protozool.* **3**, 604-610.

Smothers, J. F., Madireddi, M. T., Warner, F. D. and Allis, C. D. (1997a). Programmed DNA degradation and nucleolar biogenesis occur in distinct organelles during macronuclear development in *Tetrahymena. J. Euk. Microbiol*, **44**, 79-88.

Smothers, J. F., Mizzen, C. A., Tubbert, M. M., Cook, R. G. and Allis C. D. (1997b). Pdd1p associates with germline-restricted chromatin and a second novel anlagen-enriched protein in developmentally programmed DNA elimination structures. *Development* **124**, 4537-4545.

Sonneborn, T. M. and Schneller, M. V. (1979). A genetic system for alternative stable characteristics in genomically identical homozygous clones. *Dev. Genet.* **1**, 21-46.

Stargell, L. A., Bowen, J., Dadd, C. A., Dedon, P. C., Davis, M., Cook. R. G., Allis, C. D. and Gorovsky, M. A. (1993). Temporal and spatial association of histone H2A variant hv1 with transcriptionally competent chromatin during nuclear development in *Tetrahymena thermophila. Genes Dev.* **7**, 2641-2651.

Turksen, K., Aubin, J. E. and Kalnins, V. I. (1982). Identification of a centriole-associated protein by antibodies present in normal rabbit sera. *Nature* **298**, *763-765.*

Wenkert, D. and Allis, C. D. (1984). Timing of the appearance of macronuclear-specific histone variant hv1 and gene expression in developing new macronuclei of *Tetrahymena thermophila. J. Cell Biol.* **98**, 2107-2117.

Wolf, N., Priess, J. and Hirsh, D. (1983). Segregation of germline granules in early embryos of *Caenorhabditis elegans*: an electron microscopic analysis. *J. Embryol. Exp. Morphol*. **73**, 297-306.

Yao, M.-C. (1986). Amplification of ribosomal RNA genes. In *The Molecular Biology of Ciliated Protozoa* (ed. Gall, J. G.), pp. 179-201. Academic Press, Orlando, FL.

Yao, M.-C. and Gall, J. G. (1979). Alteration of the *Tetrahymena* genome during nuclear differentiation. *J. Protozool.* **26**, 10-13.

Yao, M.-C. and Gorovsky, M. A. (1974). Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis. Chromosoma* **48**, 1-18.

Yao, M.-C., Choi, J., Yokoyaman, S., Austerberry, C. F. and Yao, C. H. (1984). DNA elimination in *Tetrahymena*: a developmental process involving extensive breakage and rejoining of DNA at defined sites. *Cell* **36**, 433-440.

Yao, M.-C., Yao, C. H. and Monks, B. (1990). The controlling sequence for site-specific chromosome breakage in *Tetrahymena. Cell* **63,** 763-772**.**

Yokoyama R. W. and Yao, M.-C. (1984). Internal micronuclear DNA regions which include sequences homologous to macronuclear telomeres are deleted during development in *Tetrahymena. Nucl. Acids Res.* **12**, 6103-6116.