PRONUCLEI OF *HELIOPHRYA ERHARDI*
MATTHES DURING CONJUGATION AND
THEIR DIFFERENTIAL ASSOCIATION WITH
COATED AND UNCOATED MICROTUBULES

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SUMMARY

Microtubules surrounding the pronuclei during conjugation of the suctorian ciliate *Helio-
phrya erhardi* can be divided into 2 distinct classes by electron microscopy. Microtubules
around the stationary nucleus have a conventional appearance and presumably serve as a
skeleton, anchoring that nucleus in its cytoplasmic position. Microtubules surrounding the
prospective migratory nucleus are coated with electron-dense material and are in some cases
associated with 7-nm filaments. These coated microtubules supposedly function to transport
the migratory nucleus into the conjugation partner.

INTRODUCTION

During conjugation in ciliates, 2 gametic pronuclei are formed in each member of
the mating pair. One can be termed the migratory nucleus, the other the stationary
nucleus. During the nuclear exchange, the migratory nucleus from each organism
moves through an intercellular bridge and fuses with the stationary nucleus of the
conjugation partner to form a synkaryon. The obviously different behaviour of the
2 nuclei directs attention to the problem of nuclear migration during conjugation.
Light-microscopic investigations (comprehensively reviewed by Raikov, 1972) pro-
vide little to solve this question. Electron-microscopic investigations on sometimes
unfavourably preserved material supply some data for the genera *Paramecium*
(André & Vivier, 1962; Schneider, 1963; Inaba, Imamoto & Suganuma, 1966;
Stephenson, 1972; Jurand, 1976) and *Tetrahymena* (Elliott, 1973; Wolfe, 1978). This
scarcity of ultrastructural data is due to the elusiveness of these small nuclei within
the organism, and the short time span of their appearance during the life cycle. In
*Heliophrya erhardi*, the pronuclei occupy a very small defined area within an extension
of the cell, the conjugation bridge (Lanners, 1973). Therefore, these nuclei are easily
accessible for observations in living cells with the phase-contrast microscope and in
cells fixed at selected stages of the sexual cycle by electron microscopy.

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MATERIAL AND METHODS

Two strains of the suctorian ciliate *Heliophrya erhardi* (Rieder) Matthes were used in this study. One clone was isolated in 1969 from a drainage ditch at Schiphol, Holland, the other strain was kindly provided by Dr Manfred Hauser, Bochum (Germany). The organisms were cultured as described in earlier publications (Lanners, 1973, 1978). Pairs of conjugating cells were observed with an inverted dark phase-contrast microscope, and their position was marked on the underside of the Petri dish. At the desired conjugation stages, the observed organisms were fixed *in situ* with 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), postfixed with 1 % OsO₄, dehydrated in graded ethanol, and embedded in Epon 812. Collection of thin sections on grids with asymmetrical orientation marks made it possible to identify in the electron microscope those nuclei which had previously been observed with the inverted phase-contrast light microscope. Uranyl acetate-lead citrate stained sections of 8 pairs of organisms were examined with a Philips 300 electron microscope.

OBSERVATIONS

The pattern of nuclear behaviour during conjugation in *Heliophrya erhardi*, described in an earlier paper (Lanners, 1973), follows the general pattern of conjugation in other ciliates (Raikov, 1972; Grell, 1967, 1973). Figs. 1–4 show the last stages in pronuclear development after meiosis has been completed and one haploid nucleus has entered the conjugation bridge (Figs. 1, 2). In each member of the mating pair this haploid nucleus undergoes a third progamic division, such that its spindle is...
oriented parallel to a cross-wall which separates both organisms (Fig. 3). As a result
of this division, 4 haploid nuclei, the pronuclei, are located within the conjugation
bridge next to the cross-wall (Fig. 4). By light-microscopic observation it could be
seen that these pronuclei are differentiated into a stationary and a migratory nucleus
in each organism of a mating pair (Lanners, 1973). The stationary nucleus in one of
the conjugation partners is always opposed by the prospective migratory nucleus in
the other conjugant. The location of the migratory and the stationary nuclei is the
same in all organisms observed under identical conditions: looking at a conjugating
organism from the dorsal side, the stationary nucleus lies on the left side of the
conjugation bridge and the migratory nucleus on the right side, if the conjugation
process points to the top of the viewing field (Fig. 4).

Electron-microscopic observations: stationary nucleus

Both pronuclei are separated only by a septum, composed of the fused epiplasms of
both conjugants (Fig. 5). The septum has the same structure as at the beginning of
the conjugation: the epiplasmic material is interrupted by channel-like spaces and no
trace of the original cell membranes remains over the entire length of the fusion area
(Lanners, 1978). Serial sections showed that at this stage of the conjugation cycle, the
cross-wall contains no preformed large opening which might allow the passage of the
migratory nuclei. In the light microscope, and particularly in stained preparations, all
pronuclei are surrounded by a light halo. Electron micrographs show that the halo
around each pronucleus consists of 2 regions (Figs. 5, 10); an inner one (0.5 µm wide)
immediately surrounding the nucleus and containing only a few ribosomes but large
amounts of granular material, microtubules, and smooth endoplasmic reticulum, with
the latter in particular abundance in the space between the nucleus and the septum
(Fig. 5). The second or outer region (0.5-2 µm wide) consists of a semicircular zone
bordered by the septum and the first (inner) region. It contains free ribosomes —
many of them in tetrameric configuration – in addition to the organelles mentioned
for the inner region of the halo.

The microtubules surrounding the stationary nucleus exhibit the conventional
appearance of clear, hollow tubes, about 27 nm in outer diameter. They are attached
to the outer nuclear membrane by a plaque or tuft of electron-dense fibrillar material
(Figs. 5, 5B). These microtubules radiate deep (up to 30 µm) into the surrounding
cytoplasm (Fig. 5). Microtubules also connect the stationary nucleus with the septum
even over a distance of 2 µm or perhaps more (Fig. 7).

As seen by light microscopy, the stationary nucleus is in most cases elongated, with
its long axis parallel to the septum (Lanners, 1973). Intranuclear microtubules –
sometimes connected by cross-bridges (Fig. 5A) – stretch along the long axis (Fig. 6).
It is unclear whether they are actually attached to the inner nuclear membrane. The
chromatin material of the pronuclei is much less condensed than in an interphase
nucleus and is dispersed throughout the nuclear profile in 60- to 200-nm small
clusters. It is never directly attached to the inner nuclear membrane, but maintains a
constant distance of 35 to 40 nm from the nuclear envelope (Figs. 5, 5B, 6). It appears
as if the chromatin material has connexions with the electron-dense plaque material
on the outside of the nuclear envelope (Fig. 5B). Throughout the entire conjugation cycle, and therefore also at this stage, nuclear pores are present in the nuclear envelope (Fig. 5). They are always filled with electron-dense material. The perinuclear space shows extensive connexions with the smooth endoplasmic reticulum, particularly in the area between the stationary nucleus and the septum (Fig. 5).

**Electron-microscopic observations: migratory nucleus**

The appearance and the arrangement of microtubules are different around the migratory nucleus as compared to the stationary nucleus. Microtubules surrounding the prospective migratory nucleus are more numerous, run in different directions, and are associated with a fuzzy coating material (Fig. 10). This material can be seen to have a thickness of up to 30 nm. It is organized in small projections on the surface of the microtubule, 5 nm thick and up to 40 nm high (Fig. 10 inset). A directionality can be observed whereby the projections are elevated by 60–80° from the axis of the microtubule (Figs. 9, 10 inset). Occasionally small filaments (6–7 nm thick) are associated with these microtubules via the coating material (Fig. 11). The occurrence of such a configuration, however, is too inconsistent to allow a general statement of how many microtubules are associated with filaments during this stage of conjugation. The thickness of the coating material diminishes with distance from the migratory pronucleus (Fig. 8), and at a distance of 4–5 μm no coat can be seen to be associated with microtubules. The difference in the appearance of microtubules around both types of nuclei can be found in one and the same section (Fig. 5) and therefore can not be considered to be a fixation artifact.

Microtubules surrounding the migratory nucleus are attached to the nuclear envelope by electron-dense material. Unlike the case for stationary pronuclei, they not only radiate from the nuclear envelope, but also run parallel to the nuclear envelope, particularly in the immediate neighbourhood of the nucleus (Figs. 9–11). The microtubules therefore form a network surrounding the migratory nucleus.

The chromatin material of the migratory nucleus appears similar to the chromatin material of the stationary nucleus. Intranuclear microtubules are present. However, their orientation in migratory nuclei appears to be less uniformly parallel to the plane of the septum than in the stationary nuclei. The intranuclear microtubules terminate on the inner nuclear membrane without any visible attachment material (Fig. 12). A high incidence of blebbing of the nuclear envelope was encountered in the migratory nucleus. Either both membranes or only the outer nuclear membrane participate in

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**Fig. 5.** Electron micrograph of the stationary nucleus (sn). Part of the migratory nucleus (mn) can be seen at the very top of the picture. Stationary and migratory nucleus are separated by only a septum (e). A nuclear pore is indicated by an arrowhead. The 2 circles mark the attachment of microtubules to the outer nuclear membrane. The dotted line marks the 2 regions of the halo. Note the difference in appearance of the microtubules around both nuclei. ×28000. Inset A: Intranuclear microtubules connected by cross-bridges. ×60000. Inset B: Nuclear envelope of the stationary nucleus (sn) with electron-dense plaques from which microtubules radiate. ×72000.
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this phenomenon (Figs. 10, 12). This was never found to be the case in the stationary nucleus, where at the most some enlargement of the perinuclear space may occur.

Discussion

Recently a great deal of interest has focused on the high molecular weight microtubule-associated proteins (MAP), that stimulate tubulin polymerization (Dentler, Granett & Rosenbaum, 1975) and are located as periodic sidearms on the surface of microtubules assembled in vitro (Murphy & Borisy, 1975; Amos, 1977). Griffith & Pollard (1978) report evidence that interaction of actin filaments and microtubules depends upon the presence of MAP. Indirect immunofluorescence studies reveal the occurrence of proteins of high molecular weight along cytoplasmic and spindle microtubules in vivo (Sherline & Schiavone, 1977, 1978).

The coated microtubules surrounding the migratory pronucleus in _Heliophrya erhardi_ resemble very much those microtubules repolymerized in vitro with their high-molecular-weight protein fraction, although, in the case of _Heliophrya_, the coating appears to be denser. The size of sidearms on the coated microtubules from the in vivo material in _Heliophrya_ agrees fairly well with that obtained from the in vitro material (5.6 x 18.9 nm) (Murphy & Borisy, 1975).

In _Heliophrya erhardi_ the presence of both types of microtubules (coated and uncoated) can be linked with different functions. Coated microtubules surround the prospective migratory nucleus and are, in some cases, associated with 6-7-nm microfilaments. It is proposed that these microtubules and filaments are involved in providing the motive force for the migration of the pronucleus during the nuclear exchange (Lanners & Rudzinska, 1975; Lanners, in preparation). Microtubules surrounding the stationary nucleus lack any association with MAP-like structures or with filaments. They apparently serve a skeletal function of anchoring that nucleus to the surrounding cytoplasm, most probably to stabilize the stationary nucleus against the advancing migratory nucleus. During nuclear exchange a narrow segment of the dividing septum breaks down so that a passage is established for the migratory nucleus. The anchoring microtubules, radiating at an angle from the stationary nucleus towards the septum, maintain their connexion during this process (Lanners & Rudzinska, 1975; Lanners, in preparation). An anchoring of nuclei by microtubules has also

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Fig. 6. Stationary nucleus with intranuclear microtubules. The arrows point to fibrils radiating from the electron-dense material at the nuclear envelope. The arrowhead indicates a pore in the nuclear envelope. x 48000.

Fig. 7. Microtubule connecting the stationary nucleus (sn) with the epiplasmic septum (e). x 48000.

Fig. 8. Microtubules between 2 pronuclei within the same organism. The outlined arrow points towards the position of the stationary nucleus, the full arrow points towards the migratory nucleus. x 40000.

Fig. 9. High magnification of coated microtubules surrounding the migratory nucleus (mn). The arrows point to projections on the surface of the microtubule. Note the thickness of the coat material, particularly in cross-sectional microtubules. Electron-dense material connects microtubules near the nucleus with the nuclear envelope. x 80000.
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been described in *Acetabularia* (Woodcock, 1971). In addition to directly anchoring the nucleus, the radiating microtubules presumably increase the resistance to displacement of that nucleus within the cytoplasm. This, again, stabilizes this nucleus in its position.

Association of filaments and microtubules has been described for tentacle microtubules of *Heliophrya erhardi* (Hauser & van Eys, 1976), and can also be found in microtubules surrounding the contractile vacuole (Lanners, unpublished observation). All these structures have a motion-associated function. Other cytoplasmic as well as intranuclear microtubules are of the smooth type. The low incidence of observed microtubule-associated filaments in the present study might be due to the fixation conditions. Buffered glutaraldehyde fixation followed by osmium tetroxide is not favourable for the preservation of actin filaments (Forer, 1978; Maupin-Szamier & Pollard, 1978). Seagull & Heath (1979), adding tannic acid to the fixative, observed an increased number of actin-like filaments associated with cortical microtubules in radish root hairs. A different fixation protocol might also reveal a higher incidence of microtubule-associated filaments around the migratory nucleus of *Heliophrya erhardi*.

Microtubules in the vicinity of pronuclei of ciliates have been observed by Jurand (1976) in *Paramecium aurelia*. Jurand suggests that these microtubules might provide the motive force for nuclear migration. Although it has not been emphasized by this author, most of these microtubules seem to be embedded in electron-dense material [see Jurand (1976) figs. 14, 15], and it is reasonable to assume that they might be associated with MAP-like material as the microtubules in *Heliophrya erhardi* are. In an earlier paper on the same species (*P. aurelia*), Stephenson (1972) failed to show microtubules in the vicinity of a presumably migratory nucleus. For *Tetrahymena*, Wolfe (1978) reports that microtubules are attached to the external surface of gametic nuclei. Again, in an earlier paper Elliott (1973) could not demonstrate microtubules in the vicinity of the migratory nucleus of *Tetrahymena*. It appears that with improvements of electron-microscopic techniques, more and more kinetic elements can be found to be associated with gametic nuclei during conjugation.

An interesting parallel to the present observations has been reported by Fux (1974). During chromosome elimination in the gall midge *Heteropeza*, coated microtubules are associated with moving anaphase chromosomes, while normal-appearing microtubules are associated with stationary, eliminated, chromosomes.

Microtubules show the heaviest coating in the immediate vicinity of the migratory

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Fig. 10. Migratory nucleus surrounded by the 2 regions of the halo (dotted lines). Blebs involving one (arrowhead) or both membranes (arrow) of the nuclear envelope can be seen. (e, epiplasmic septum.) × 25700. Inset: Coated microtubule with side-arms. × 69,700.

Fig. 11. High magnification of coated microtubules in cross- and longitudinal section near the migratory nucleus. The arrowheads indicate a filament associated with a microtubule via the coat material. × 39,500.

Fig. 12. Bleb formation of the nuclear envelope of a migratory nucleus (mm) involving both membranes of the nuclear envelope. The termination of an intranuclear microtubule on the inner nuclear membrane is marked by a circle. × 43700.
nucleus. The electron-dense material (probably MAP) diminishes with distance away from the nucleus. This gradient effect calls for a factor which operates in the vicinity of the migratory nucleus, and which might promote the association of MAP or of the coat material with microtubules. The observed high incidence of blebbing in the nuclear envelope of the migratory nucleus could support the viewpoint of a highly active migratory nucleus in contrast to the stationary nucleus. However, as Franke (1974) pointed out, it is hard to assess the significance of blebbing, and it might merely reflect a reaction of the nuclear envelope to stress, including fixation. But, even in this case, the blebbing shows, in the 2 types of nuclei, a different behaviour or reaction to the same stimulus.

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REFERENCES


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