

KINETOCHORES ASSOCIATED WITH THE NUCLEAR ENVELOPE IN THE MITOSIS OF A DINOFLAGELLATE

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Mitosis in free-living dinoflagellates is unusual in that the nuclear envelope remains intact but a number of cytoplasmic channels containing microtubules run completely through the nucleus (8, 9, 13). The chromosomes are known to be attached to the nuclear envelope (1, 8, 13) and during division this connection appears to take the form of a narrow stalk of chromosome material which fits into a depression in the nuclear membrane lining the channels. No centrioles or basal bodies have been observed adjacent to the dividing nuclei and until recently no connection had been seen

between the microtubules and any other structure. It has been suggested that the microtubules merely provide a structural framework for mitosis and that the segregation of daughter chromatids might be brought about by movement of the nuclear envelope (8, 10, 12, 13). More recently it has also been suggested (6) that there are probably connections between the microtubules and the chromosomes, just as there are in most eucaryotic organisms and also in the parasitic dinoflagellate *Oodinium* (2).

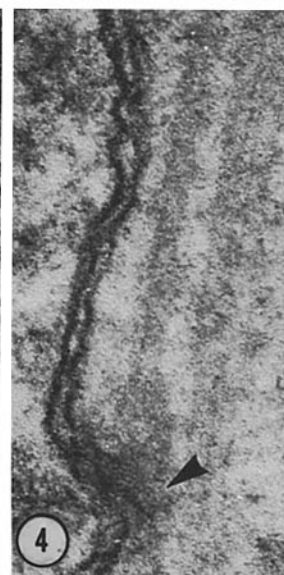
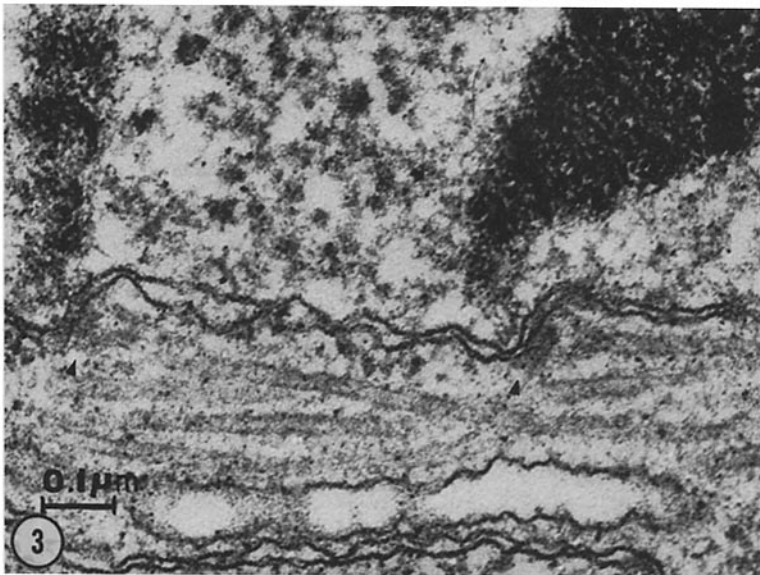
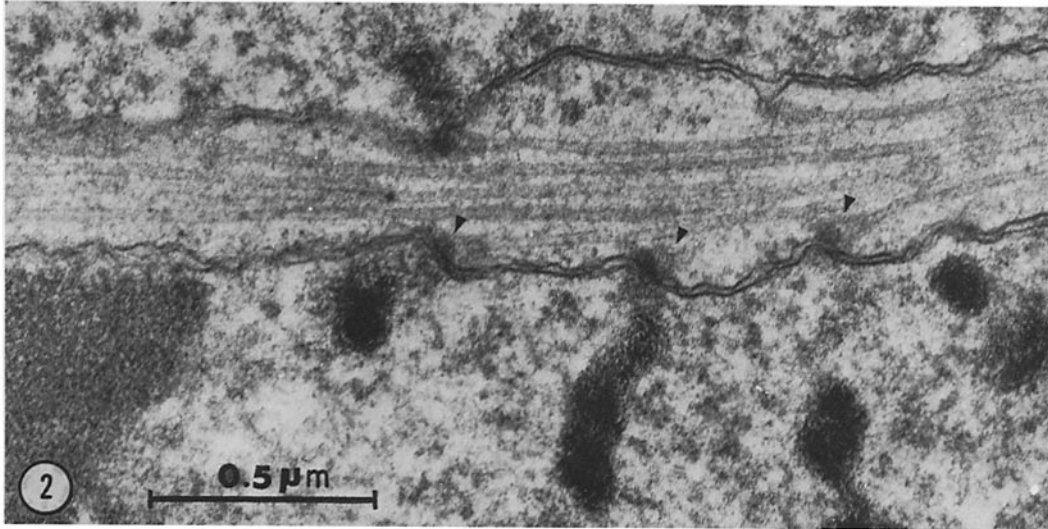
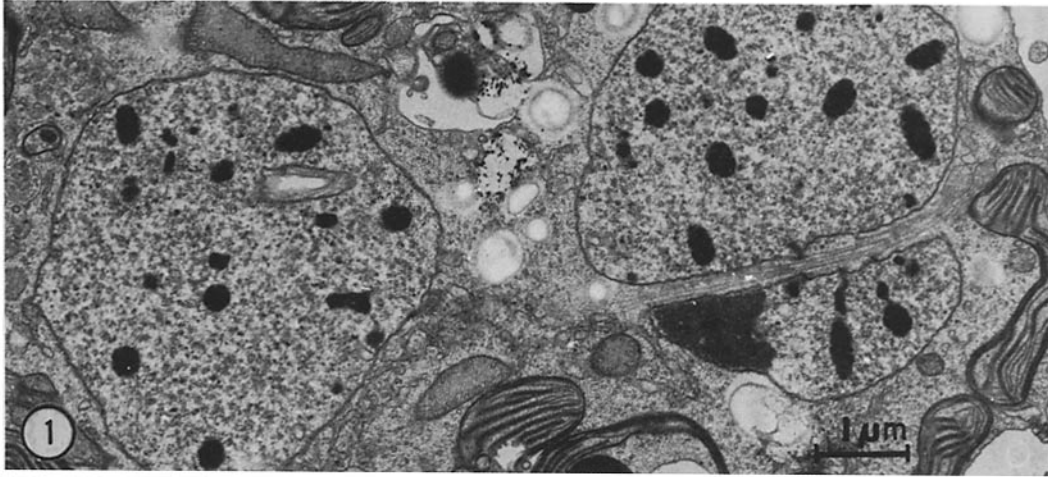
In the present note we report evidence for the

FIGURE 1 Late anaphase-telophase stage of *Amphidinium* showing the two daughter nuclei. Each contains scattered chromosomes and a single channel: that in the left-hand nucleus is sectioned obliquely and that in the right-hand nucleus, longitudinally. The right-hand nucleus also has a nucleolus. $\times 12,000$.

FIGURE 2 Enlargement of the lower half of the right-hand nucleus in Fig. 1. Note the microtubules in the channel and the three "kinetochores" (arrowheads) at the points where chromosomes are attached to the nuclear envelope. $\times 60,000$.

FIGURE 3 Two kinetochore-like structures (arrowheads) from another dividing nucleus. Note the arrowed portion of chromosome which contacts the nuclear envelope. $\times 100,000$.

FIGURE 4 High magnification of a kinetochore (arrowhead), showing the area of electron-dense material between the nuclear envelope and the microtubule. $\times 140,000$.



presence of definite kinetochore-like structures in a free-living dinoflagellate. These appear to connect together channel microtubules and the nuclear envelope immediately adjacent to the positions of attachment of chromosomes on the nuclear side of the envelope.

MATERIAL AND METHODS

The dinoflagellate used was isolated by Dr. J. A. West from Puerto Penasco, Sonora, Mexico in March 1968. The taxonomy of the smaller members of the genus *Amphidinium* is a subject of some controversy so that the exact species is uncertain. It was originally identified as *Amphidinium massarteeae* Biecheler. Our electron and light microscope observations suggest, however, that it is *A. carterae* Hulburt. Regardless of the species, however, it is clearly a free-living organism and to date no *Amphidinium*-like stages have been implicated in the life histories of any parasitic dinoflagellates.

The organism was maintained in Guillard's f/2 medium in a growth cabinet programmed to induce a partial synchronization of division. During the 18-h light period the temperature was increased in five steps from 15° to 25°C and decreased in seven steps back to 15°C. Light was similarly increased in three steps to 6,000 lx and back in three steps to darkness.

For electron microscopy, material was fixed in 1% redistilled glutaraldehyde in 0.125 M phosphate buffer, with sucrose to 0.3 M, for 40 min. This was followed, after washing, by 1 h in 1% osmium tetroxide in similar buffer. The specimens were embedded in Spurr resin, and sections were stained in uranyl acetate and lead citrate before examination in Zeiss EM9A or AEI 801 electron microscopes. The fixation schedule used gave a very good preservation of cell organelles and microtubules and, in particular, caused little osmotic distortion in this "naked" dinoflagellate.

RESULTS

Fig. 1 shows a median longitudinal section of a late anaphase or early telophase stage. The chromosomes appear to be scattered through the daughter nuclei and a nucleolus is visible in one of them. The nuclear envelope is intact, and this can also be seen to line a channel through one of the nuclei. From serial sections it is known that channels pass through both nuclei. In the channel sectioned longitudinally (Fig. 2) there are several 20-nm diameter microtubules some of which pass completely through the nucleus and run some distance into the space between the two daughter nuclei (Fig. 1). In places the envelope which lines the channels is depressed into the channel, generally in the form of a V- or U-shaped hollow. On the nuclear side of the envelope each of the chromo-

somes appears to be attached to its own depression. In the channel some of the microtubules, which always come from the side of the cell to which the nucleus is moving, connect with the depressions. From the sections there only appears to be one microtubule per depression (= per chromosome?) but there may be more. Some 100 nm away from the membrane the microtubule becomes splayed out to form a rather more dense conical structure which then connects with a pad of electron-dense material. This is approximately 25 × 75 nm in section but not very clearly defined and it appears to be firmly attached to the nuclear envelope (Figs. 3, 4). The kinetochore-like structure seems to sit on the outside of the membrane and is not embedded in it. No definite connections have been observed across the perinuclear space although on occasion rather diffuse electron-dense material is seen in this area (Fig. 3). In earlier division stages (possibly equivalent to metaphase) the depressions in the walls of the channels are more rectangular in shape (see Fig. 9 in reference 5), and at this stage it would seem likely that both chromatids are attached to the depression as well as microtubules from both sides of the nucleus. The anaphase separation would then change the shape of the depressions to the U or V shapes with microtubules only attached at one side. If this is true, then the microtubules would clearly appear to be involved in the segregation and separation of the chromatids.

DISCUSSION

As predicted by Hollande (6) it would now seem clear that the free-living dinoflagellates do have spindle attachments or kinetochores. The association of kinetochores with the nuclear membrane is thus shared with the parasitic dinoflagellate *Oodinium* (2) and with certain protozoans such as *Syndinium* (11, 12), which may or may not be a dinoflagellate, and *Trichonympha* (7). The kinetochores are not all structurally identical, however. In *Amphidinium* the nuclear envelope remains entire while in *Syndinium* (12) and *Trichonympha* (7) the kinetochores rest in perforations in the envelope. At present we have no information concerning the formation of the kinetochore so we cannot say whether it forms within the nucleus and migrates more or less through the nuclear envelope as in *Trichonympha* (7), although this would seem unlikely. In *Amphidinium* the dense kinetochore-like body is completely outside the membrane and

it probably forms in step with the assembly of the microtubules.

The present work clearly shows that, contrary to what the earlier light microscope work appeared to show (3, 4), the dinoflagellates do have a type of spindle with both chromosomal fibers and interzonal fibers. One feature still to be established is what happens to the spindle at the "pole" ends. In our micrographs the microtubules often seem to collect into bundles but they simply terminate, often near a group of microbodies (see right side of Fig. 1), but without definitely connecting to any structure. No centrioles, basal bodies, or spindle-organizing structures have been seen in this region. One thing is clear; although the nuclear envelope may be involved in some way in the segregation of chromatids (at the very least new envelope must presumably be synthesized to enable the kinetochores of sister chromatids to be pulled apart), this process cannot be entirely due to membrane changes in the way that has been postulated (8, 10). The microtubules must surely be actively involved in the mitotic processes. It follows from this that the dinoflagellate mitosis is probably not so primitive as the histoneless chromosomes and postulated membrane-induced segregation have been thought to suggest.

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