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NUCLEAR DIVISION IN THE MARINE DINOFLAGELLATE *OXYRRHIS MARINA*

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SUMMARY

The nuclear division of *Oxyrrhis marina* is a very distinct one among the mitoses of dinoflagellates that have been studied. Using synchronized populations, we have investigated the ultrastructural changes in this nuclear division. In interphase, nuclei can be classified into two groups on the basis of the shapes of the chromosomes. Y- and U-shaped chromosomes have been observed in both types of interphase nuclei. By prophase the nucleus becomes oval, many nuclear plaques appear on the nuclear envelope, and many microtubules radiate from these nuclear plaques within the nucleus. Metaphase can be identified by the characteristic arrangement of the chromosomes; an equatorial metaphase plate is absent. As in many higher organisms, anaphase includes two stages: anaphase A and anaphase B. During anaphase A the nucleus does not apparently elongate and the chromosomes migrate towards the poles by a combination of the shortening of the chromosome-associated microtubules and the elongation of those located between daughter chromosomes. During anaphase B the nucleus elongates to about twice its former length. This elongation may result from growth of the interzonal nuclear envelope. Dividing nucleoli are associated with microtubules, which suggests that microtubules may play an active role in the division of the nucleolus.

The evolution of mitosis and the phylogenetic relationships between *Oxyrrhis*, typical dinoflagellates and *Syndinium* are discussed.

INTRODUCTION

The position of *Oxyrrhis marina* among dinoflagellates has not been clearly determined; some of its characteristics are very different from those of typical dinoflagellates. The arched structure of typical dinoflagellate chromosomes has not been observed in *O. marina* (Dodge & Crawford, 1971). When typical dinoflagellates were fixed and treated with hot trichloroacetic acid or DNase to remove DNA, and stained with acid dyes to demonstrate basic proteins, the chromosomes were found to have been completely dissolved away (Dodge, 1973). However, after the same treatment the chromosomes of *Oxyrrhis* were stained, just as were the metaphase chromosomes of higher eukaryotes (Li *et al.* 1978). In addition, Triemer (1982) has shown that the mitosis of *Oxyrrhis* is very different from that of other dinoflagellates that have been studied. In typical dinoflagellates (Leadbeater & Dodge, 1967; Bouligand *et al.* 1968; KuBai & Ris, 1969; Soyer, 1969; Schmitter, 1971) and the special dinoflagellate *Syndinium* (Ris & KuBai, 1974), the spindle is extranuclear and the microtubules traverse the nucleus in cytoplasmic channels lined with the nuclear envelope, which remains unbroken during mitosis. In *Oxyrrhis* the spindle is intranuclear, the microtubules lie in the nucleoplasm and the organization of the

Key words: mitosis, dinoflagellate, *Oxyrrhis*, evolution.

spindle is very distinct from those in other organisms investigated to date (Triemer, 1982). However, owing to his failure to synchronize *O. marina*, Triemer was unable to make more detailed observations on this organism. In the present study, using synchronized populations, we have made further investigations on mitosis in *Oxyrrhis marina*.

MATERIALS AND METHODS

Materials

O. marina was collected in Qingdao by Dr Li Mingren, and cultured in our laboratory at 20 °C on a 12 h : 12 h, light/dark cycle in modified Droop's medium (modified by Zhang Zhe-fu *et al.*, unpublished): sea water, 1 l; KNO_3, 100 mg; ferric citrate, 1 mg; L-alanine, 10 mg; L-valine, 5 mg; L-proline, 0·03 μg; cholesterol, 0·5 μg; V8 solution (vitamin mix), 1 ml; soil extract, 50 ml.

Synchronization

When cells had been cultured for a week after inoculation they were centrifuged at 570 ***g*** for 5 min. Then the deposited cells were discarded and the cells still suspended were treated with alternating normal (20 °C) and low (4 °C) temperature cycles (Fig. 1A). During the fourth normal-temperature treatment the number of cells per ml increased by 72–98 %.

Electron microscopy

Cells were sampled at different times after synchronization (Fig. 1B), and were fixed with 2·5 % glutaraldehyde in 0·1 M-phosphate buffer (pH 6·8) for 2 h, washed and post-fixed with 0·1 M-phosphate buffered (pH 7·4), 1 % osmium tetroxide for 1 h. The samples were infiltrated and embedded with Araldite CY 212. Thin sections were stained with uranyl acetate and lead citrate and examined in an H-300 transmission electron microscope at 75 kV.

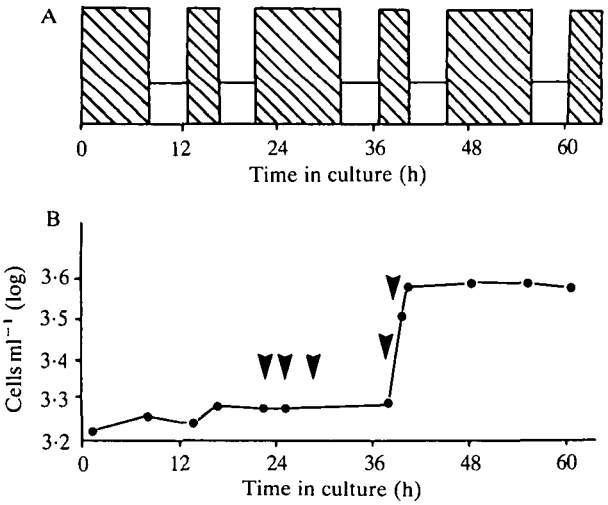


Fig. 1. A. The normal (20 °C)/low (4 °C) temperature cycles. B. The growth curve of the synchronized populations. The arrowheads show the times at which the cells were sampled.

RESULTS

Interphase

If the appearance of the nuclear plaques is taken as a criterion for distinguishing prophase from interphase, the nuclei during interphase can be classified into two groups, type I and type II. Type I nuclei were commonly seen. In these nuclei the chromosomes were rod-shaped (Fig. 2A,B). Type II nuclei were seen mainly in cells fixed at 26 h after synchronization began and were characterized by having thicker, irregular-shaped chromosomes (Fig. 3A,B). Both types of nuclei were spherical. Within the nuclei most of the chromosomes were evenly dispersed, but a few were attached to the nuclear envelope and several of them were Y- or U-shaped.

Prophase and metaphase

When cells enter prophase the shape of the nuclei changes from round to oval. The nucleolus begins to elongate at the same time. Many nuclear plaques are formed on the nuclear envelope. These plaque-like structures consist of dense amorphous material (Fig. 4). Microtubules radiate from the nuclear plaques into the nucleus.

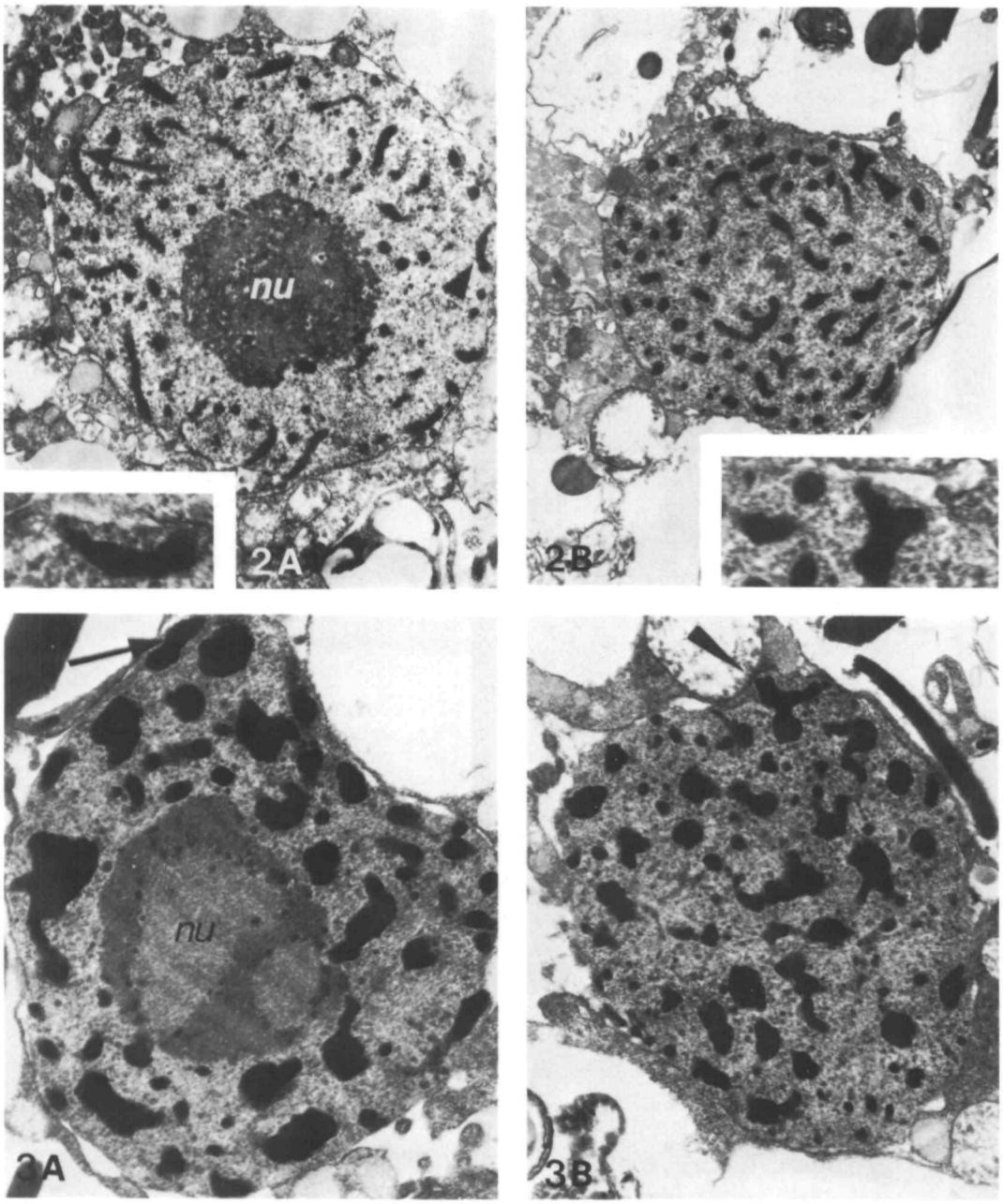
In metaphase the nucleus becomes elliptical and the spindle has formed, consisting of scattered microtubules that radiate from the nuclear plaques (Fig. 6). The axis of the spindle is perpendicular to the long axis of the elliptical metaphase nucleus. The chromosomes are arranged loosely. None is attached to the nuclear envelope. An equatorial plate has not been observed at metaphase.

Anaphase

Anaphase in *O. marina* can be divided into two successive stages: anaphase A, characterized by the poleward movement of the chromosomes; and anaphase B, characterized by the elongation of the nucleus. In Fig. 7 a nucleus is shown in early anaphase A, when the chromosomes begin to migrate. As mitosis proceeds, the chromosomes separate into two groups (Fig. 8) and finally come to lie in the pole regions. Then anaphase B begins (Fig. 15). During anaphase B the nucleus apparently elongates. As a result, at the end of anaphase B and the beginning of telophase the length of the nucleus is about twice that of the nucleus in anaphase A.

In the spindle of *O. marina* some of the microtubules form continuous fibres and others chromosomal fibres (Figs 8, 10). Kinetochores or kinetochore-like structures have not been observed at the points where the chromosomal fibres are connected to the chromosomes. In Fig. 9 the nuclear plaques located on the opposite poles are shown. Fig. 11 shows the distribution of the microtubules in part of the anaphase A nucleus. In the interzonal region of the anaphase A nucleus several microtubules occur in bundles (Fig. 12). Some microtubules run between the two groups of chromosomes and are associated with them (Fig. 14A,B). These may play a role in the mechanism of the poleward movement of the chromosomes.

In early anaphase B the nucleus is somewhat rectangular in outline, the chromosomes are arranged in the two polar regions, and few microtubules are seen in the interzonal region of the nucleus. As division proceeds, the nucleus changes in shape



Abbreviations used in figures: *m*, mitochondrion; *mt*, microtubules; *ne*, nuclear envelope; *np*, nuclear plaque; *nu*, nucleolus.

Fig. 2. Type I interphase nuclei. Some chromosomes are closely appressed to the nuclear envelope (arrow and arrowheads). Insets: higher magnifications of U-shaped (A) and Y-shaped chromosomes (B) attached to the envelope (see arrowheads). A. ×10 000; inset, ×33 000; B, ×7000; inset, ×12 800.

Fig. 3. Type II interphase nuclei with irregular chromosomes. Arrow in A indicates the chromosome attached to the nuclear envelope; arrowhead in B shows Y-shaped chromosome. A. ×14 000; B, ×11 000.

and elongates. There are two large mitochondria in the cytoplasm close to the interzonal region of the anaphase B nucleus. In Fig. 16 at each pole region there is a mass of dense material intruded into the nucleus, but the significance of this is not clear. When telophase begins the length of the nucleus is about twice its length in anaphase A. It is impossible to conceive that microtubules play an active role in the

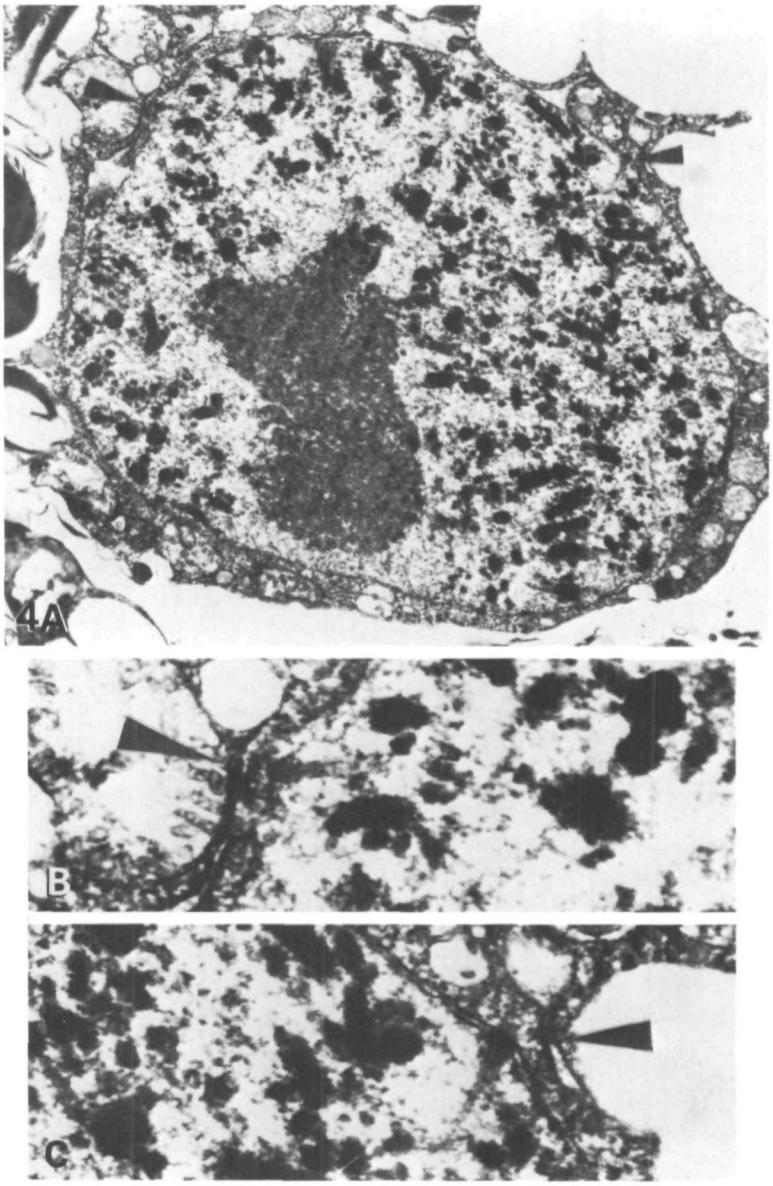


Fig. 4. Prophase; the nucleus becomes oval and the nucleolus elongates. The chromosomes are distributed as in Fig. 2, but on the nuclear envelope the nuclear plaques have developed from which microtubules radiate (arrowheads). A, ×11 600; B,C, ×30 000.

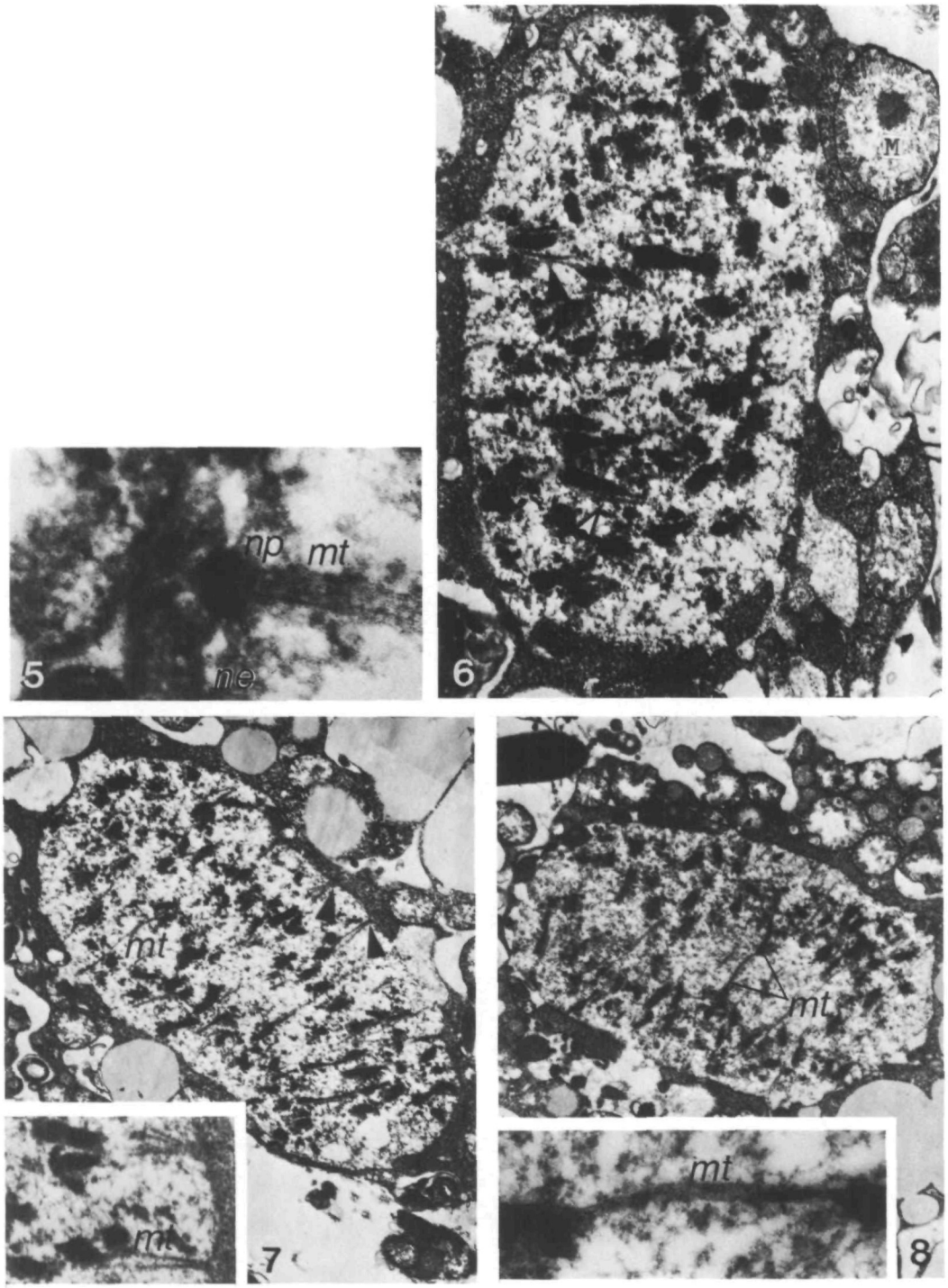


Fig. 5. A magnified view of a nuclear plaque. ×105 000.

Fig. 6. Metaphase. The nucleus becomes elliptical. Chromosomes are not separated into two groups. Arrowheads indicate microtubules. ×16 000.

Fig. 7. Early anaphase A. Chromosomes begin to separate into groups at this stage. Inset: a magnified view of the region indicated by arrowheads. ×7000; inset, ×13 800.

Fig. 8. Mid-anaphase A. Chromosomes have separated into two groups. Inset: a magnified view of the region indicated by arrows, which shows the microtubules located between and associated with the chromosomes moving toward the poles. ×7000; inset, ×40 000.

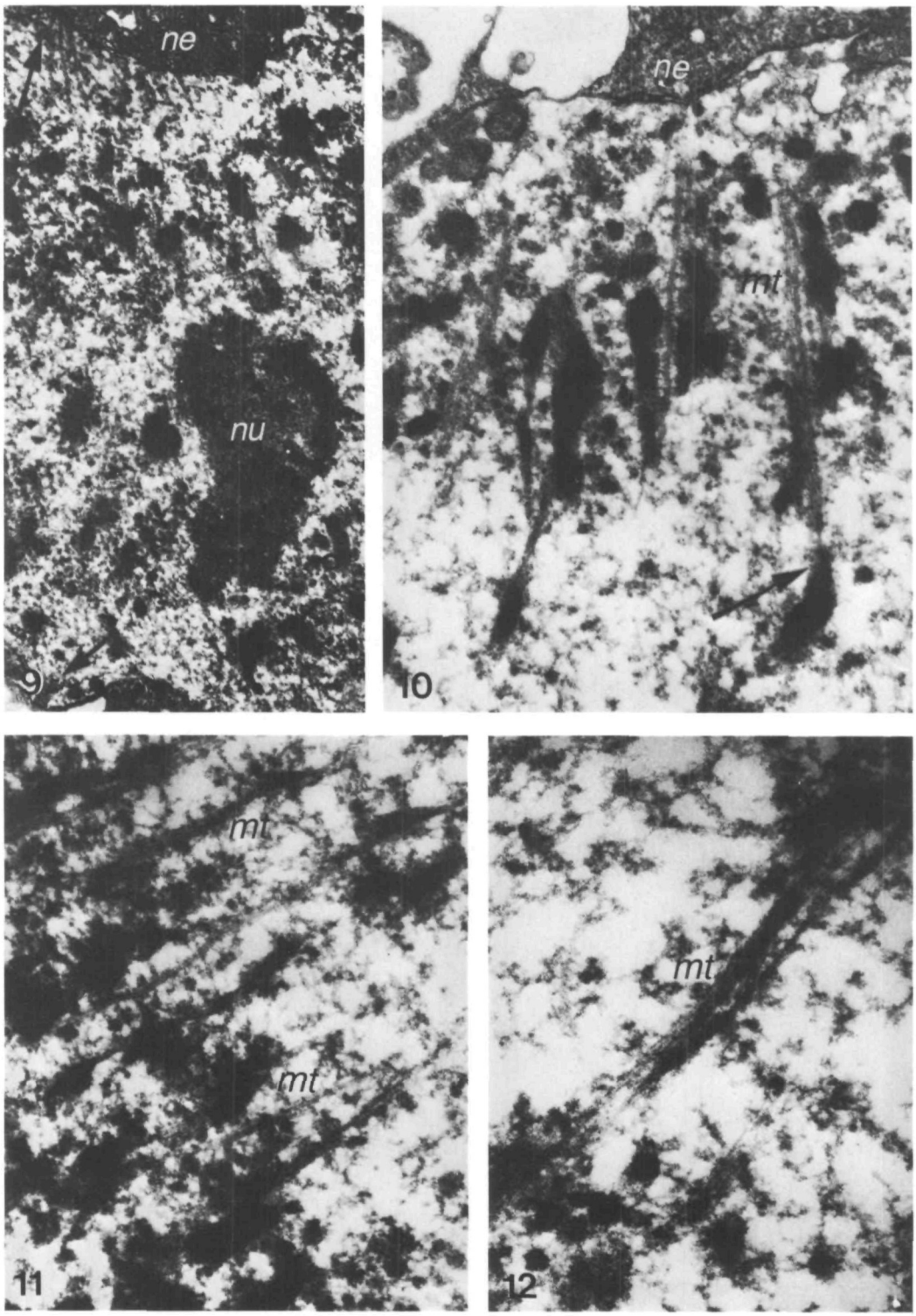
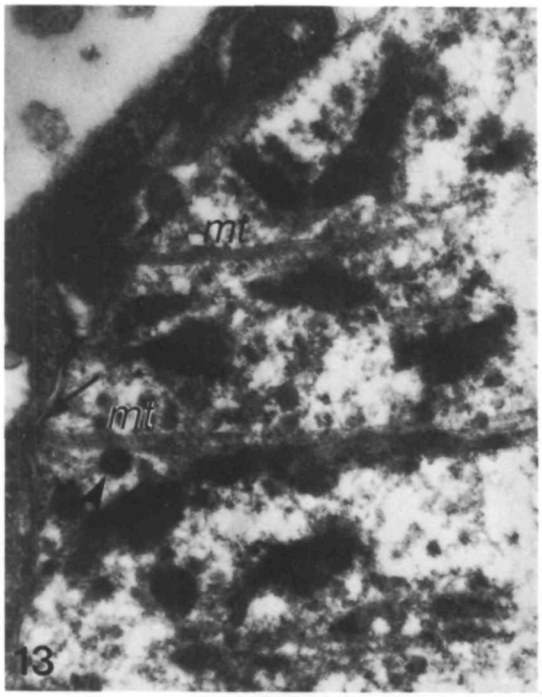


Fig. 9. A pair of nuclear plaques on the nuclear envelope (arrows). ×22 500.

Fig. 10. Mid-anaphase A. Microtubules associated with chromosomes (arrow). ×29 400.

Fig. 11. Microtubules in an area of a mid-anaphase A nucleus. ×30 000.

Fig. 12. Mid-anaphase A. A bundle of microtubules in the interzone of the nucleus. ×50 000.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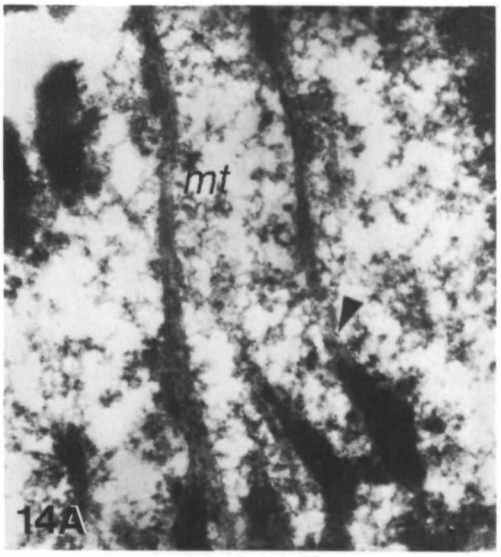
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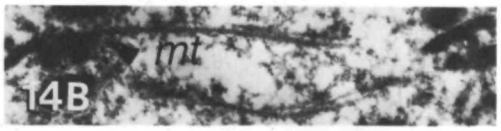
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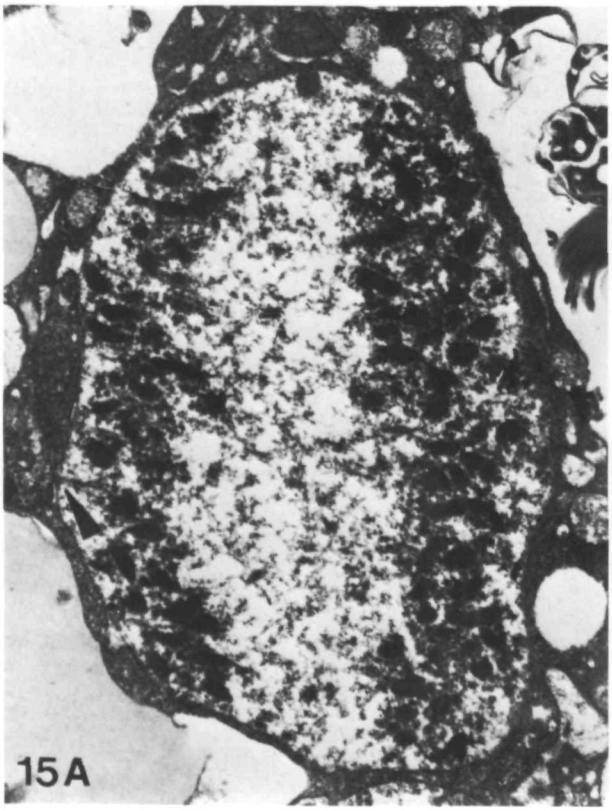
15A


15B

elongation of the nucleus at this stage, because there are very few microtubules seen in the interzonal region of the nucleus. It is possible, therefore, that the growth of the interzonal envelope of the nucleus is an important factor in this process.

Telophase

Telophase begins with the invagination of the interzonal envelope of the nucleus, and ends in the formation of two daughter nuclei (Figs 17, 20). In Fig. 19 a later telophase nucleus is shown in which large cavities appear in its interzonal region; the continuity of the nuclear envelope in this area seems to have been destroyed, and many dense cytoplasmic masses have invaded the nucleus. Unfortunately, this appearance was observed in only two sections of the same nucleus. Hence two explanations are possible: either the continuity of the nuclear envelope is truly destroyed at a certain stage of telophase or the phenomenon results from unnatural (i.e. pathological) factors. The latter could be the more reasonable explanation because of the appearance of large cavities in only one side of the nuclear interzone.

Microtubules and the division of the nucleolus

The nucleolus divides at the same time as the nucleus. During nuclear division microtubules are associated intimately with the nucleolus (Fig. 18A,B). Although it is not clear to which constituent of the nucleolus the microtubules are attached, the fact of their association does suggest that the microtubules might play an important role in the division of the nucleolus.

DISCUSSION

Interphase nuclei

In non-synchronized populations of *O. marina* only one kind of nucleus is usually seen, which corresponds to our type I nucleus. However, in synchronized populations there are two types of interphase nuclei. In type I nuclei the chromosomes are rod-shaped and in type II they are thicker and irregular. In a colourless euglenoid, *Astasia longa*, Chaly *et al.* (1977) classified the interphase nuclei into three groups: types I, II and III, the differences among them being mainly in their chromosome conformation. They suggested that the three types of nuclei corresponded to G_1, S and G_2 phases, respectively. In *O. marina*, type I is also different from type II in its chromosome conformation, but we have not been able to relate the two types of the nuclei to G_1, S or G_2 phases.

Fig. 13. Mid-anaphase A. Microtubules radiating from the nuclear plaques (arrows). Arrowhead shows microtubules associated with a chromosome. ×30000.

Fig. 14. Microtubules (arrowheads) located between and associated with the chromosomes moving towards the two polar regions. A. ×30 000; B, ×20 000.

Fig. 15. Early anaphase B. All the chromosomes grouped in two polar regions. Arrowhead shows the nuclear plaque and the microtubules radiating from it. A, ×11 600; B, ×30 000.

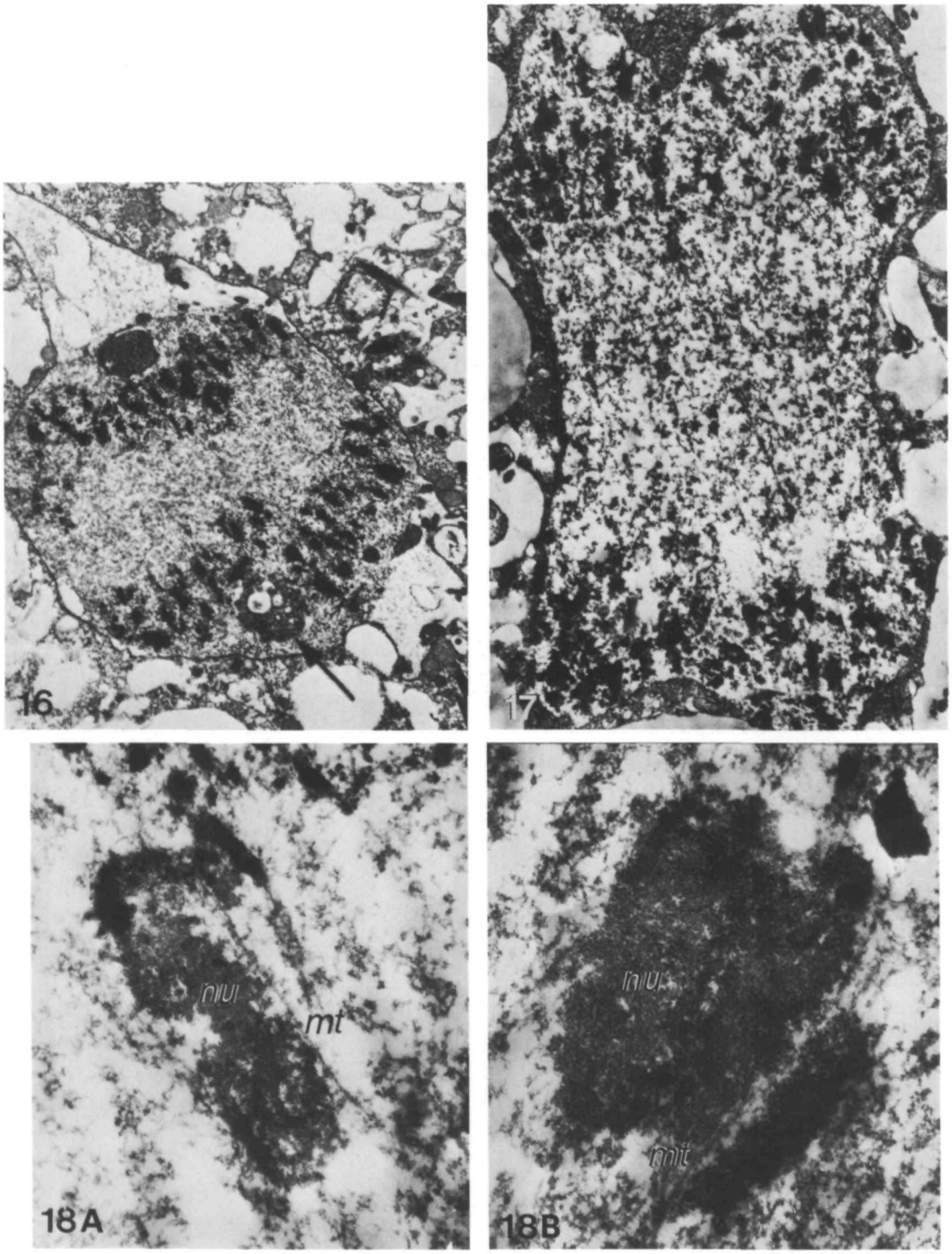

Fig. 16. Mid-anaphase B. The nucleus elongates. There are two masses of dense cytoplasmic material within the polar nucleoplasm (arrows). ×7000.

Fig. 17. Early telophase. The nuclear envelope begins to invaginate around the interzone of the nucleus. ×11 600.

Fig. 18. Microtubules associated with dividing nucleoli. A. Anaphase B nucleolus; B, telophase nucleolus. A. ×20 000; B, ×30 000.

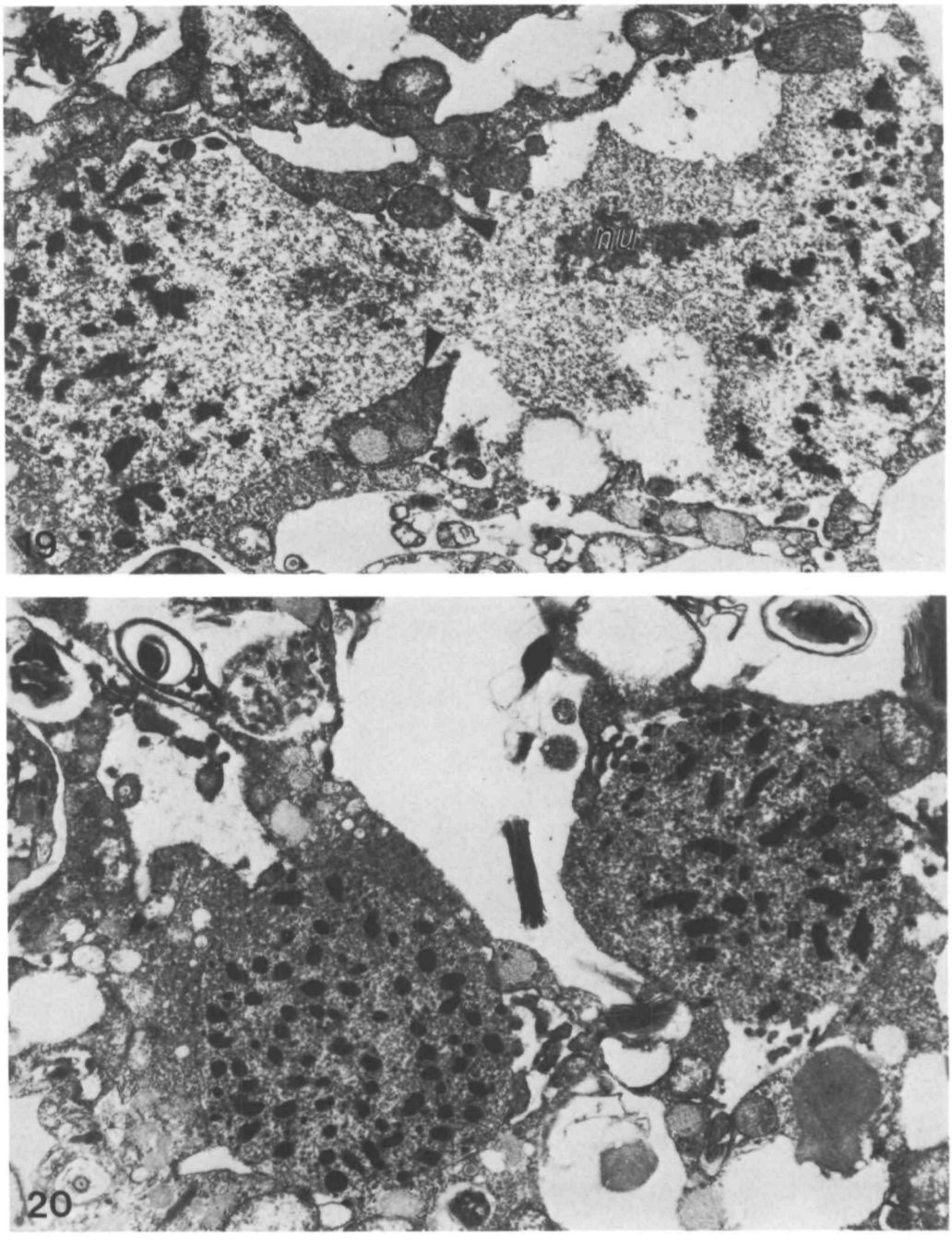

Fig. 19. Mid-telophase. There are masses of cytoplasmic material (arrowheads) and several cavities in the interzonal region of the nucleus. ×12 500.

Fig. 20. Late telophase. Two daughter nuclei have formed. ×8400.

The early studies on nuclear division in *O. marina* were done by Dunkerly (1921) and Hall (1925). Cachon *et al.* (1979) first investigated this nuclear division by electron microscopy. Cachon *et al.* have shown that the nuclear envelope plays an important role in the division of chromosomes in *Oxyrrhis*, and considered the Y-, V- and U-shaped chromosomes as dividing ones. Our observations show that the Y- and U-shaped chromosomes appear before the nuclear plaques form on the nuclear envelope. This suggests that, although the division of chromosomes in *O. marina* is similar to that in other dinoflagellates, the period at which the chromosomes divide is different. In typical dinoflagellates and *Syndinium* the Y- and V-shaped chromosomes appear in the course of nuclear division (Leadbeater & Dodge, 1967; Ris & KuBai, 1974), but in *Oxyrrhis* they appear in interphase. It is interesting, furthermore, that the Y-shaped chromosomes are seen in both types of interphase nuclei. This perhaps suggests that division of chromosomes may occur throughout most of interphase in *O. marina*.

Nuclear plaques as microtubule organizers

In *O. marina* nuclear plaques appear in prophase and consist of dense amorphous material. They can be regarded as 'microtubule organizers'. Although this precise kind of structure has not been discovered in other organisms studied to date, plaque-like microtubule-organizing centres made up of dense amorphous material are often seen in protozoa, algae and fungi. A well-known example is the microtubule-organizing centre in the mitotic apparatus of *Saccharomyces cerevisiae* (Robinow & Marak, 1966; Peterson *et al.* 1972; Moens & Rapport, 1971). The microtubule organizers in *O. marina* are ultrastructurally similar to those in *S. cerevisiae*. The difference between them is that there is only one pair of microtubule-organizing centres in yeast, but many pairs in *O. marina*. The centres in *Oxyrrhis* are very small and do not unite to form a single pair of plaque-like microtubule-organizing centres. It is possible that this special kind of polar structure represents an ancestral form of the microtubule-organizing centre in the evolution of mitosis.

Movement of chromosomes and elongation of the nucleus

Anaphase in most eukaryotic organisms can be divided into two stages: anaphases A and B. In anaphase A chromosomal fibres shorten and chromosomes move towards the poles. In anaphase B the spindle elongates as a result of the rearrangement and elongation of the continuous fibres. Anaphase in mitosis in *Oxyrrhis* can also be divided into these two stages. In anaphase A some of the microtubules of the spindle form continuous fibres, while others are associated with chromosomes and form chromosomal fibres. In addition, there are some microtubules located between and attached to the chromosomes that are moving towards the poles. During this stage the chromosomes migrate towards the poles and finally become grouped in the polar regions, without any apparent elongation of the nucleus. We suggest that the poleward movement of the chromosomes in anaphase A could result from both the shortening of the chromosomal fibres and the elongation of the fibres located between the separating chromosomes.

McDonald & Eutenauer (1983) have shown that, in both the interzone of the spindle of diatoms and that of mammalian cells, the spindle microtubules from the opposite poles overlap each other, and they suggested that a mechanochemical system in the overlap is responsible for the pole separation. However, in *Oxyrrhis* very few microtubules can be seen in the interzonal region of the anaphase B nucleus. Thus it is impossible to suggest that microtubules are responsible for the elongation of the nucleus. It is more reasonable to suggest that the elongation of the *Oxyrrhis* nucleus at this stage results from the growth of the nuclear envelope in its equatorial region.

Microtubules and nucleolus division

The association of microtubules with the dividing nucleolus has been observed in *A. longa* and *Euglena gracilis* (Chaly *et al.* 1977; Gillot & Triemer, 1978). Our observations confirmed this association in *O. marina*. These facts suggest that microtubules may play an active role in the division of the nucleolus, perhaps providing the force that the division requires.

Mitosis in Oxyrrhis *and other dinoflagellates*

Cachon *et al.* (1979) reported that during the nuclear division of *O. marina* the nuclear envelope, as in other dinoflagellates, invaginates to form channels in which no microtubules are present. Triemer's (1982) and our observations show that is not the case. The mitosis of *O. marina* is very different from the previously studied mitoses of dinoflagellates, including that of *Syndinium*. There are three basic differences in the mitoses among typical dinoflagellates and *Syndinium* on the one hand, and *Oxyrrhis* on the other: (1) during division, in typical dinoflagellates (e.g. *Crypthecodinium cohnii*) the nuclear envelope invaginates to form several cytoplasmic channels in which microtubules are present or, in *Syndinium*, to form only one channel in which the mitotic apparatus exists, but in *Oxyrrhis* the nuclear envelope does not invaginate and the microtubules are seen to be entirely within the nucleus; (2) in *Oxyrrhis* there are nuclear plaques appearing on the nuclear envelope that act as microtubule organizers, but no such structures appear in typical dinoflagellates or *Syndinium*; (3) the movement of chromosomes during anaphase is driven by microtubules through the nuclear envelope in typical dinoflagellates and *Syndinium*, but in *Oxyrrhis* it is driven by microtubules directly. In *Syndinium*, unlike the situation in typical dinoflagellates and *Oxyrrhis*, centrioles participate in the formation of the mitotic apparatus.

Triemer (1982) suggested that a *Syndinium*-like mitosis might have given rise to a mitosis like that of *Oxyrrhis*. However, a more reasonable relationship among them would be that mitoses in *Oxyrrhis* and in *Syndinium* have evolved from mitosis in typical dinoflagellates. If mitosis is taken as a phylogenetic criterion, the relationship among typical dinoflagellates, *Syndinium* and *Oxyrrhis* should be that the latter two have evolved from typical ones.

The early evolution of mitosis

The mitosis of *Oxyrrhis* perhaps represents an important step in the evolution of mitosis. On the basis of our knowledge we propose one possible sequence in the early evolution of mitosis. (1) In the early stages of evolution, microtubules in the spindle were present within many cytoplasmic channels lined with the nuclear envelope, which remained unbroken during mitosis. Some microtubules in the channels played a static role, determining the direction of division, and others were attached to the nuclear envelope at those points where chromosomes were associated with the envelope, by means of kinetochore-like structures. The movement of the chromosomes was driven by microtubules through the nuclear envelope, just as in typical dinoflagellates. The *Syndinium*-type mitosis is just a modification of the typical dinoflagellate mitosis. (2) Later, many pairs of microtubule organizers developed on the nuclear envelope. The microtubules that radiated from the microtubule organizers were present in the nucleus and directly drove the movement of the chromosomes as in *Oxyrrhis*. (3) Later still in evolution, the multiple pairs of microtubule organizers on the nuclear envelope united to form only one pair of plaque-like microtubule-organizing centres, which are found in many fungi, algae and protozoa (e.g. the so-called 'centriolar plaques').

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