Conical Kinetochores with Single Microtubules in the Micronuclear Mitosis of the Ciliate *Colpoda steinii*

A. Kuck and A. Ruthmann

Lehrstuhl für Zellmorphologie, Ruhr-Universität Bochum, Postfach 10 21 48, D-4630 Bochum 1

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During micronuclear metaphase, the decondensed chromosomes bear conical kinetochores connected by a single microtubule to the tapered ends of the spindle-shaped nucleus. Using Bernhard's procedure for the preferential staining of ribonucleo-protein-containing structures, the whole kinetochore cone retains its electron density. Chromosome separation at anaphase involves shortening of the kinetochore microtubule and elongation of the interpolar region.

Introduction

While the kinetochores of higher animal and plant cells are either trilaminar and discoidal or of the cup and ball-type [1], usually connected to the spindle pole region by a number of microtubules, the spindle attachment of protozoan chromosomes is more varied [2]. In the "closed" micronuclear spindle of ciliates, discrete kinetochores could be identified in only few not closely related species. In the gymnostome *Loxodes magnus* the kinetochore has the disc-like structure observed in most animal cells [3] and in the hymenostome *Tetrahymena* it is of the cup and ball-type [4] as in most plant cells. In *Stentor coeruleus* the kinetochores also appear to be discs but their trilaminar organization is not as clear as in *Loxodes* [5]. Recently, huge trilaminar kinetochores covering the entire poleward surfaces of peculiar composite chromosomes have been studied in the micronuclear mitosis of *Nyctotherus ovalis*, a heterotrichous ciliate [6]. Lewis *et al.* [7] found microlamellae which assume the function of kinetochores in the micronuclear spindle of *Paramecium bursaria*. In other ciliates, the microtubules terminate at the chromosomes without any recognizable contact structure [8]. In the present communication the unusual conical kinetochores of *Colpoda steinii* are described.

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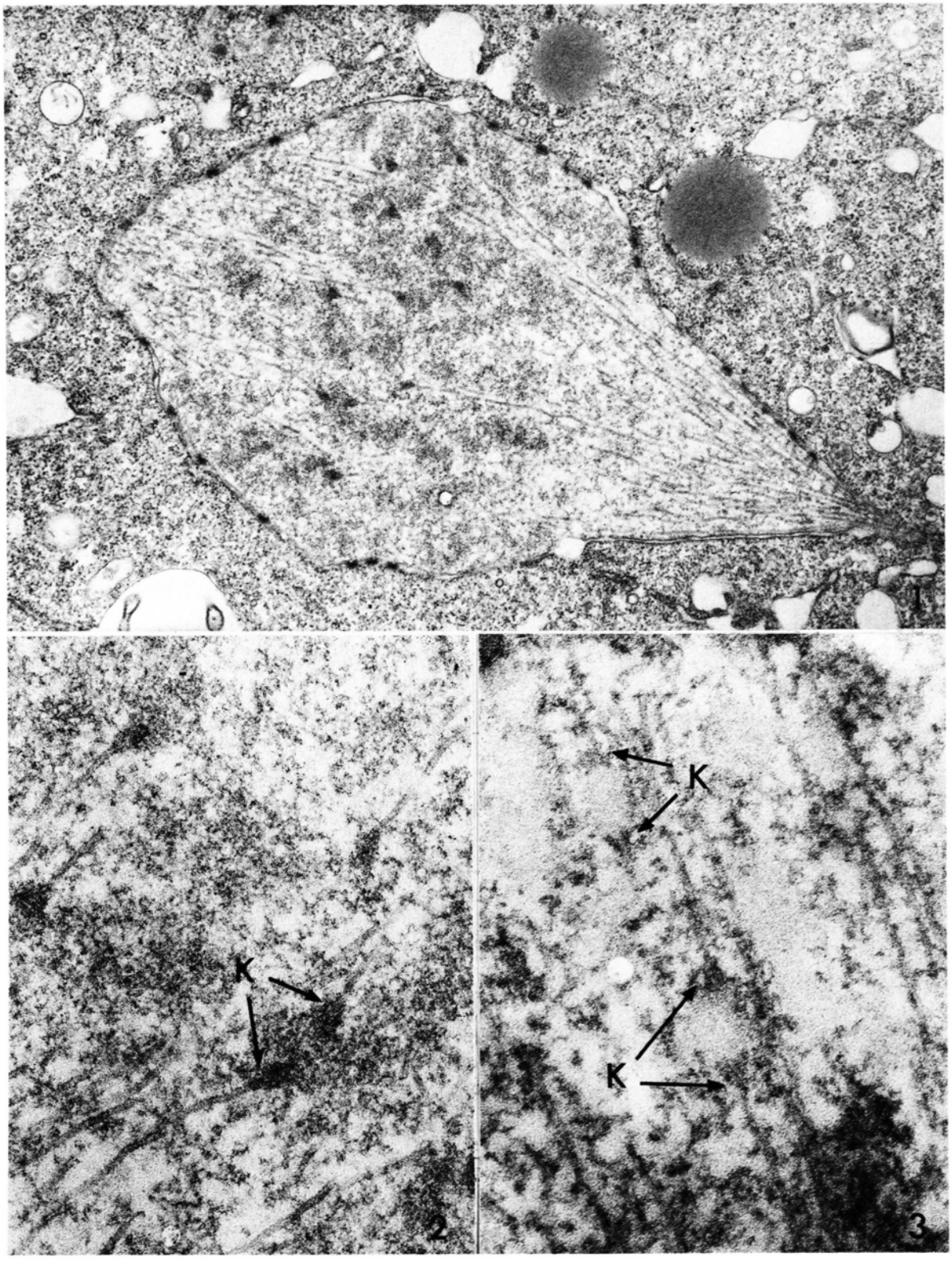
Materials and Methods

Colpoda steinii was isolated from moss and cultured in Erdschreiber medium containing 0.9% NaCl, 0.01% $NaHCO_3$ and 0.2% casamino acids (DIFCO) at room temperature. For electron microscopy, division cysts were collected in centrifuge vials, washed and fixed first in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 (60 min) and then in 1% OsO_4 in the same buffer. Sections of Epon-embedded cells were stained with uranyl acetate and lead citrate and viewed with a JEM 100 B electron microscope at 80 kV. For the preferential electron staining of ribonucleoproteins the method of Bernhard [9] was applied. After glutaraldehyde fixation, the sections were stained with uranyl acetate, treated for 40 min at 40 °C with 0.1 M EDTA and poststained with lead citrate.

Results

Colpoda steinii forms thin-walled reproductive cysts and divides twice in succession before excystment. During interphase, the oval micronucleus lies at one side of the single macronucleus. Its chromatin is condensed and forms meandering loops surrounding fibrillar material of low electron density. In early prophase the chromatin mass separates into small lumps and single microtubules appear below the nuclear envelope. During prometaphase the micronucleus elongates and the chromatin lumps separate into small chromosomes. The microtubules increase in number and extend into the direction of nuclear elongation. At metaphase the chromosomes are decondensed and appear as fibrillar masses in the center of the spindle-shaped micronucleus (Fig. 1). An equatorial plate in the strict sense is not formed. The microtubules extend from this central chromosome mass and seem to insert at the pointed ends of the nuclear envelope. There are only few interpolar microtubules which reach from one halfspindle into the other. The kinetochores which are not seen before the chromosomes are assembled in the central region of the micronucleus are conical and homogeneous. The flat base of each cone is in contact with the chromosome while a single microtubule emerges from its pointed end (Fig. 2). Chromosome separation at anaphase is accompanied by a stretching of the interpolar region and a shortening of the kinetochore microtubules. As the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distance to the poles decreases, the micronucleus assumes an elongated cylindrical shape. Finally, the dividing nucleus traverses the whole length of the reproductive cyst, its bulbous ends being close to the pellicle. At telophase the ends of the micronucleus bend over the macronucleus and the connecting stem body is pinched off at both sides. It is resorbed during cytokinesis. The chromatin condenses again into compact granules and the microtubules disappear. Since ribonucleoprotein has been shown to be a component of the inner layer of trilaminar kinetochores of higher eukaryotes [10] and of the ciliate *Nyctotherus ovalis* [6] we examined the *Colpoda* kinetochore using Bernhard's method for the preferential preservation of RNP contrast. After 40 min treatment with EDTA, the chromatin of metaphase nuclei was destained but the kinetochores had retained their contrast. Their form and homogeneity was unchanged (Fig. 3). The contrast of macronuclear nucleoli and ribosomes in the cytoplasm was also retained.

Discussion

Conical kinetochores have not been reported from ciliates as yet. The peculiar shape may be due to the fact that only a single microtubule is anchored in each kinetochore. This is not rare in yeasts and lower fungi which also have small chromosomes with decondensed chromatin at metaphase [11]. The distribution of presumed ribonucleoprotein after the application of Bernhard's procedure suggests that the cone corresponds to the inner RNP-layer in trilaminar kinetochores.

In organisms with a single microtubule emanating from the kinetochore, the microtubule is reported to reach from the chromosome to the pole for the whole duration of metaphase and anaphase [12]. Although we cannot document this by serial sections in the case of *Colpoda steinii*, the enormous length of kinetochore microtubules is evident in Fig. 1. Since the distance between the kinetochores and the poles decreases during anaphase, the single microtubule which connects a chromosome to the pole must depolymerize and shorten at the same rate as the chromosome progresses.

There are no special polar structures in the spindle such as the polar caps seen in *Didinium nasutum* [8] and *Loxodes* [3]. The possible role of these structures as microtubule organizing centers is still discussed. Special wide tubules of 35–40 nm which originate from a persisting nuclear crystalloid become continuous with the inner nuclear membrane in the suctorian *Paracineta limbata* [13]. In *Colpoda steinii* normal microtubules terminate at the inner nuclear membrane mostly at a small tapered part of the nucleus as in *Nyctotherus ovalis* [6].

[1] C. L. Rieder, J. Cell Biol. **80,** 1–9 (1979).
[2] I. B. Raikov, The Protozoan Nucleus. Morphology and Evolution, Wien 1982.
[3] I. B. Raikov, Tsitologiya (Leningrad) **18,** 144–151 (1976).
[4] I. R. La Fountain and L. A. Davidson, Chromosoma (Berl.) **75,** 293–308 (1979).
[5] N. Raikhel, J. J. Paulin, and S. O. Skarlato, J. Protozool. **28,** 103–107 (1981).
[6] U. Eichenlaub-Ritter and A. Ruthmann, Chromosoma (Berl.) **84,** 701–716 (1982).
[7] L. M. Lewis, E. R. Witkus, and G. M. Vernon, Protoplasma **89,** 203–219 (1976).
[8] B. P. Karadzhan and I. B. Raikov, Protistologica **13,** 15–29 (1977).
[9] W. Bernhard, J. Ultrastruct. Res. **27,** 250–265 (1969).
[10] C. L. Rieder, J. Ultrastruct. Res. **66,** 109–119 (1979).
[11] I. B. Heath, Int. Rev. Cytol. **64,** 1–80 (1980).
[12] I. B. Heath, in: Nuclear Division in the Fungi (I. B. Heath, ed.), 89–176, New York 1978.
[13] M. Hauser, Z. Zellforsch. **106,** 584–614 (1970).

Fig. 1. Longitudinal section through the micronuclear spindle. The microtubules converge towards the tapered ends of the nucleus (lower right) × 24000.

Fig. 2. A single microtubule emerges from each of the conical kinetochores (K) × 75000.

Fig. 3. Preferential preservation of RNP contrast after EDTA-treatment. Chromatin bleached, K: kinetochores × 75000.