MITOSIS IN *CHLAMYDOMONAS REINHARDTII* BASAL BODIES AND THE MITOTIC APPARATUS

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Cell division in *Chlamydomonas reinhardtii* was described by Johnson and Porter (1968). In their paper, they state (p. 409): "... they (*referring to basal bodies*) are not found at the poles of the mitotic apparatus as are the centrioles of animal cells and certain plant cells" (present author's

italics). However, the reported absence of basal bodies[1] at the spindle poles of *C. reinhardtii* contrasts markedly with the spindles of other volvocalean algae which have been studied recently. Basal bodies are found at one pole, at least, of the mitotic apparatus of *Tetraspora* (Pickett-Heaps, 1973), and basal bodies are found near the spindle poles in vegetative cells (Pickett-Heaps, unpublished observations) and developing sperm packets of *Volvox* (Deason and Darden, 1971). All green algae (except *Chlamydomonas*) so far found to have persistent centrioles or basal bodies separate these organelles to daughter cells via mitosis; the centrioles/basal bodies become associated with the polar organizers of the spindle and are evenly distributed to daughter cells as a consequence of this association (Dietz, 1966; Nakao et al., 1968; Pickett-Heaps, 1969, 1971; Friedländer and Wahrman, 1970). Pickett-Heaps (1973) points out that the absence of basal bodies at the poles of the spindle apparatus of *C. reinhardtii* is, therefore, somewhat of an anomaly, which, if true, has considerable significance. He suggested that the basal bodies might be closer to the poles than Johnson and Porter (1968) reported. The relationship of the basal bodies to the poles of the mitotic apparatus could easily have been misinterpreted after examination of insufficient numbers of thin sections. Consequently, mitosis in *C. reinhardtii* was investigated using 0.25 and 0.5 μm thick sections and the High Voltage Electron Microscope (HVEM) at the University of Colorado to determine the location of basal bodies with respect to the spindle apparatus in this alga.

MATERIALS AND METHODS

Wild type 137c of *C. reinhardtii* was used in this investigation. This alga was grown and prepared for electron microscopy by the laboratory of Dr. D. J. L. Luck at The Rockefeller University, New York.

The cultures were grown on the medium of Sager and Granick (1953) supplemented with 3% CO_2 on a 12 h light/dark cycle.

Cells were fixed 3 h into the dark cycle. 1 ml of 12% glutaraldehyde in 0.02 M sodium cacodylate buffer (pH 7.5) was added to 4 ml of cells in suspension. After 10 min at room temperature the cells were pelleted and resuspended in 2 ml of 5% glutaraldehyde (0.01 M sodium cacodylate, pH 7.5) in the cold. 2 ml of 1% OsO_4 (0.01 M sodium cacodylate, pH 7.5) were then added to the cells and the mixture was left in the cold for 1 h. The cells were then pelleted and rinsed three times in 0.01 M sodium cacodylate buffer. The final pellet was dehydrated in ethanol and embedded in Epon. 0.25 and 0.5 μm thick sections were cut with either a glass or diamond knife, picked up on Formvar-coated slot grids, and stained for 30 min in 2% methanolic uranyl acetate and for 20 min in lead citrate. The sections were viewed in a JEM 1000 electron microscope operating at 1,000 kV.

RESULTS AND DISCUSSION

When mitotic nuclei were examined in the HVEM, basal bodies were commonly found at least at one of the polar fenestrae of the spindle (Figs. 1–3). Two basal bodies were found near each pole of the metaphase spindle when thick serial sections were examined. The mitotic nucleus is bent and the nuclear envelope is extended and hooked at the poles at metaphase, and a thick section that included both sets of polar basal bodies was not encountered. However, during anaphase the poles are not as hooked as during metaphase, and a thick section through an anaphase spindle which included both sets of polar basal bodies was encountered (Fig. 4 B). During anaphase, the basal bodies are not located directly at the foci of the spindle microtubules, but slightly to one side. Consecutive serial sections through this spindle show its bent nature (Fig. 4 A–C).

If the colonial members of the Volvocales, the Chlorococcales, and some of the Ulothrichales evolved from a *Chlamydomonas*-like progenitor that utilized a phycoplast (and not a phragmoplast) (Pickett-Heaps and Marchant, 1973), and if some mitotic features are conservative (Pickett-Heaps, 1974), then the mitotic apparatus of all these organisms would be expected to be reasonably similar to each other. Thus, *Chlamydomonas* should possess basal bodies at the poles of its mitotic apparatus. The results of this study indicate that the spindle of *C. reinhardtii* is similar to those of the other volvocalean genera. This finding is further supported by the work of Triemer and Brown (1974). They have described mitosis in *Chlamydomonas moewusii*, a closely related species that also possesses basal bodies near the poles of its spindle. Possession of basal bodies at the spindle poles by both *C. reinhardtii* and *C. moewusii* removes *Chlamydomonas* from the anomalous position it would otherwise occupy in the phylogeny of volvocalean organisms, on the

[1] By *basal bodies*, I am referring to the basal bodies that served as the nucleating centers for the microtubules of the flagella during interphase, but which during cell division become detached from the axonemes, lose their transition regions, replicate, and are then ultrastructurally similar to the centrioles commonly found at the poles of centric spindles.

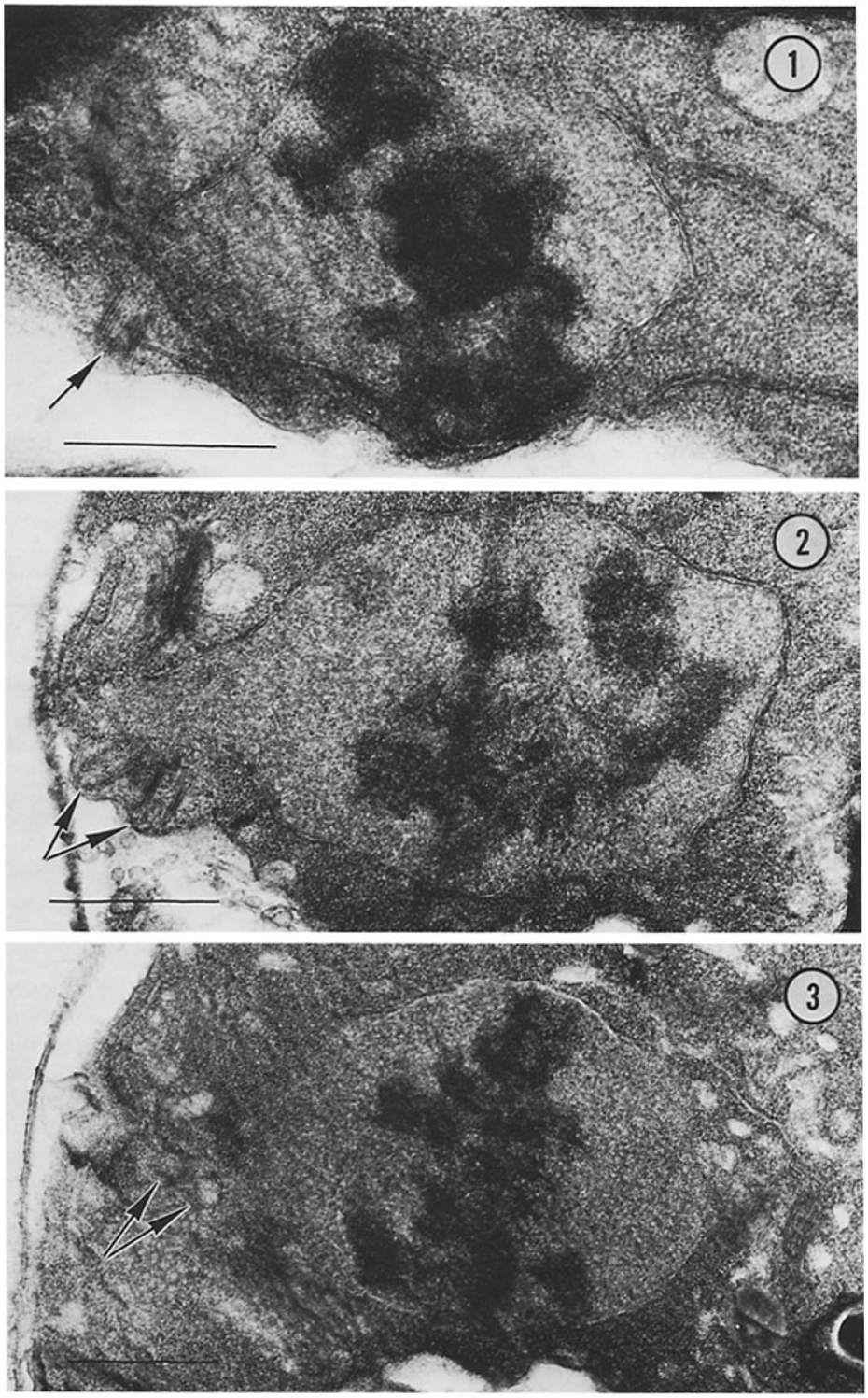
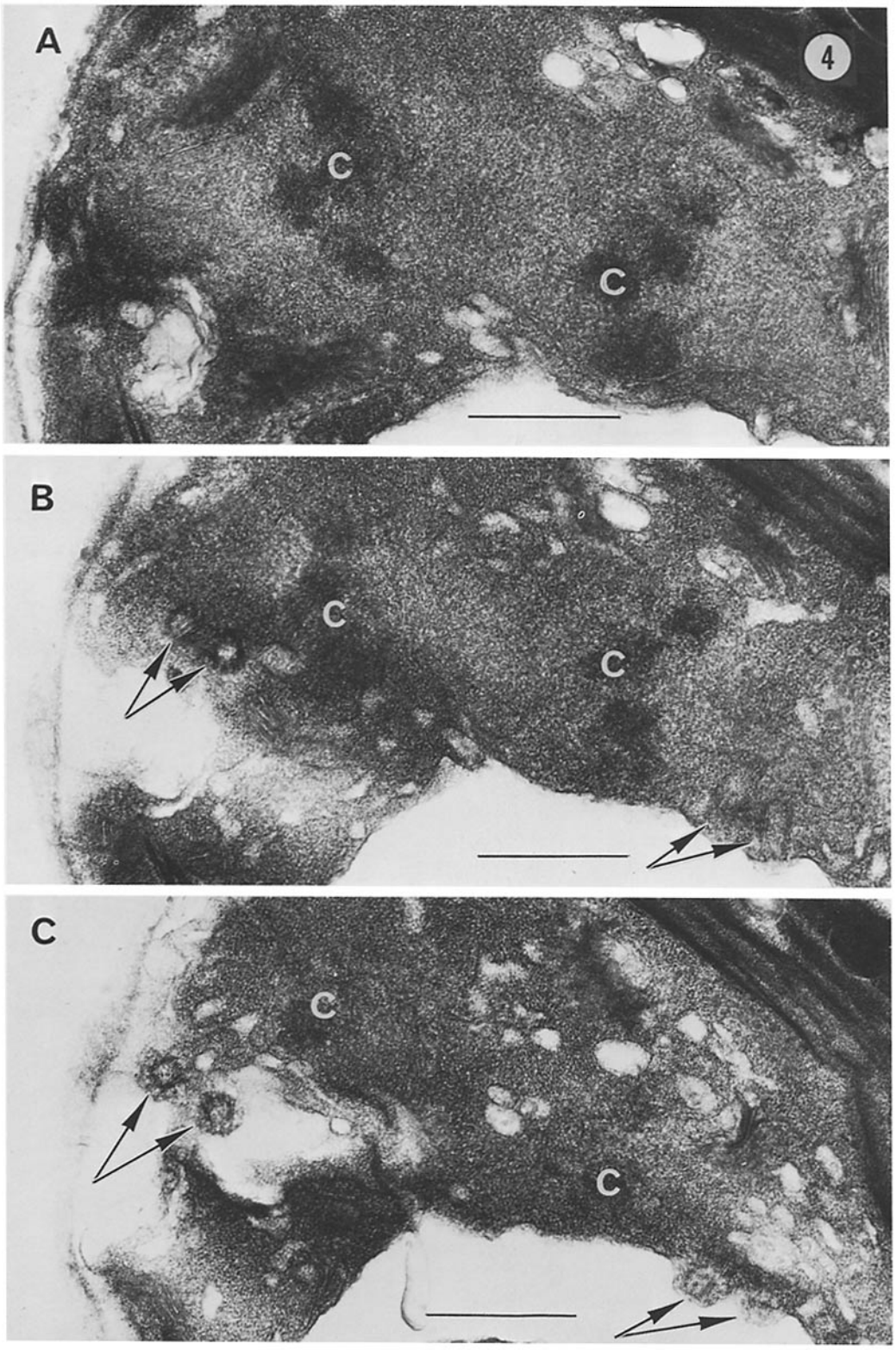


FIGURE 1 High voltage electron micrograph of a 0.5 μm thick section through a metaphase nucleus. The arrow points to a basal body at one of the poles of the spindle. $\times$ 30,000.

FIGURE 2 High voltage electron micrograph of a 0.25 μm thick section through a nucleus in early anaphase. The paired arrows show the two basal bodies near one of the poles. $\times$ 24,000.

FIGURE 3 High voltage electron micrograph of a 0.25 μm thick section through a metaphase nucleus. The paired arrows point out the two basal bodies near one of the poles. $\times$ 21,000.



FIGURES 4 A–C High voltage electron micrographs of three consecutive 0.25 μm thick sections through a nucleus in late anaphase showing the bent nature of the spindle. *C*, chromosomes. The spindle is bent slightly down and toward the reader. Fig. 4 A: No basal bodies are present in this micrograph although the chromosomes and spindle microtubules are well defined. × 23,000. Fig. 4 B: Both pairs of polar basal bodies are present (paired arrows) in this micrograph. × 23,000. Fig. 4 C: The four basal bodies are still recognizable (paired arrows) in this micrograph, although little of the nucleus is present. × 23,000.

basis of Johnson and Porter's report (1968). These results further show that *Chlamydomonas* evenly distributes its basal bodies to daughter cells by the same mechanism utilized by all other green algae with persistent centrioles or basal bodies.

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