Microtubule Nucleating Sites in Higher Plant Cells Identified by an Auto-Antibody against Pericentriolar Material

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ABSTRACT Human scleroderma serum 5051, which is known to recognize the amorphous pericentriolar microtubule organizing center material of a variety of vertebrate cells, was found to immunostain spindle poles of meristematic higher plants from pre-prophase to late anaphase. Subsequently, during cytokinesis, staining was redistributed around the reforming telophase nuclei, but was not evident in the cytokinetic phragmoplast. At the transition between telophase and interphase, before the typical cortical interphase microtubule array was established, short microtubules radiated from the nucleus and in such cells the material recognized by 5051 was located around the daughter nuclei and not the cortex.

These observations have led us to propose that the perinuclear region, or the nuclear surface, may function as a nucleation center for both spindle and interphase microtubules in higher plant cells.

Four successive microtubule arrays are present during the cell cycle in meristematic higher plant cells: the interphase cortical array (which is involved in orienting fibrils in the wall), the pre-prophase band (which predicts the division plane), the spindle, and, finally, the cytokinetic phragmoplast (12, 20, 23). The functions of these arrays and the control of their rearrangements are fundamental to tissue patterning and morphogenesis (7); very little is known, however, about the nature or location of the components which nucleate and/or organize microtubules in plant cells. It is not known, for example, whether each array has its own nucleating sites or whether common sites successively nucleate all arrays (16).

The origins of the cortical interphase array are not clear. Concentrations of electron-dense material, thought to be microtubule nucleating sites, have been observed in the cell cortex, and this has led to the idea that this microtubule array may be generated by sites at cell edges (8). However, it is not known whether this concept applies generally to higher plant cells, nor is it known if these putative interphase sites are related to those active during cell division.

In most animal cells, a pair of centrioles serves as a convenient marker for the associated amorphous pericentriolar material (PCM)[1] which functions as the microtubule nucleating site both in interphase and at mitosis (6, 18). Higher plant cells do not contain centrioles, but osmiophilic material resembling PCM has been observed at the poles of plant spindles (19). This led Pickett-Heaps (19) to propose the term "microtubule organising centres" for similar material in both plants and animals.

[1] *Abbreviations used in this paper:* PCM, pericentriolar material.

Recently, a human auto-antibody has been described (1) which recognizes PCM in a variety of vertebrate cells, but also immunostains the spindle poles of mouse eggs which, like plant cells, do not possess a centriole. The ability to detect PCM in the absence of centrioles has led us to investigate the staining pattern of this serum in plant cells, and we report here that the serum recognizes material at the spindle poles of *Allium* root-tip cells, and we have followed its distribution during the cell cycle.

MATERIALS AND METHODS

Immunofluorescence Procedures: Seeds of *Allium cepa* Linnaeus were germinated on moist filter paper at 20°C; the terminal 1 mm of 3-d-old radicles was prepared for immunofluorescence staining using a modification of the method of Wick et al. (23) as described in detail elsewhere (2). Fixed cells were released from the root-tip onto the wells of multitest slides (Flow Laboratories Ltd.) by gentle squashing with a plastic rod, and air dried. These cells retained their shape, as in the intact root tip, and were permeable to antibodies without further treatment. Detergent extraction, with 0.1% Nonidet P-40, did not significantly alter the pattern of staining. Incubations with antibodies were performed at 37°C for 45 min. Where required, nuclei were stained with 1 μg/ml propidium iodide (Sigma Chemical Co., Poole, UK) after the antibody treatments.

Antibodies: Human scleroderma serum 5051 was the generous gift of Drs. T. Mitchison and M. Kirschner, University of California Medical Center, San Francisco). The serum was used diluted 1/100 (vol/vol) in phosphate-buffered saline (PBS) or PBS containing 1% bovine serum albumin. Scleroderma sera were initially tested by immunofluorescence staining of PtK_2 cells prepared by fixation at −20°C in methanol followed by acetone. Cells were rehydrated at room temperature in PBS containing 2% (wt/vol) bovine serum albumin.

Microtubules were stained using a monoclonal antibody raised against yeast tubulin (11). FITC-conjugated goat anti-human IgG and rhodamine-conjugated

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rabbit anti-rat IgG were obtained from Miles Laboratories Ltd. and used at dilutions of 1/150 and 1/100, respectively.

Microscopy: Slides were examined using a Zeiss Universal II microscope equipped with an HBO 50 high pressure mercury vapor lamp for epifluorescence illumination, and filter sets for rhodamine and FITC fluorescence detection. A planapo 40x objective numerical aperture 1.0 was used, and photographs were recorded on Ilford FP4 or XP1 35-mm film.

RESULTS

Squashes of fixed root tips contain representative cells from all stages of the cell cycle. Preservation of the microtubule arrays in these cells was good and double staining with anti-tubulin was used to compare microtubule distribution and 5051 staining in most preparations.

The earliest stage of mitosis where material could be detected with 5051 was in cells where a preprophase band was present—recognized by double staining with anti-tubulin, or by the characteristic chromatin condensation pattern in these cells, visualized with propidium iodide (Fig. 1, *a* and *b*). Cells in this stage invariably possess microtubules at the nuclear surface, focused upon the forming spindle poles (22, 23). The 5051 staining material was present close to the nucleus, marking the incipient poles (Fig. 1*a*). The immunostaining at this stage was not so intense as it appeared during stages of mitosis, particularly at prophase (Fig. 1, *c* and *d*) where brightly staining material is clearly present at the two broad poles. No 5051 staining material was detected in the cortical zone marked by the pre-prophase band microtubules.

At metaphase the polar staining was spread over the broad, typically "barrel-shaped" spindle (Fig. 2, *a–d*). In particular, the micrograph pair that shows the double staining with anti-tubulin (*c* and *d*) reinforces the impression of many small concentrations of material contributing to the spindle pole. During anaphase, especially in later stages (*e* and *f*), staining was still present at the pole but also showed some redistribution around the outside of the spindle and chromosomes.

At telophase—when the cytokinetic phragmoplast is present—this redistribution was more marked; the poles no longer showed bright staining, whereas clusters of material appeared at the edges of the re-forming telophase nucleus facing the phragmoplast (Fig. 3, *a* and *b*). In double-stained preparations, the phragmoplast microtubules often appeared to end in close proximity to concentrations of 5051 staining (Fig. 3, *a* and *b*). No staining of the mid-zone of the phragmoplast with 5051 was observed.

Later in cytokinesis, when the phragmoplast microtubule array had almost disappeared and the cell plate was nearly complete, a new microtubule array was apparent in the daughter cells. We termed this array "early interphase"; it was comprised of short microtubules, or bundles of microtubules, apparently radiating from the nuclear surface (Fig. 3*e*). No pattern intermediate between this and the fully developed cortical interphase array could be detected in our preparations; this suggests that this transition is relatively rapid, and/or unstable to fixation. In cells at this stage, 5051 staining was distributed around the surface of the daughter nuclei (Fig. 3*c*); chromatin in these cells demonstrated stages of decondensation (Fig. 3*d*). Fig. 3*f* shows the complete cortical microtubule array; no 5051 staining could be detected in interphase cells possessing such a mature microtubule configuration. The use of auto-antisera such as 5051 has the disadvantage that it is not possible to compare staining patterns with those of pre-immune control sera. However, we have used non-scleroderma human serum on *Allium* cells in order to confirm that the staining we observed in 5051 was not due to components generally present in human serum (data not shown). In addition, we have screened a range of human scleroderma sera for reaction with centrosomes and kinetochores. None of those staining animal cell kinetochores cross-reacted with plant nuclear components; one serum, however,

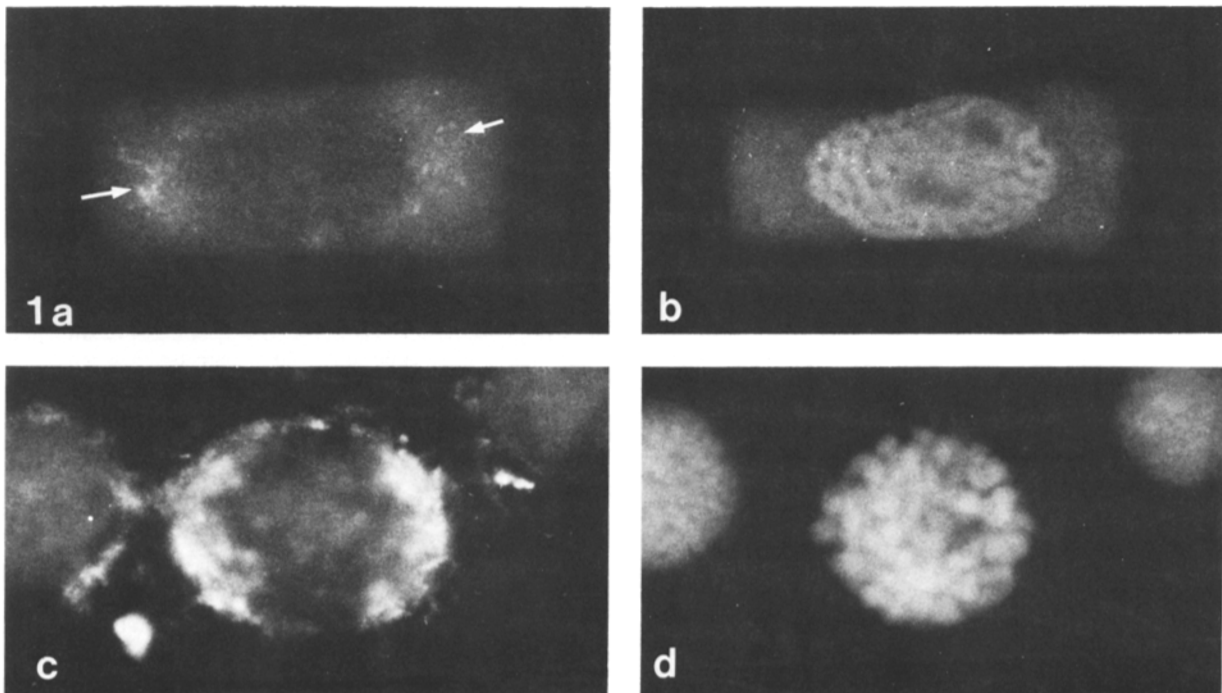

FIGURE 1 *Allium* root tip cells stained with anti-centrosome serum 5051; chromatin was counterstained with propidium iodide. (*a*) Pre-prophase: stained material is present close to the nucleus at polar locations (arrowed); (*b*) counterstaining of cells in *a* with chromatin condensation pattern; (*c*) prophase spindle poles are brightly stained with 5051; (*d*) chromatin condensation pattern in c. × 1,100.

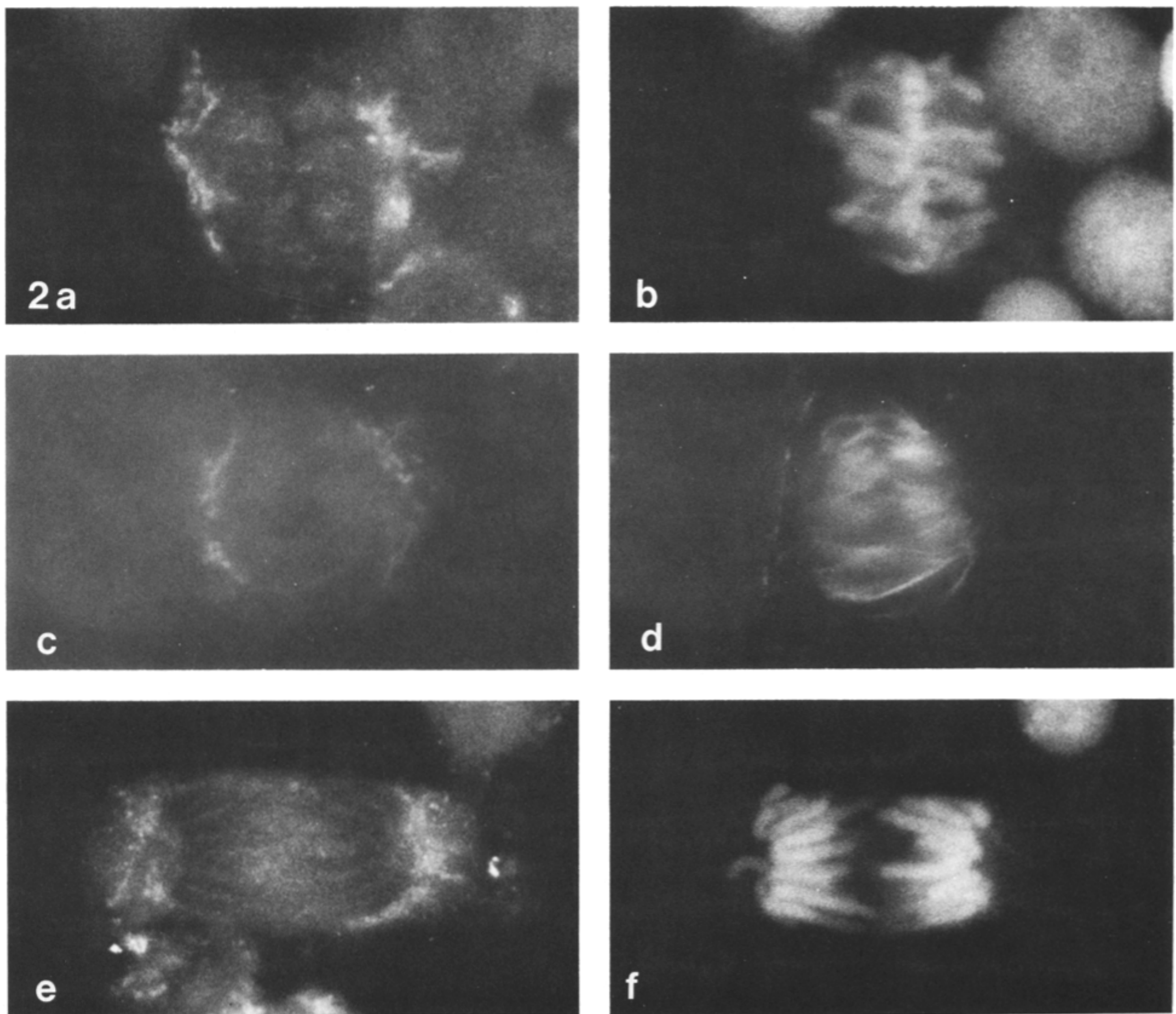

FIGURE 2 (*a* and *b*) Micrograph pair showing a cell in metaphase stained with (*a*) 5051 and (*b*) propidium. The spindle poles are broad and flat compared with the "cap-shaped" poles in prophase (cf. Fig. 1 *c*). (*c* and *d*) Metaphase cell double-stained with (*c*) 5051 and (*d*) anti-tubulin. Spindle poles appear to consist of numerous small foci. (*e* and *f*) Anaphase cell stained with (*e*) 5051 and (*f*) propidium. × 1,100.

(designated WMH/JI.01) which stained animal centrosomes demonstrated a similar pattern of staining of *Allium* cells to that shown by 5051 (Fig. 4).

The precise biochemical nature of the antigen reacting with these antibodies is unknown, but may range from a nucleic acid, protein, or a protein modification, such as a phosphorylation (21). The staining data presented here, using two independent antisera which recognize centrosomal material, strongly suggest that animal and plant mitotic microtubule organizing centers possess one or more common antigenic components.

DISCUSSION

A major difference between higher plant cells and most animal cells is the absence from the former of a conspicuous centrosome. In animal cells, this consists of a pair of centrioles with associated amorphous material, and it is this PCM that nucleates microtubules both during interphase and mitosis (15). The centrioles are not essential for spindle formation but they "probably help the proper distribution and organization of the pericentriolar cloud" (14). At a practical level, centrioles provide an obvious landmark for locating the PCM.

The scleroderma serum, 5051, stains the poles of vertebrate cells lacking centrioles (1), and it is shown here that it also stains plant spindle poles. The presence of common antigenic sites supports Pickett-Heap's prediction (16) that plant and animal spindle poles should have similarities.

The staining pattern throughout the cell cycle of onion cells shows close parallels to that seen in the earliest stages of mouse development, where PCM-like material is only detectable with serum 5051 in dividing or recently divided cells.

Preparation for mitosis is often indicated in plant cells by the formation of a pre-prophase band of microtubules (which predicts the plane of division). At this stage microtubules may be seen focused on the forming poles using anti-tubulin staining (22, 23). It is in such cells that staining of the poles with 5051 is first detected. The stained material then undergoes several re-arrangements during mitosis: resembling "polar caps" at prophase, flat plates at metaphase, leading to a more dispersed arrangement surrounding the half-spindle and the chromatin by late anaphase. Such a pattern is consistent with Mazia's idea (17) of a flexible centrosome in which the microtubule organizing centers may take different forms in-

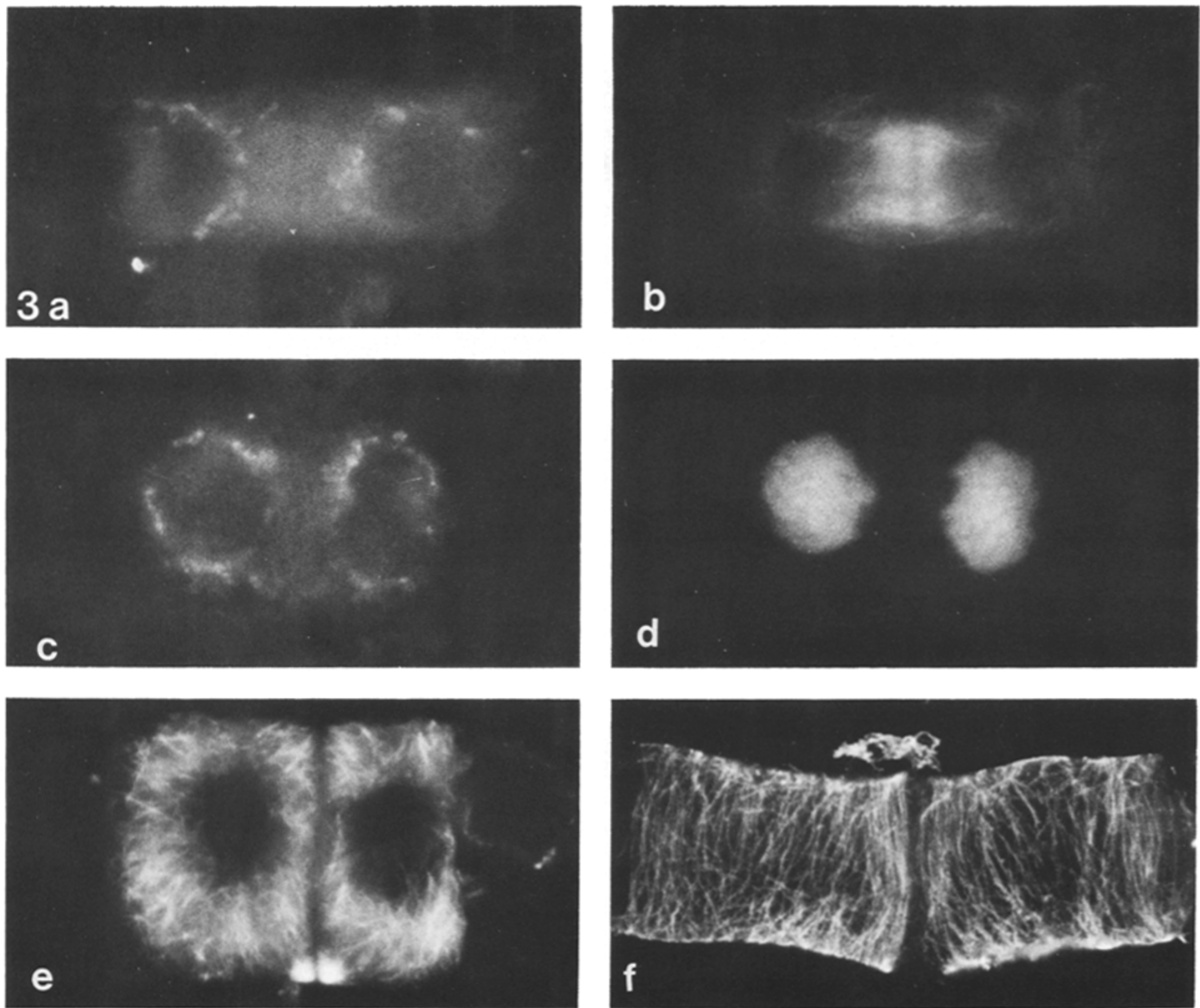

FIGURE 3 Cytokinesis and early interphase in *Allium* cells. (*a* and *b*) A cell double-stained with (*a*) 5051 and (*b*) anti-tubulin showing the re-distribution of 5051 staining around the telophase nuclei—particularly the region facing the phragmoplast. (*c* and *d*) Daughter cells in early interphase—5051 staining (*c*) is present around the reforming nuclei (*d*). (*e*) Anti-tubulin staining of cells in early interphase demonstrates microtubules that radiate from the nucleus. In this cell the remnants of the phragmoplast are visible at one edge of the cell plate. (*f*) Anti-tubulin staining of cells later in interphase, with a mature cortical microtubule array. × 1,100.

dependent of the centrioles; the pattern is also consistent with the idea that spindle and interphase microtubules are formed as the mutually exclusive activities of a common set of microtubule nucleating sites (13).

During late telophase/early interphase, the 5051 staining surrounds the nucleus, concomitant with the appearance of short microtubules which radiate from the nuclear surface. This implies that the nucleating sites for the interphase array are located around the nucleus and not (as has been suggested by Gunning et al. [8] and Hardham et al. [9]) at the cell edges. It is of course possible that cortical microtubule nucleating sites are present in *Allium* but go unrecognized by the two sera used here. However, if the present observations concerning perinuclear microtubule nucleating sites are correct, then the subsequent development of mature, transverse cortical arrays from an initially radial array may be independent of cortical sites and depend instead upon the interaction of long and growing microtubules with each other and with the plasma membrane (15). This concept of microtubules winding around the cortex is supported by recent immunofluorescence studies showing interphase microtubule arrays to be helices (14)—a conformation not easily constructed from microtubule organizing centers aligning the vertical cell edges. Other workers have also reported a perinuclear origin for interphase microtubules in endosperm (3) and lilly pollen meiocytes (4), although in these cases there is no typical higher plant cell wall and the later organization of microtubules around the cortex was not seen.

The origin of the phragmoplast microtubules remains more problematical in that no immunostaining of the mid-zone was observed with 5051, despite the reported presence there of amorphous, electron-dense material presumed to be the nucleating sites (10). However, 5051 staining was present around the phragmoplast-facing edges of the re-forming telophase nuclei, suggesting a perinuclear origin for these microtubules also. Such a model is consistent with the microtubule polarity data from *Haemanthus* phragmoplasts (5) which showed the plus ends of each set of microtubules directed away from the nuclei and towards the cell plate. In all systems examined so far, the plus ends are distal to the nucleating site.

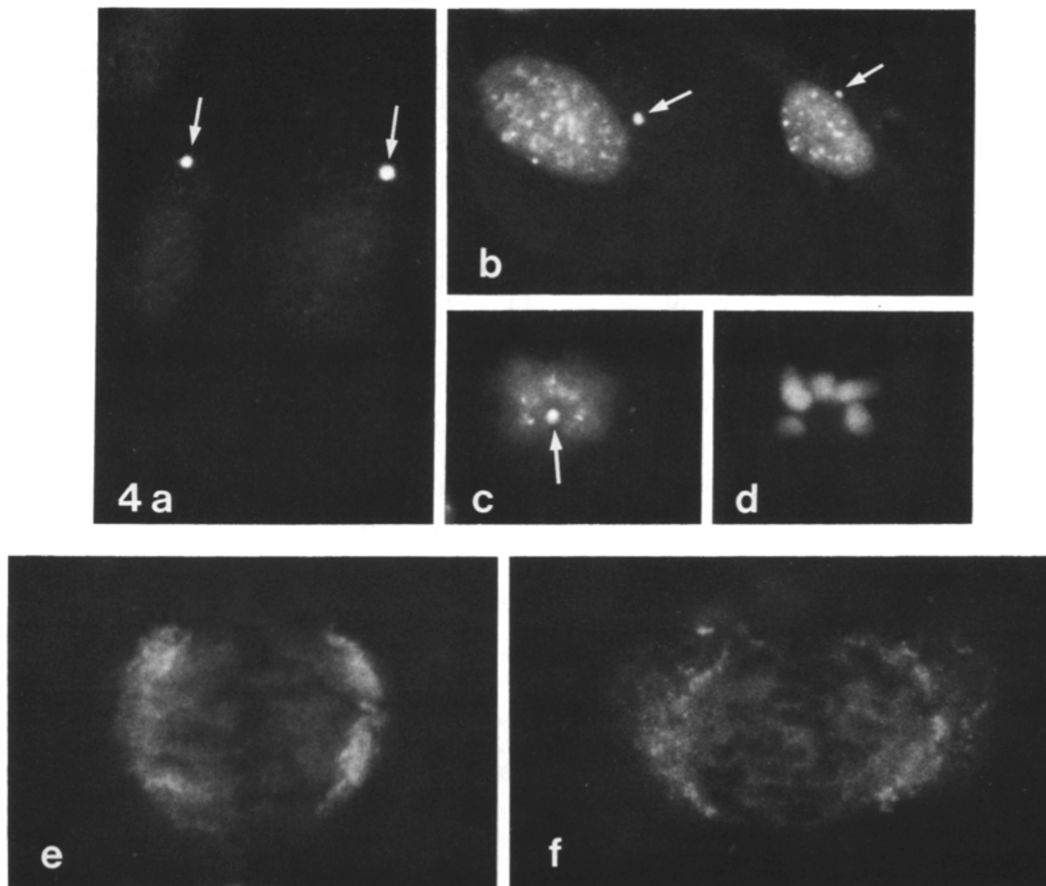

FIGURE 4 (*a*) PtK_2 cells stained with serum 5051 show bright staining of the centrosome. (*b*) PtK_2 cells at interphase, stained with WMH/JI01. (*c* and *d*) Mitotic PtK_2 cell viewed from one spindle pole. (*c*) Immunofluorescence staining with WMH/JI.01. Arrow points to centrosome. (*d*) Chromosomes stained with propidium iodide WMH/JI.01 stains the centrosomes and also the kinetochores of animal cells but does not appear to recognize plant kinetochores (G. Creissen, personal communication). × 830. (*e* and *f*) *Allium* cells in (*e*) metaphase and (*f*) prometaphase stained with WMH/JI.01 demonstrate staining of the mitotic poles similar to that shown by 5051. × 1,100.

The problem is, however, more complex as a perinuclear origin does not easily explain the centrifugal spread of the phragmoplast to meet side walls often far from the nucleus and the original field of the mitotic apparatus.

Similarly, 5051 does not stain the preprophase band and from the present data its origins are not clear. A separate nucleating site for this array need not be invoked if it is formed, as suggested by Pickett-Heaps (20), by a contracting or "bunching together" of the existing interphase microtubules before division.

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