

Minus End-Directed Kinesin-Like Motor Protein, Kcbp, Localizes to Anaphase Spindle Poles in *Haemanthus* Endosperm

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Microtubule-based motor proteins assemble and reorganize acentrosomal mitotic and meiotic spindles in animal cells. The functions of motor proteins in acentrosomal plant spindles are unknown. The cellulosic cell wall and relative small size of most plant cells precludes accurate detection of the spatial distribution of motors in mitosis. Large cell size and absence of a cellulosic cell wall in *Haemanthus* endosperm make these cells ideally suited for studies of the spatial distribution of motor proteins during cell division. Immunolocalization of a kinesin-like calmodulin-binding protein (KCBP) in *Haemanthus* endosperm revealed its mitotic distribution. KCBP appears first in association with the prophase spindle. Highly concentrated within the cores of individual kinetochore fibers, KCBP decorates microtubules of kinetochore-fibers through metaphase. By mid-anaphase (when a barrel-shaped spindle becomes convergent), the protein redistributes and accumulates at the spindle polar regions. In telophase, KCBP relocates toward the phragmoplast and cell plate. These data suggest a role for KCBP in anaphase spindle microtubule convergence, which assures coherence of kinetochore-fibers within each sister chromosome group. Increasing coherence of kinetochore-fibers prevents splitting within each sister chromosome group and formation of multinucleated cells. *Cell Motil. Cytoskeleton* 41:271–280, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION

The basic task of mitosis is the fail-proof equipartition of chromosomes to opposite spindle poles. Early observations [Wilson, 1928] showed that the organization of polar areas in different organisms is variable. Recent studies on the function, organization, and composition of different types of spindle poles demonstrate their ultimate function in chromosome segregation [reviewed in Merdes and Cleveland, 1997; Waters and Salmon, 1997]. One of the basic features shared by spindle poles, independent of their organization, is that they focus chromosomes during anaphase progression. This prevents the sister chromosomes from splitting into smaller subgroups and forming micronuclei, an aberration that would forfeit the very purpose of equal chromosome segregation.

Prevalent concepts of the organization and function of the spindle poles are based on animal somatic tissue cells or eggs of marine organisms. The organization of the spindle poles in these cells is dominated by centrosomes, which are major microtubule (MT) organizing centers (MTOCs). The centrosome is composed of centrioles surrounded by pericentriolar material. The latter functions as MTOC, nucleating and organizing MT arrays.

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Various developed asters emanate from the centrosome and focus spindle fibers toward the center of the pole. However, some meiotic and early embryonic cells lack typical centrosomes. Mouse oocytes have pericentriolar material but lack centrioles [reviewed in Balczon, 1996]. Furthermore, it has been shown that *Drosophila* oocytes do not have a centrosome in any form [Matthies et al., 1996]. However, in prophase, these cells form spindles with convergent poles and spontaneous mitotic aberrations are virtually non-existent. However, aberrations in spindle morphogenesis and loss of chromosomes are common in oocytes of *Drosophila* mutants lacking a minus-end MT motor [Hatsumi and Endow, 1992a,b; Endow et al., 1994]. Thus, even in the absence of a centrosome at the spindle poles, proper chromosome segregation is related to the convergence of spindle MTs and is precisely controlled.

The majority of angiosperms have a clearly different spindle organization. They do not have a localized organelle that is structurally and functionally equivalent to a typical centrosome at any stage of their growth [reviewed in Smirnova and Bajer, 1992; Vaughn and Harper, 1998]. Furthermore, polar regions of the spindle undergo a profound transformation during mitosis. In *Haemanthus* endosperm, the metaphase spindle is predominantly barrel-shaped, but usually becomes convergent in anaphase [Smirnova and Bajer, 1994]. The change of spindle shape in anaphase is an important feature of mitosis, preventing formation of multinucleated cells. In other plant cells, such as maize microsporocytes, a convergent spindle is formed earlier, during the prophase-metaphase transition. Maize mutants, defective in genes responsible for focusing of the spindle poles, display various abnormalities [Staiger and Cande, 1990, 1991]. Thus, an increasing convergence of the spindle poles plays an important role in preventing mitotic aberrations. However, it is still unknown how the angiosperm spindle becomes convergent in the absence of a focusing structure (centrosome).

It has been proposed that formation of the spindle is an expression of self-assembly/organization of the MT cytoskeleton, regulated by MT motor proteins [Mitchison, 1992; Hyman and Karsenti, 1996]. This concept is supported by numerous in vitro and in vivo observations. Under certain conditions, MT arrays form convergent or radial (astral) arrays in the absence of the centrosome/MTOC [Matthies et al., 1996; Merdes et al., 1996; Gaglio et al., 1996, 1997; Heald et al., 1996, 1997; Endow and Komma, 1997; Nédélec et al., 1997; Rodionov and Borisy, 1997]. It appears that MT motor proteins play an essential role in this process [reviewed in Merdes and Cleveland, 1997; Compton, 1998]. Observation of MT reorganization in anucleated cytoplasmic fragments from endosperm of *Haemanthus* suggests that self-organiza-

tion of MT arrays is the basic mechanism directing spindle assembly in the absence of a centrosome/MTOC [Bajer and Molè-Bajer, 1986; reviewed in Smirnova and Bajer, 1992]. However, direct evidence on the role of motor proteins in angiosperm spindle assembly and reorganization is lacking [reviewed in Asada and Collings, 1997].

Recently a novel member (KCBP, kinesin-like calmodulin binding protein) of the kinesin family was isolated from *Arabidopsis*, potato and tobacco [Reddy et al., 1996a,b; Wang et al., 1996]. This protein is unique among kinesin-like proteins (KLPs) in having a calmodulin-binding domain adjacent to its motor domain. KCBP is a minus end-directed motor, and the motor domain of KCBP, like other KLPs, binds to MTs and tubulin subunits in an ATP-dependent manner [Song et al., 1997; Narasimhulu et al., 1997; Narasimhulu and Reddy, 1998; Deavours et al., 1998]. Furthermore, the binding of KCBP to either MTs or tubulin subunits is regulated by calcium/calmodulin [Narasimhulu et al., 1997; Narasimhulu and Reddy, 1998; Deavours et al., 1998]. The N-terminal tail of KCBP contains a second MT binding domain that is insensitive to ATP [Narasimhulu and Reddy, 1998]. KCBP has recently been colocalized with the MTs of the spindle and phragmoplast in *Arabidopsis* cells and tobacco suspension culture [Bowser and Reddy, 1997]. These studies allowed general localization of KCBP, but cell size and inherent light microscopy limitations associated with diffuse immunoreactive material did not permit detailed analysis of the spatial distribution of KCBP. Cells of *Haemanthus* endosperm lack cellulosic cell walls and have mitotic spindles 4–6 times larger than those of other angiosperms. Additionally, kinetochore fibers (K-fibers) of the spindle are organized as highly autonomous structural units, MT converging centers (MTCCs) [Smirnova and Bajer, 1994]. These combined features make the *Haemanthus* spindle uniquely suited for comprehensive analysis of MT convergence and localization of KCBP during mitosis. Our major finding is that KCBP, colocalizing with the prophase and metaphase MT spindle, concentrates at the spindle poles during mid-anaphase-early telophase. In mid telophase, it relocates to the phragmoplast and the cell plate. Our data suggest that KCBP may play a major role during anaphase spindle reorganization by focusing broad polar regions into convergent ones.

MATERIAL AND METHODS

Materials

Haemanthus katherinae Bak. (*Scadoxus multiflorus* ssp. *katherinae*) was used as material. The procedure for handling endosperm is described elsewhere [Bajer and Molè-Bajer, 1986].

Electrophoresis and Western Blotting

Extraction of proteins from endosperm cells was previously described [Smirnova et al., 1995]. Proteins were separated by 7.5% SDS-PAGE, transferred to nitrocellulose membranes and probed with pre-immune serum and affinity purified anti-KCBP antibodies. Antibodies to a synthetic peptide corresponding to a region in the C-terminus of *Arabidopsis* KCBP were prepared and purified as described [Bowser and Reddy, 1997]. Protein blots were incubated with anti-KCBP antibodies (1:400 dilution). Cross-reacting proteins were detected with a secondary antibody conjugated to alkaline phosphatase.

Immunolocalization

Cells were fixed using two different protocols: (1) the cells were fixed in either methanol for 24 hours or methanol containing 15 mM of EGTA for 1 hour, at -20°C . Then the cells were either processed for immunostaining or extracted for 1 hour in 1% BSA and 1% Nonidet P-40 in 10 mM TBS, pH 7.4, containing 5 mM EGTA. (2) Cells were permeabilized in PHEM buffer (60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 2 mM MgCl_2 , pH 6.8) and 0.5% Triton X-100 for 3 min, then fixed in 3.7% paraformaldehyde (30 min) or methanol at -20° for 24 hours. Protease inhibitors, as described by Bowser and Reddy [1997] were added to those solutions. Cells were double stained with monoclonal antibody against β -tubulin (Amersham, Arlington Heights, IL) and affinity-purified anti-KCBP antibodies. Secondary antibodies were goat antimouse conjugated to FITC and donkey antirabbit conjugated to Texas Red (Amersham). As a control, cells were also incubated with preimmune serum and Texas Red conjugated antirabbit antibody.

Microscopy and Micrographs

Preparations were analyzed and photographed on Tri-X film (Kodak, Rochester, NY) and Ektachrome Elite 200, using a Zeiss microscope with double fluorescence and 63x Plan-Apo filter sets. 1.4 N.A. Zeiss objective. Slides and b/w prints were scanned with Polaroid Sprint Scan 35, minimally processed with slight contrast enhancement in Adobe PhotoShop and printed on a Tektronix Phaser 440 Dye-sublimation printer.

RESULTS

KCBP is a novel minus-end MT motor protein found only in plants. Antibodies to a synthetic peptide (23 amino acid residues) corresponding to a calmodulin-binding region unique to KCBP were used to test for the presence of KCBP in *Haemanthus* endosperm. It has been previously shown that anti-KCBP antibodies against the synthetic peptide are highly specific and do not cross-react with other KLPs or calmodulin-binding proteins

[Bowser and Reddy, 1997]. As shown in Figure 1, a protein of about 140 kDa was detected with anti-KCBP antibodies. The size of the protein on Western blot is similar to those of KCBP in *Arabidopsis* and tobacco [Bowser and Reddy, 1997]. In addition to a 140 kDa band, a 75 kDa polypeptide was also detected. The intensity of the second band varied in different samples. This is likely to be a degraded product of KCBP since it is highly susceptible to proteolysis. Extraction of proteins in the absence of protease inhibitors or delay in preparing blots resulted only in a smaller band with increased intensity, suggesting that full-length 140 kDa protein degrades into a 75 kDa protein. Similar results were obtained with extracts made from other plants [Bowser and Reddy, 1997] (Bowser and Reddy, unpublished data). Until now, KCBP has been reported only in dicot species [Reddy et al., 1996a,b; Wang et al., 1996; Bowser and Reddy, 1997; Song et al., 1997]. Detection of KCBP in cells of the monocot *Haemanthus* suggests the ubiquitous presence of KCBPs in phylogenetically diverse angiosperms.

Immunolocalization

Antibody raised against KCBP was applied to cells fixed in methanol and permeabilized prior to fixation. Methanol fixation resulted in prominent staining of mitotic MT arrays coupled with background staining of the cytoplasm (Fig. 2A). No signal was detected if antibody was replaced by pre-immune serum (Fig. 2B). Thus, labeling of MT arrays and cytoplasm was due to cross-reaction with anti-KCBP antibody. Permeabilization of cells prior to fixation considerably reduced the level of background staining and provided better resolution of KCBP localization within the metaphase spindle and phragmoplast.

KCBP association with mitotic MT arrays was detected in mid-late prophase. Independent of prophase spindle morphology [Smirnova and Bajer, 1998] and type of fixation, antibody diffusely stained the area occupied by the spindle. Double labeling against tubulin and KCBP revealed nearly identical patterns of stain (Fig. 3A, B).

During prometaphase, variably shaped prophase spindles transformed into barrel-shaped, laterally expanded metaphase spindles composed of distinct K-fiber complexes. Typically, polar areas of metaphase spindles were broad and undefined [Bajer and Molè-Bajer, 1986]. Fixation types and processing regimes affected KCBP localization within the metaphase spindle. Thus, after methanol fixation, whole K-fibers were labeled by antibody (Fig. 3C, D). Permeabilization before fixation resulted in distinct punctate staining, confined to the central part of K-fibers, adjacent to the kinetochore (Fig. 4A, B). Occasionally, however, the core of K-fibers was stained along its entire length (Fig. 4B).

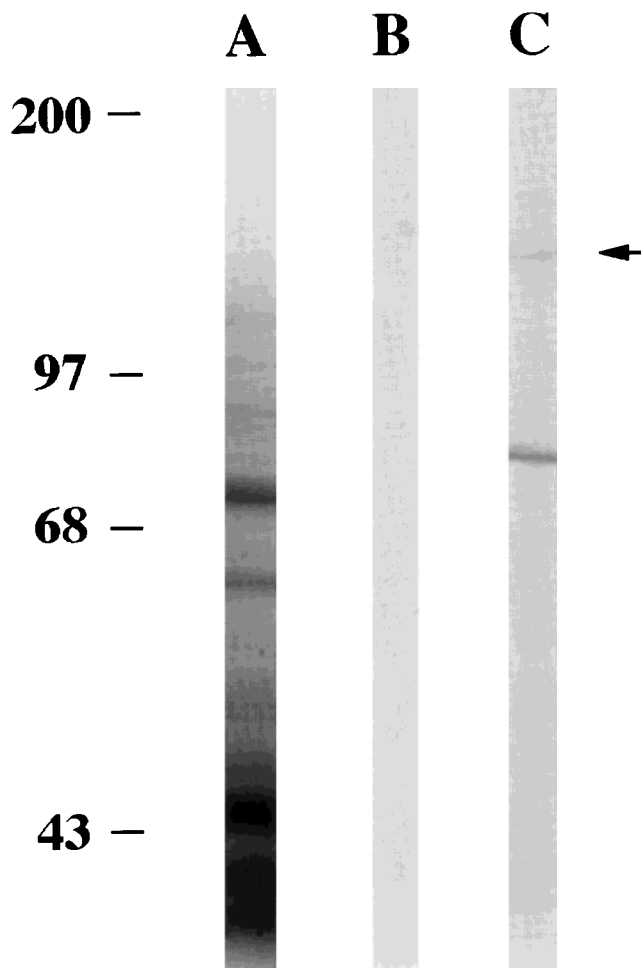


Fig. 1. Immunodetection of KCBP with antibody raised to a synthetic peptide corresponding to a unique C-terminal region in *Arabidopsis* KCBP. Proteins from *Haemanthus* endosperm were separated by SDS-PAGE and stained with Coomassie blue (lane A), immunodetected using preimmune serum diluted 1:400 (lane B), or affinity purified anti-KCBP diluted 1:400 (lane C). Arrow indicates 140 kDa protein corresponding to KCBP.

During early-mid anaphase, when the spindle remained barrel-shaped and poles unfocussed, anti-KCBP antibody stained shortened K-fibers and, concurrently, the MTs of the interzone (Fig. 4C, D). In mid-late anaphase, chromosome movement toward polar areas was often accompanied by focusing of K-fibers and compacting of broad polar areas into convergent poles. Double staining demonstrated that independent of the type of fixation, KCBP predominantly accumulated at the convergent spindle poles (Figs. 3E,F, 4E,F, 5A,B), whereas MTs of the interzone and the developing phragmoplast were faintly stained. In most cells, the intensity of staining in the interzone was similar to other cellular areas. Yet in methanol fixed cells (Figs. 3 E,F, 5A,B), diffuse background staining of the cytoplasm did not obscure pronounced labeling of the convergent spindle poles.

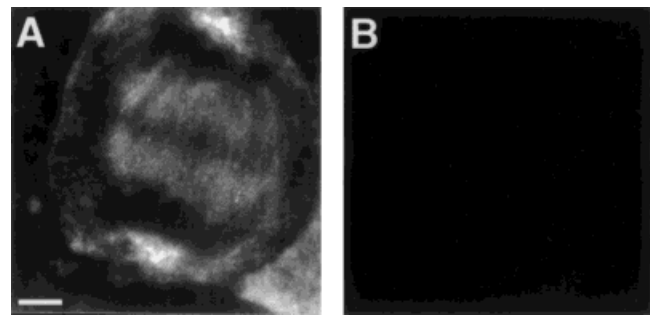


Fig. 2. Methanol fixed cells were incubated with (A) affinity-purified anti-KCBP antibodies and (B) pre-immune serum as primary antibodies. Telophase cell shown in A had accumulation of KCBP-positive material at the polar areas of the spindle. KCBP also stained MTs of the phragmoplast. Cell in B was at the same stage of mitosis, and had no detectable staining. Bar = 10 μ m.

Conspicuous staining of polar areas persisted until mid-telophase. However, in early telophase, diffuse KCBP-immunoreactive material was also detected in the region between sister chromosome groups. Double labeling for tubulin and KCBP revealed localization of the motor in the area occupied by the phragmoplast. Additionally, KCBP predominantly accumulated within the external (facing the chromosomes) part of the phragmoplast (Fig. 5C,D). In mid-late telophase, KCBP was detected in association with MTs of the phragmoplast and the nucleus. At this stage, phragmoplast staining was very intense and uniform (Fig. 5E,F). Permeabilization of cells prior to fixation reduced the intensity of phragmoplast labeling, and facilitated visualization of KCBP within the cell plate (Fig. 4G,H).

DISCUSSION

The centrosome is a major MTOC of eukaryotic animal cells. It nucleates and organizes MT arrays, thus determining their polarity and contributing to the stability of MT arrays by holding MT minus ends together. These important features assure coherence of MT arrays at the polar region and thus prevent formation of micronuclei. A typical centrosome is composed of centrioles surrounded by pericentriolar material, which functions as MTOC. Centrosomes lacking centrioles retain MT organization function [Balczon, 1996].

Since most angiosperms lack typical centrosomes, it has been proposed that they have either a universal diffuse centrosome [Mazia, 1987] or multiple specialized MTOCs [Lambert and Lloyd, 1994]. However, neither permanent diffuse centrosomes nor specialized MTOCs have been detected at spindle poles of higher plants. This has led to the notion that their mitotic spindle is formed in a centrosome/MTOC-independent manner via self-organization of MTs [Smirnova and Bajer, 1992]. This

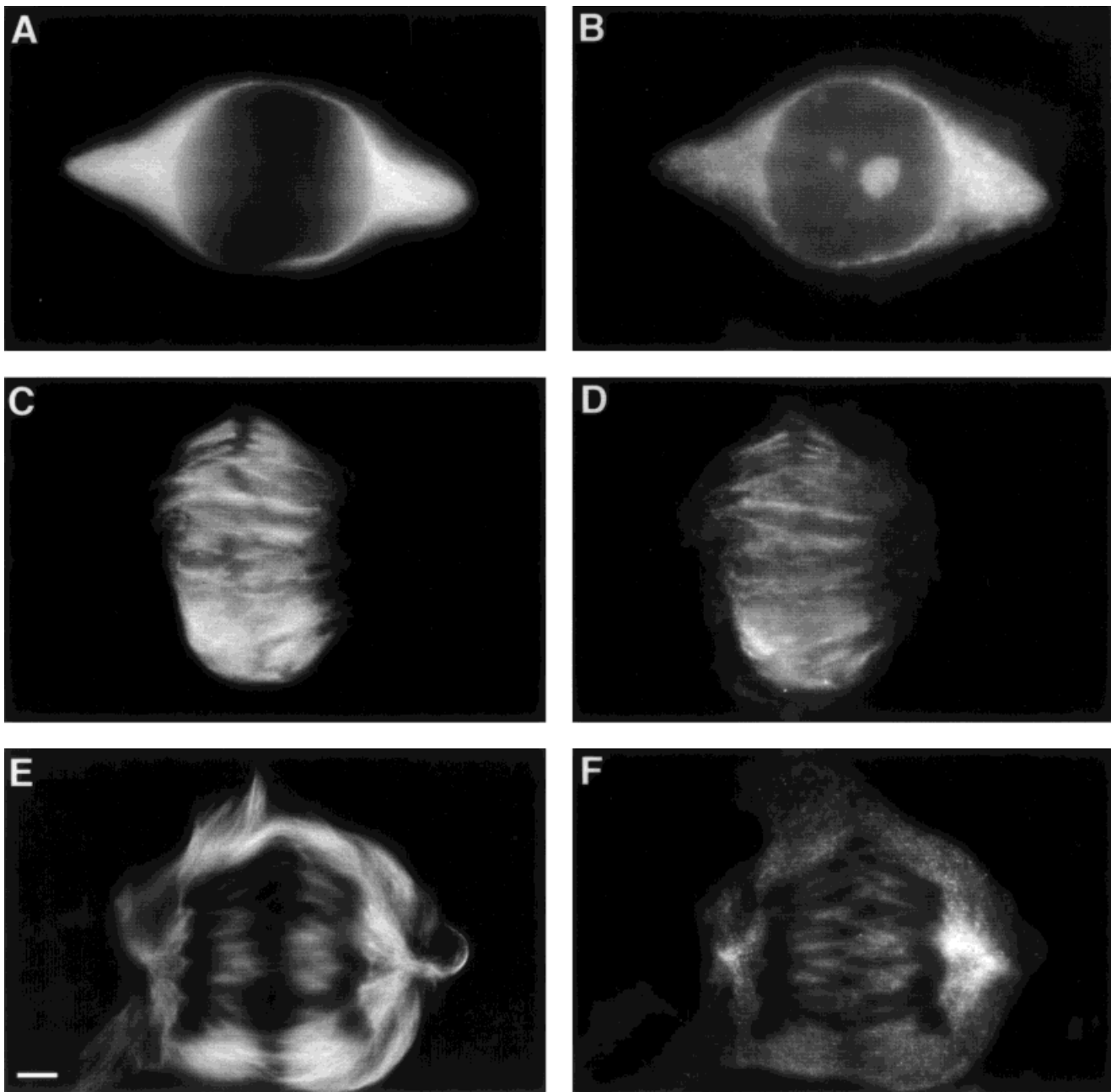


Fig. 3. Immunolocalization of tubulin (A, C, E) and KCBP (B, D, F) in methanol fixed cells. **A,B:** Bipolar prophase spindle has pronounced convergent poles. KCBP uniformly stains the spindle in a diffuse manner. **C,D:** Barrel-shaped metaphase spindle consists of distinct

K-fibers. KCBP colocalizes with MTs of K-fibers. **E,F:** Spindle in mid-late anaphase is composed of interzonal and polar MTs. KCBP accumulates at the focused polar areas, while interzone is depleted of stain. Bar = 10 μ m.

assumption was primarily based on observations of MT reorganization in spontaneously formed cytoplasts of *Haemanthus* endosperm [Bajer and Molè-Bajer, 1986]. Recent experimental data show that spindle-like MT arrays can be formed in the absence of MTOCs and motor proteins are instrumental in this process [Matthies et al., 1996; Merdes et al., 1996; Gaglio et al., 1996, 1997; Heald et al., 1996, 1997; Nédélec et al., 1997]. MT

motors have been implicated in various functions, including MT polarity sorting and focusing of MTs at the spindle poles [Mitchison, 1992; Hyman and Karsenti, 1996]. Precise regulation of MT focusing is crucial for cells lacking a centrosome at the spindle pole [Compton, 1998]. Otherwise, there would be a very high probability of irregular chromosome segregation during anaphase [Clark, 1940; Staiger and Cande 1991].

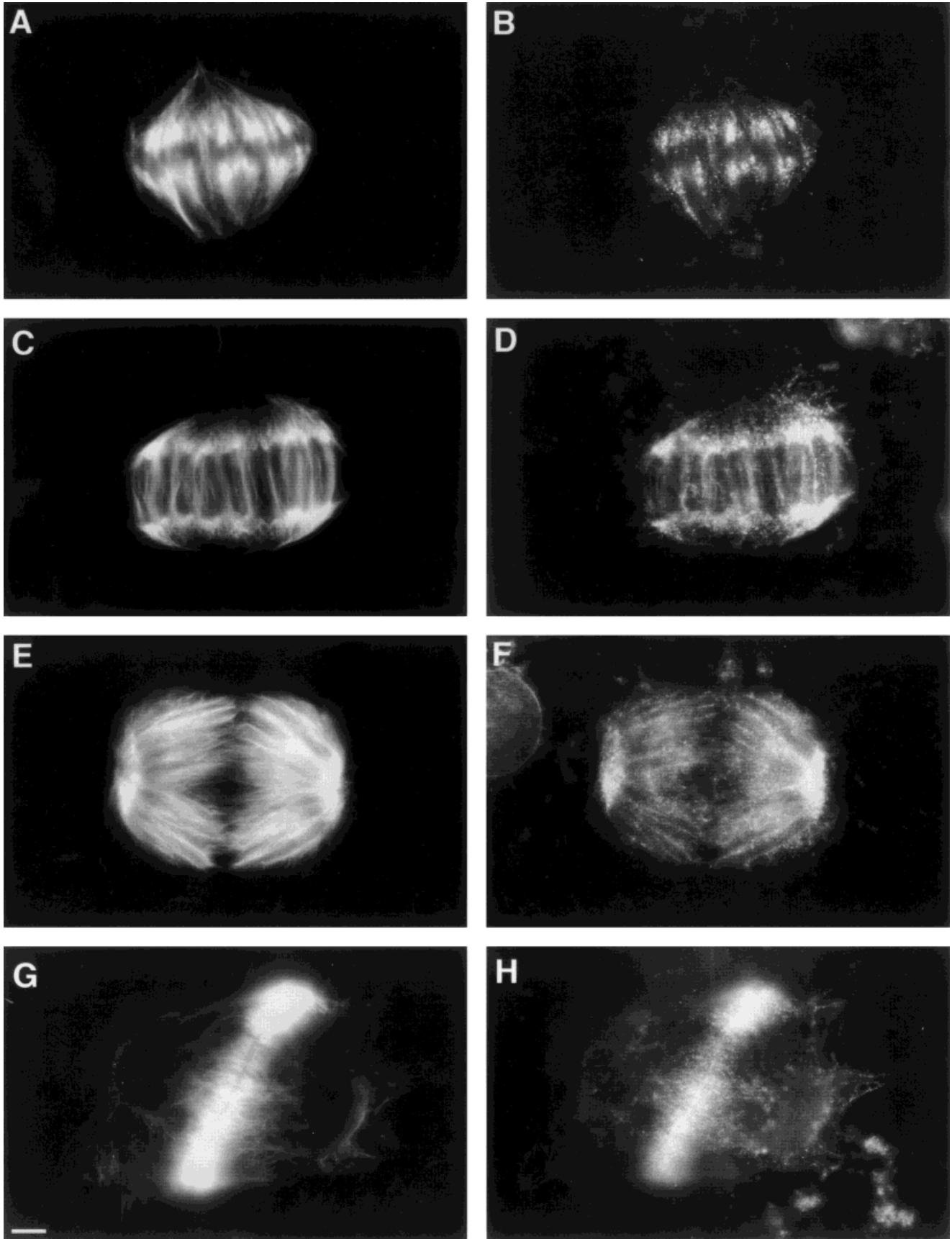


Fig. 4. Immunolocalization of tubulin (A, C, E) and KCBP (B, D, F) in cells permeabilized prior to fixation. **A,B:** In metaphase spindle, KCBP decorates the core of K-fibers in punctate manner. **C,D:** In early anaphase, the spindle retains its barrel-like shape. KCBP decorates K-fibers and MTs of the interzone. Broad polar areas are not labeled by

KCBP. **E,F:** In late anaphase, KCBP accumulates at the convergent spindle poles, and slightly decorates MTs, tracing the chromosome arms. **G,H:** In telophase, KCBP decorates MTs of the phragmoplast and the cell plate. Bar = 10 μ m.

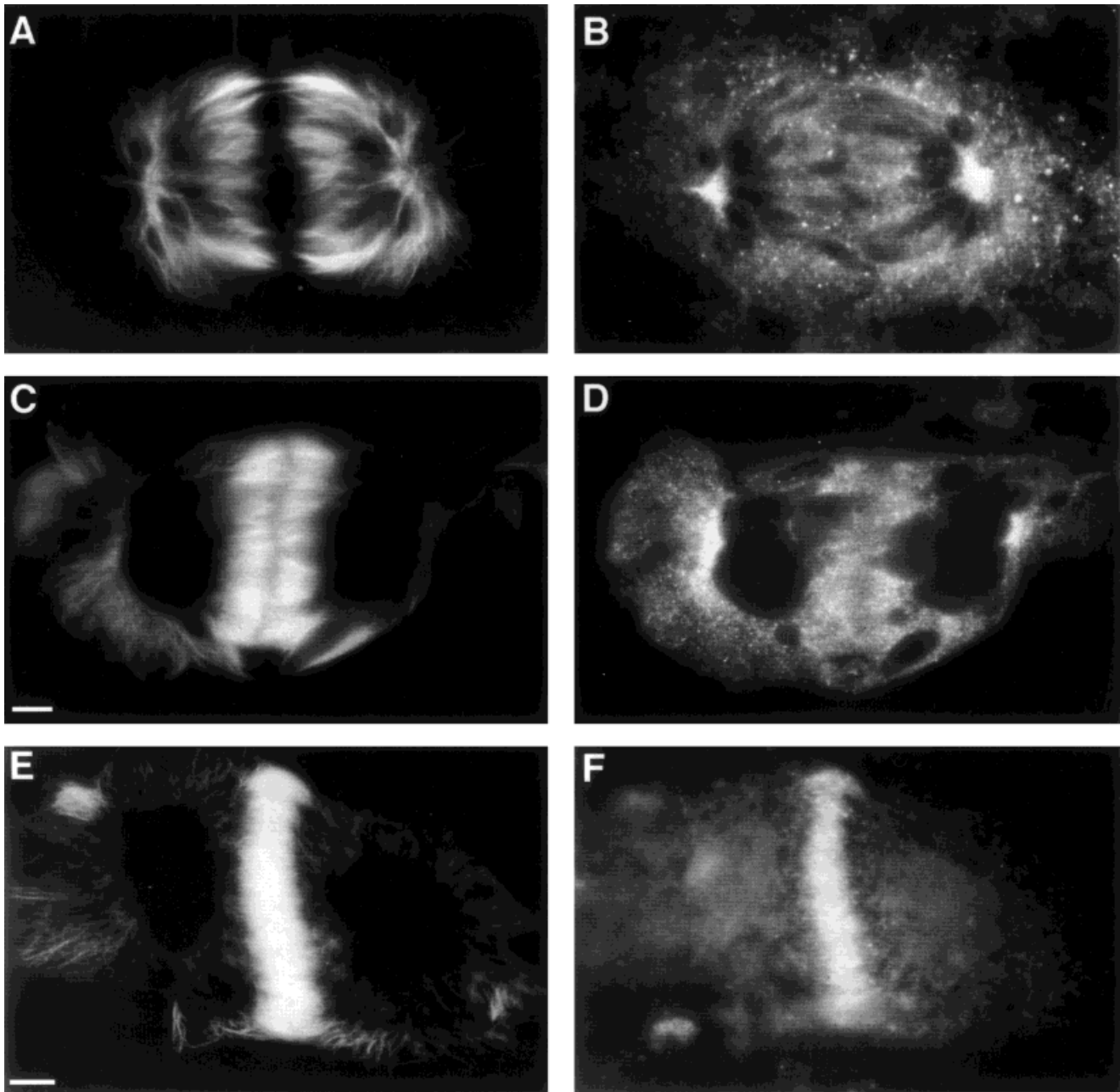


Fig. 5. Immunolocalization of tubulin (A, C, E) and KCBP (B, D, F) in methanol fixed cells. **A,B:** In late anaphase, distal parts of K-fibers converge and form focused poles. KCBP accumulates at the polar areas, whereas the interzone is depleted of KCBP. **C,D:** In early telophase the cell has a well-developed phragmoplast. KCBP staining

concentrates at the spindle pole areas, the nuclei, and at the area of the nuclei adjacent to the interzone. Proximal to the cell plate area of the phragmoplast is devoid of stain. **E,F:** Cell in late telophase has matured phragmoplast, which is narrower than shown in C. KCBP uniformly decorates MTs of the phragmoplast (F). Bar = 10 μ m.

The spindle of *Haemanthus* endosperm undergoes profound transformations during mitosis. In prophase, the spindle may be apolar, bipolar, or multipolar [Smirnova and Bajer, 1998], and polar regions, if present, are usually convergent. During prometaphase, the spindle becomes bipolar, and K-fibers terminate at the broad and ill-defined polar regions. However, in anaphase, when

chromosomes approach the polar regions, spindle poles regain their convergence. Thus, spindle reorganization occurs during prometaphase, when the prophase spindle transforms from multiform to bipolar, and in anaphase, when the spindle converges. Our present data draw attention to a possible regulatory mechanism of MT convergence in anaphase.

The exceptionally large spindle size and lack of rigid cellulosic cell walls in endosperm cells allowed detailed analysis of the spatial distribution of KCBP during mitosis. Immunodetection of KCBP on blots revealed two bands corresponding to 140 and 75 kDa proteins. We believe that the smaller polypeptide is a degraded form of KCBP. The KCBP is highly susceptible to proteolysis [Bowser and Reddy, 1997] and this feature seems to be common for other plant kinesin-like proteins [Liu and Palevitz, 1996]. Contamination of endosperm extracts with cells damaged during extrusion from young seeds is unavoidable, due to preparatory techniques. The content of the young seed is under internal pressure and mere opening of the ovule results in abrupt pressure release, which deforms majority of the cells. Endosperm samples used for biochemical analysis, contain cells from at least 20 different fruits [see also Smirnova et al., 1995]. The number of damaged cells varies and, therefore, overall assessment of the degree of contamination is impossible. It is expected, however, that the amount of degraded protein in endosperm extracts would be higher than in other plant cell types. Does our preparatory technique affect immunostained cells? Preparations for immunostain were made from a single ovule, and those that contained a large number of damaged cells were always discarded. We argue also that our fixation and processing regimes did not cause degradation or redistribution of KCBP within the cell as reported by Melan and Sluder [1992]. The absence of cellulosic cell walls and the nature of endosperm membrane, facilitate rapid penetration of fixative into living cells [Bajer et al., 1986]. Furthermore, no basic differences in KCBP distribution between methanol fixed and pre-permeabilized cells were detected, and addition of protease inhibitors did not change the pattern of the staining. Permeabilization of cells prior to fixation, however, decreased background staining of the cytoplasm and enhanced visualization of KCBP within dense MT arrays (K-fibers and phragmoplast). We conclude, therefore, that labeling of the cells is related to specific cross-reaction of antibodies with KCBP but not its degraded product.

The pattern of KCBP redistribution during *Haemanthus* mitosis is different from KLPs reported for other plant cells. The *Arabidopsis* KLP encoded by the *KatA* gene was detected within the metaphase spindle, in the interzone in anaphase and in the phragmoplast [Liu et al., 1996]. A similar distribution was reported for the tobacco kinesin-related polypeptide of 125 kDa, involved in translocation of MTs, elongation of the spindle during anaphase B and formation of phragmoplast MT arrays [Asada et al., 1997]. However, the distribution of KCBP in *Arabidopsis* and tobacco cell cultures differs between both types of cells and from that of other KLPs [Bowser

and Reddy, 1997]. The significance of those differences remains to be understood.

It appears that distribution of KCBP within the *Haemanthus* spindle changes during metaphase-telophase (Fig. 6). This was not noticeable in several plant suspension culture cells [Bowser and Reddy, 1997]. A few factors may account for this difference. One of them may be the size of the mitotic spindles and intrinsic limitation of the light microscope resolution. The spindles of *Haemanthus* endosperm are 2–3 times larger than the spindles of cells in suspension culture. Thus, the fluorescent signal in the latter is weaker and more difficult to detect. The other factor may be the differences in development/differentiation of the spindle in endosperm and the other higher plant cells. Endosperm cells lack preprophase bands, displaying a variety of multiform MT configurations around the nucleus in prophase, yet they always form flawless bipolar metaphase spindles [Smirnova and Bajer, 1998]. Moreover, in anaphase-telophase, endosperm cells often assemble aster-like polar MT arrays [Bajer and Molè-Bajer, 1986], which have not been reported in any other plant cells. Although polar accumulation of KCBP was observed in cells with and without such “asters,” it is conceivable that KCBP may be instrumental in enhanced “gathering” of MTs at the anaphase spindle poles.

We did not detect any major differences in the pattern of early phragmoplast staining in *Haemanthus* (Fig. 5C, D) and BY-2 suspension culture cells, reported by Boswer and Reddy [1997] (Fig. 3 J-I). In *Haemanthus*, however, in late telophase, KCBP is localized predominantly in the central region of the phragmoplast. Additionally, permeabilization of *Haemanthus* cells prior to fixation revealed uniform staining of phragmoplast MTs, accompanied by distinct staining of the cell plate, the site of MT plus ends [Eutenauer et al., 1982]. It is noteworthy, however, that KCBP appears within the phragmoplast in mid-late telophase when the phragmoplast differentiates and expands laterally. This coincides with an increase in birefringence in the center and then at the edges of the phragmoplast, suggesting nucleation and sideways growth of new MTs [Inoué and Bajer, 1961]. Thus, KCBP accumulation in the phragmoplast in the later stages of its development may be related to these processes and the distinctive features of phragmoplast differentiation in endosperm (Bajer and Smirnova, unpublished data). It should be noted, however, that regulatory mechanisms of phragmoplast formation and differentiation during endosperm development are not clearly understood [Brown and Lemmon, 1997]. Various patterns of KCBP localization may reflect differences in phragmoplast morphogenesis between plant tissues with cellulosic cell walls [Stahelin and Hepler, 1996] and coenocytic endosperm.

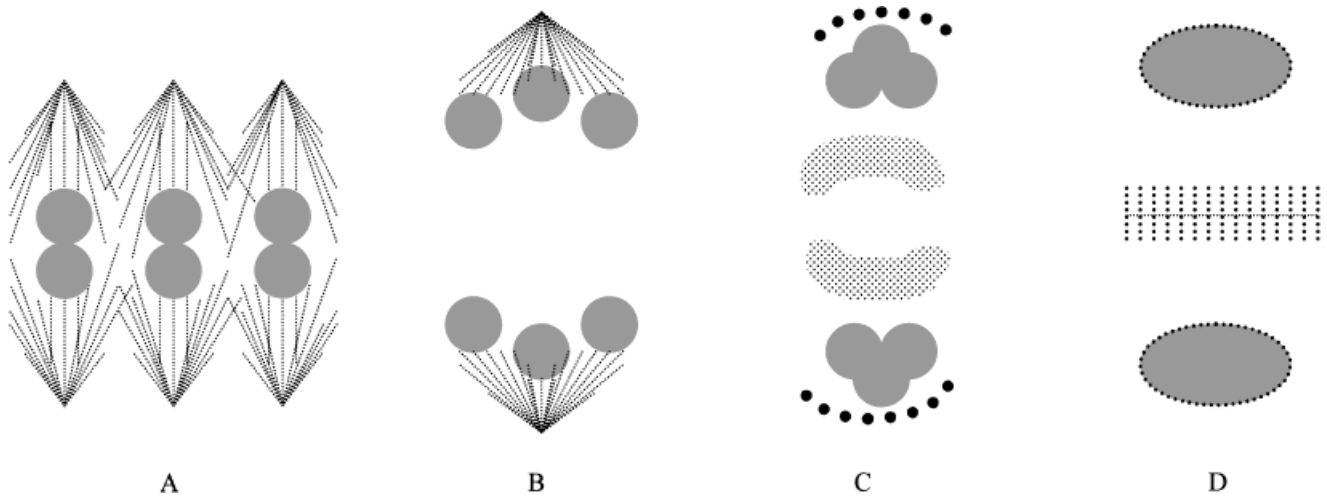


Fig. 6. Redistribuition of KCBP within the spindle and phragmoplast during mitosis in *Haemanthus* endosperm. **A:** In metaphase, KCBP colocalizes to MTs of the spindle. **B:** In late anaphase, the motor accumulates at the convergent polar areas. **C:** In early telophase, KCBP

distributes around the nuclei and at the phragmoplast area. **D:** In mid-late telophase, KCBP localizes within the phragmoplast, cell plate, and nuclear surface. Chromosomes and nuclei are gray; sites of KCBP localization are dashed black.

Relocation of KCBP toward polar areas in anaphase is consistent with the established MT polarity of the *Haemanthus* spindle [Eutenauer et al., 1982; Vantard et al., 1990] and localization of dynein in mammalian tissue culture cells [Pfarr et al., 1990; Steuer et al., 1990]. The time of relocation coincides with the transformation of a barrel-shaped spindle into a focused one. We believe, therefore, that in *Haemanthus*, KCBP is involved in focusing of spindle poles during anaphase. The proposed role of KCBP in converging MTs is consistent with its biochemical and motility properties. KCBP is a minus-end directed motor [Song et al., 1997] and contains two distinct MT binding sites: one in the N-terminal tail region and a second one in the C-terminal motor domain [Narasimhulu and Reddy, 1998]. Such properties might allow the KCBP to cross-link adjacent MTs, translocate them toward the MT minus-ends, and thereby converge MT arrays. These data [Song et al., 1997; Narasimhulu et al., 1997; Narasimhulu and Reddy, 1998; Deavours et al., 1998], combined with our present observations suggest the involvement of this motor in focusing of anaphase MT arrays. Since the spindle is composed of distinct K-fibers, which are modified MTCCs, KCBP may also be involved in assembly of those structural units. Proposed explanations are consistent with the present understanding of the role of KCBP. Further studies are needed, however, to correlate conspicuous increase of anaphase spindle convergence and KCBP redistribution at the spindle poles in endosperm of *Haemanthus*.

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