Isolated Plant Nuclei Nucleate Microtubule Assembly: The Nuclear Surface in Higher Plants Has Centrosome-like Activity

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In most eukaryotic cells, microtubules (MTs) are assembled at identified nucleating sites, such as centrosomes or spindle pole bodies. Higher plant cells do not possess such centrosome-like structures. Thus, the fundamental issues of where and how the intracellular plant MTs are nucleated remain highly debatable. A large body of evidence indicates that plant MTs emerge from the nuclear periphery. In this study, we developed an in vitro assay in which isolated maize nuclei nucleate MT assembly at a tubulin concentration (14 μM of neurotubulin) that is not efficient for spontaneous MT assembly. No MT-stabilizing agents, such as taxol or dimethyl sulfoxide, were used. Our model provides evidence that the nuclear surface functions as a MT-nucleating site in higher plant cells. A monoclonal antibody raised against a pericentriolar antigen immunostained the surface of isolated nuclei, and a 100-kD polypeptide in 4 M urea-treated nuclear extracts was detected.

INTRODUCTION

In most eukaryotes, the microtubule-organizing center (MTOC) is the site of nucleation and spatially organizes the microtubule (MT) arrays in vivo as well. In vitro, MTs can be nucleated by isolated centrosomes at pure tubulin concentrations below those required for spontaneous MT assembly (Mitchison and Kirschner, 1984). Centrosome activity is thought to be cell cycle regulated by cyclin-dependent protein kinases (Buendia et al., 1992; Ohta et al., 1993). Structures other than centrosomes, such as spindle pole bodies in fungi, can ensure MTOC function (Fuller et al., 1992). In higher plants, neither centrosomes nor spindle pole bodies are present. The mechanism of MT nucleation has yet to be elucidated.

In most eukaryotic cells, a unique, large, radial MT array emerges from the centrosome, whereas in higher plant cells, distinct MT arrays coexist (Baskin and Cande, 1990; Goodbody et al., 1991; Staiger and Lloyd, 1991): these include the cortical nuclear-associated MTs in interphase, the preprophase band (PPB) in G2 phase, and the mitotic spindle and the phragmoplast MTs during mitosis. No structurally defined MTOC is detected at the spindle poles. Such a MT organization is unique to higher plant cells and raises some fundamental questions. Is there a main MTOC that may change configuration during the cell cycle, as suggested by the concept of a "flexible centrosome" (Mazia, 1987)? Such a situation has been observed in mouse oocytes (Schatten et al., 1986). Are there multiple MTOCs with defined locations? For example, are cortical MTs nucleated at the nuclear envelope and then translocated, or are they nucleated in the cortex (Hasezawa and Nagata, 1993)? Several experiments suggest that the plant nuclear surface serves as an MTOC (Lambert, 1993). Microinjection of fluorescent brain tubulin into living higher plant cells (Zhang et al., 1990, 1993; Wasteneys et al., 1993) and incorporation of a reporter tubulin into lysed endosperm cells (Vantard et al., 1990) indicate that the plant nuclear surface is the preferred site for MT assembly during interphase and during telophase when daughter nuclei are being formed. In addition, antigenic determinants of the pericentriolar material of isolated animal centrosomes have been detected on the plant nuclear surface at sites where MTs are anchored (Chevrier et al., 1992).

Recently, a γ-tubulin-related protein of 58 kD has been found to be associated with all plant MTs arrays; these include the cortical MTs, the PPB, and the phragmoplast. This γ-tubulin-related protein was distributed around the nucleus of higher plant cells, mainly during G2 phase when the number of nuclear-associated MTs is higher (Liu et al., 1993). Recently, two γ-tubulin genes of Arabidopsis have been sequenced; the predicted proteins show 70% amino acid identity with the γ-tubulins of animals and fungi (Liu et al., 1994). γ-Tubulin, considered as a universal component of the MTOCs, is thought to be essential for MT nucleation and/or a minus end MT marker (Oakley, 1992). Recently, it has been reported that MTs originating from taxol-mediated tubulin assembly form a radiating distribution pattern around disrupted nuclei and nuclear...
particles isolated from cultured tobacco cells (Mizuno, 1993). These data reinforce the hypothesis that the plant nucleus participates in MT organization.

The essential question remains unanswered: Does the plant nuclear surface function like the centrosome? If so, it should respond to the criteria that characterize the centrosomal activity in all other eukaryotes (Mitchison and Kirschner, 1984; Bornens et al., 1987). These criteria include (1) the capacity to nucleate the assembly of MTs under conditions where spontaneous assembly is inefficient, and (2) the capacity to establish and/or anchor the minus end of MTs.

We developed a functional assay in which MT assembly was strictly dependent on isolated plant nuclei, as was demonstrated for purified centrosomes (Mitchison and Kirschner, 1984; Bornens et al., 1987). The validity of our experimental procedure is based on three points: (1) the isolated nuclei used were intact and showed no endogenous MT fragments at their surface; (2) no MT-stabilizing agents were added; and (3) purified brain tubulin, free of MT-associated proteins, was used at concentrations where spontaneous MT assembly is inefficient.

Under these conditions, isolated plant nuclei were able to nucleate the assembly of purified neurotubulin. MTs were anchored at the nuclear surface in a sunlike distribution pattern that resembled the radial cytoplasmic MT array emerging from the plant nuclear surface in the living cell. Using a monoclonal antibody raised against the pericentriolar material of purified mammalian centrosomes (Chevrier et al., 1992), we detected a 100-kD protein associated with the nuclear envelope. Our in vitro model sheds light on the nucleation process of MT assembly in higher plants and opens new perspectives for studying plant MT dynamics.

RESULTS

Isolation of Intact Plant Nuclei from Maize Cultured Cells

To test the ability of the plant nuclear surface to nucleate MTs, we developed a technique for isolating plant nuclei without damaging the nuclear surface. Mechanical shearing, nonionic detergents, and Percoll gradients were not used because these treatments may alter the nuclear envelope, especially the outer membrane (Willmitzer and Wagner, 1981; Saxena et al., 1985; Hendrix et al., 1989). In the procedure we developed, Triton X-100 was used at a low concentration (0.025% [v/v]). At this concentration, the nuclear envelope is not altered (Saxena et al., 1985). To isolate nuclei, cell cultures were grown in L32 medium (Maas and Werr, 1989) without sucrose for 4 days before harvesting. This procedure reduces the amount of starch grains that cosediment with nuclei. Under these conditions, the cells continue to divide, although more slowly than in L32 medium with sucrose. Using these conditions, we obtained an enriched nuclei fraction that is ~70% pure (Figure 1); starch grains represent the most abundant contaminant. Approximately 45 to 50% of the isolated nuclei were in G1 phase while 15% were in G2, as assessed by DNA cytofluorometry after 4',6-diamidino-2-phenylindole staining. The integrity of both nuclear membranes and nuclear pores was verified on serial sections of nuclei by electron microscopy (Figure 2).

To determine whether fragments of plant MTs remained attached at the nuclear surface after the isolation procedure, immunocytochemical labeling of the isolated nuclei was done using anti-tubulin antibodies in addition to electron microscopic observations. No MT fragments were detectable at the nuclear surface within the detection limits of these techniques.

Isolated Maize Nuclei Nucleate MTs

Because purified plant tubulin is difficult to obtain in large quantities, purified pig brain tubulin was used for the nucleation assays. In our tubulin preparations, no MT-associated proteins were visible on overloaded SDS gels stained with Coomassie Brilliant Blue R 250 (Figure 3A, lane 3). Undetected residual Tau proteins would represent less than 0.1% of the total protein; such contamination is considered negligible for the nucleation of MT assembly on isolated centrosomes (Mitchison and Kirschner, 1984). The capacity of the purified tubulin to polymerize was checked in vitro (Figure 3B). Neither taxol nor any other stabilizing agent was used in the nucleation buffer. As a control, MT nucleation assays were simultaneously done on isolated calf centrosomes (Figure 3C).

Isolated maize nuclei were incubated with different concentrations (10, 14, 18, and 23 μM) of purified neurotubulin. MT assembly was analyzed by immunofluorescence microscopy after anti-tubulin labeling and by differential interference contrast microscopy. At 10 μM, neither spontaneous tubulin assembly was strictly dependent on isolated plant nuclei, as was demonstrated for purified centrosomes (Mitchison and Kirschner, 1984; Bornens et al., 1987). The validity of our experimental procedure is based on three points: (1) the isolated nuclei used were intact and showed no endogenous MT fragments at their surface; (2) no MT-stabilizing agents were added; and (3) purified brain tubulin, free of MT-associated proteins, was used at concentrations where spontaneous MT assembly is inefficient.

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Isolated Plant Nuclei Nucleate Microtubules

Figure 2. Ultrastructure of the Isolated Maize Nuclei. 
(A) Electron micrograph of a nucleus. The inset shows a differential interference contrast micrograph of a similar nucleus. 
(B) Detail of the same nucleus shown in the inset in (A). Both outer and inner membranes are preserved (arrowheads). 
\( n \), nucleus. Bar in (A) = 1 \( \mu \text{m} \); bar in (B) = 0.1 \( \mu \text{m} \).

assembly nor MT nucleation was observed. Very little spontaneous assembly occurred with 14 \( \mu \text{M} \) tubulin, whereas assembly of this tubulin at the plant nuclear surface was very efficient (Figure 4). Increasing the tubulin concentration to 18 or 23 \( \mu \text{M} \) did not increase the length or number of MTs at the nuclear surface. However, at 18 and 23 \( \mu \text{M} \), numerous autoassembled MTs were visible. For this reason, 14 \( \mu \text{M} \) tubulin was chosen for our nucleation assays.

After testing different incubation times (10, 20, and 30 min), 20 min was found to be optimal. In the experiments described above, results were comparable using either Piperazine-N, N'-bis-(2-ethanesulfonic acid) (Pipes) or 2-(N-morpholino)ethanesulfonic acid (Mes) buffers.

The results obtained under our standard nucleation conditions (14 \( \mu \text{M} \) for 20 min) showed that all MTs emerge from the nuclei in a radiating fashion (Figures 4 and 5). We estimated that \(~\sim 20\%\) of the nuclei in the preparation were able to nucleate MTs. The length of MTs emerging from a single nucleus varied from 2 \( \mu \text{m} \) to \(~\sim 20 \mu \text{m} \). In some cases, MTs nucleate only on a portion of the nuclear surface, as seen in Figure 5. This could be a result of partial damaging of the nuclei during the isolation procedure. DNA cytofluorometric measurements of the isolated nuclei that nucleated MTs revealed that nuclei in \( G_1 \) or \( G_2 \) phases are able to nucleate MT assembly in our assay conditions.

To confirm the specificity of plant nuclei to nucleate MTs, similar assays using purified tubulin incubated with animal nuclei isolated from rat liver and brain were done. No MT nucleation on those animal nuclei was obtained.

Nucleation of Neurotubulin in the Presence of Oryzalin

Although no MTs were visible on the isolated nuclei, it is possible that very short endogenous MT fragments could remain. Because such MT stubs could serve as seeds for neurotubulin assembly during the nucleation assays, isolated nuclei were treated with oryzalin to eliminate any residual plant MT fragments. Oryzalin is one of the most potent inhibitors for plant MTs. It has been shown that within a few minutes, \( 10^{-6} \text{ M} \)
oryzalin inhibits plant tubulin polymerization and depolymerizes plant MTs both in vivo and in vitro but has no effect on brain MT assembly (Morejohn et al., 1987). Isolated maize nuclei were preincubated with $10^{-6}$ M oryzalin at 4°C, and no plant MTs were detected at the surface of these oryzalin-treated nuclei after anti-tubulin labeling. After 20 min, purified neurotubulin and GTP were added to the desired concentrations and then the mixture was incubated at 30°C to initiate the MT assembly. Results obtained using oryzalin-treated nuclei were similar to those obtained with untreated nuclei.

Detection of Centrosomal-like Components on the Surface of Isolated Plant Nuclei

We previously reported that a monoclonal antibody (mAb6C6) raised against purified calf thymus centrosomes stains the pericentriolar material of animal centrosomes and labels the nuclear surface in maize cells (Chevrier et al., 1992). Here, we showed that this antibody also reacts with the surface of isolated nuclei. A fine dotted pattern on the nuclear surface was obtained (Figures 6B and 6C), suggesting multiple reactive sites. These data indicated that at least one protein located at the plant nuclear surface shares common antigenic determinants with the nucleating domain of mammalian centrosomes.

In addition, to investigate the proteins responsible for the MT nucleation activity of the plant nuclei, isolated nuclei were treated with increasing concentrations of urea. This procedure has been used to inactivate parthenogenetic activity of isolated centrosomes (Klotz et al., 1990) and is based on the hypothesis that plant MT-nucleating material may be at least partially anchored in the nuclear envelope. Isolated nuclei were incubated with 2, 4, and 6 M urea for 30 min at 4°C. Both soluble and insoluble protein extracts were immunoblotted, using mAb6C6 as primary antibody. A polypeptide with a molecular mass of 100 kDa was detected (Figure 6A). This polypeptide, which is totally extracted from the nuclear surface using 4 M urea, was not detectable on immunoblotted crude protein extract from maize cells. Treatments of the nuclei with 2 and 4 M urea abolished both their MT nucleation capacity and the mAb6C6 labeling. These results actually corroborate the presence of MT-nucleating material at the nuclear surface.

Figure 4. MT Nucleation on One Isolated Maize Nucleus.

Isolated nuclei were incubated with purified tubulin (14 μM) and GTP (1 mM) for 20 min at 30°C.

(A) Differential interference micrograph of an isolated nucleus.

(B) Fluorescence microscopy after anti-tubulin labeling of the same nucleus.

Arrows, microtubules; stars, guide marks. Bar in (A) = 10 μm.

Figure 5. MT Nucleation on Two Different Isolated Maize Nuclei Observed by Differential Interference Contrast Microscopy.

Isolated nuclei were incubated with purified tubulin (14 μM) and GTP (1 mM) for 20 min at 30°C.

(A) In some cases, the MT nucleation is restricted to parts of the nuclear surface. Free MT fragments above the nucleus (arrows) were found to be connected to the nuclear surface as seen in other optical sections.

(B) MTs are nucleated primarily around the nuclear surface. Bar in (A) = 10 μm.
Figure 6. Detection of the mAb6C6 Plant Antigen.

(A) Immunoblot of the supernatant obtained after treatment of isolated maize nuclei with 4 M urea. Molecular mass markers are given at left in kilodaltons.

(B) and (C) Immunolocalization of the mAb6C6 antigen at the periphery of isolated maize nuclei. (B) is a focal view of the nuclear surface. (C) is an optical section through the nucleus.

(D) 4',6-Diamidino-2-phenylindole staining. Bar in (D) = 10 μm.

DISCUSSION

In the in vitro assay we developed, neurotubulin is used instead of plant tubulin as a source of purified tubulin. The assembly of neurotubulin is not affected by oryzalin, which we used to depolymerize any residual plant MT fragments.

In our in vitro model, it was essential to check that no endogenous, stable MT seeds could serve as templates for MT elongation and/or be a source of artifactual MT capping. To ensure that plant MTs were depolymerized, we incubated the nuclei with oryzalin. We assumed that the long action of the drug combined with cold treatment was sufficient to depolymerize any plant MT fragments. However, whereas one cannot exclude the possibility that some drug- and cold-resistant MTs remain attached to the nuclear envelope, it seems unlikely that such rare MTs could account for the dense sunlike MT distribution pattern that we observed around the isolated nuclei. These data strongly suggest that the vast majority of the MTs that were observed around the plant nucleus resulted from nucleation of exogenous neurotubulin.

Recently, it has been shown that in the presence of taxol and dimethyl sulfoxide plant tubulin could be assembled on non-intact isolated tobacco nuclei, suggesting that in vitro the plant nucleus could be a selective site of formation and/or attachment of stabilized MTs (Mizuno, 1993). However, agents such as taxol and dimethyl sulfoxide are known to lower both the critical concentration for tubulin assembly and to increase the stability of the MTs. In the presence of these agents, the assembly of stable MTs may mimic a nucleation activity (De Brabander et al., 1981; Verde et al., 1991). Therefore, to probe the MT nucleation activity of the plant nuclear surface, it is essential to perform assays under conditions in which (1) spontaneous tubulin assembly is controlled, and (2) MTs can polymerize and depolymerize.

In our assays, intense MT assembly around intact plant nuclei occurred at a purified tubulin concentration of 14 μM. Free MTs, which may still polymerize at this tubulin concentration, are known to depolymerize quasi instantaneously (Mitchison and Kirschner, 1984). Our model provides evidence that proteins involved in the plant MT nucleation are localized at the nuclear periphery. Ultrastructural data already indicated an association between cytoplasmic MTs and the plant nuclear envelope in situ (Lambert, 1980; Panteris et al., 1991).

Previous results based on exogenous tubulin incorporation in lyzed Haemanthus endosperm cells (Vantard et al., 1990) suggest that in higher plants the minus ends of nuclear-associated MTs are located at the nuclear periphery, as is known for other MTOCs. In our model, we suppose that the nucleated MTs exhibit a comparable polarity, with their plus end distal from the nuclear envelope and capable of elongation. Further studies are required to define the polarity of the nuclear-associated MTs in higher plant cells.

In this study, we report that a monoclonal antibody raised against isolated calf thymus centrosomes and that labeled the plant nuclear surface within the cell (Chevrier et al., 1992) immunostains the periphery of isolated maize nuclei. This antibody recognized a 100-kD polypeptide that can be solubilized by urea. Although numerous proteins have been described as centrosome components (Fuller et al., 1992; Sellitto et al., 1992), no purified proteins have yet been characterized as putative MT-nucleating components in higher plants. It remains to be determined whether the mAb6C6 antigen is involved in the MT nucleation and/or MT organization in both mammalian and higher plant cells. Previous observations showed labeling of the plant nuclear periphery by human auto-antibodies (Clayton et al., 1985; Wick, 1985), but the significance of this cross-reactivity has been questioned (Harper et al., 1989).

MT-nucleating activity of animal centrosomes can be inactivated by urea treatment (Klotz et al., 1990). Similarly, after
urea treatment of isolated plant nuclei, no MT nucleation is detected, although no morphological differences are observed. Our data suggested that plant nucleating components can be extracted from the nuclear surface by urea and solubilized. Human centrosomes inactivated by urea can be functionally complemented by Xenopus egg extracts (Buendia et al., 1992). It will be relevant to develop such assays on isolated plant nuclei.

In vivo, the intense nucleating activity of the daughter plant telophase nuclei precedes cortical MT distribution. During G1 phase, when cortical MTs are spatially organized, the density of nuclear-associated MTs is strikingly reduced. After S phase, in G2, this nucleating activity is recovered around the nucleus, while the PPB progressively appears in the cortex, and cortical MTs are disorganized (Lambert and Lloyd, 1994). It is not known whether the oscillation of MT-nucleating activity of the plant nucleus during the cell cycle is either the result of a variation of the amount of soluble tubulin or is the consequence of a regulation by cell cycle–dependent protein kinases (Katsuta and Shibaoka, 1992; Traas et al., 1992; Colasanti et al., 1993).

In our in vitro assays, we observed that nuclei in G1 or G2 phase were able to nucleate MT assembly from purified tubulin. Further studies are needed to evaluate MT nucleation capacity of plant nuclei at specific stages of the cell cycle. It will be of interest to study the effect of cell cycle–specific extracts of animal or plant cells and/or cell cycle–dependent protein kinases on the nucleating activity of synchronized isolated plant nuclei.

We previously defined the MTOCs as possessing both MT nucleation and MT-organizing functions. Our model suggests that the plant nuclear surface nucleates MTs, but the putative role of the nuclear envelope in the organization, the activity, and the duplication of the nucleating components has yet to be determined. Are there multiple MTOCs with defined locations and whose individual nucleation capacity would be regulated by coordinated cell cycle and developmental signals? One may assume that the principal MTOC is located on the plant nuclear surface and changes configuration during the cell cycle, analogous to the “flexible centrosome” model proposed by Maizel (1987). A structural continuity has been shown between nuclear-associated MTs and the PPB (Lloyd et al., 1992) or cortical MTs (Goodbody et al., 1991). All these data favor the view that all plant MTs, whatever their final intracellular distribution, may originate from the nuclear surface (Lambert, 1993). The spatial reorganization of structurally independent and/or distinct MT arrays could occur after MT severing and translocation, as is thought to be the case in other systems (Joshi and Baas, 1993; Karsenti, 1993).

Recently, a γ-tubulin–related protein has been detected in all plant MT arrays and at the nuclear envelope (Liu et al., 1993). Therefore, the MT nucleation capacity of the plant nuclear surface in our in vitro assay can be correlated with the presence of γ-tubulin–related protein within the plant cell. Nevertheless, the widespread distribution of anti-γ-tubulin staining in plant cells remains surprising because in most animal cells, γ-tubulin is found at the centrosome.

In conclusion, our in vitro assay provides insights into the understanding of the nucleation of plant MT assembly in the absence of defined centrosomes. This model now provides a means of investigating the regulation of plant MT nucleation in vivo during the cell cycle and development.

METHODS

Plant Material

Maize (Zea mays) suspension cultures established from immature embryo explants of the Black Mexican Sweet line were cultivated at 30°C in the dark in L32 liquid medium adapted from Maas and Werr (1989). The medium contained 10% coconut water, 100 mg/L myoinositol, 0.5 mg/L 2,4-D, and 6% sucrose. The medium was changed every 3.5 days. Cells were subcultured in L32 medium without sucrose for 4 days before the isolation of nuclei.

Isolation of Nuclei

Cells were suspended (v/v) in an enzyme mixture comprising 0.05% Pectolyase Y23 (Seishin Pharmaceutical, Tokyo, Japan), 1% macerozyme R10 (Serva, Heidelberg, Germany), and 4% Caviase 345 (Cayla, Toulouse, France) in 8 mM CaCl2, 600 mM mannitol, 25 mM 2-(N-morpholino)ethanesulfonic acid (Mes), pH 5.5, 720 mosmol/kg. Protoplasts were obtained by incubation in the dark for 90 min at 28°C with agitation. Nuclei were isolated using a procedure modified from Hendrix et al. (1989). All buffers contained the following protease inhibitors: 10 μM pepstatin, 10 μM leupeptin, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1 mM aprotinin. Protoplasts were filtered through a 100-μm nylon mesh. They were collected by centrifugation and washed twice in nuclei isolation buffer (NIB; 5 mM MgCl2, 5 mM NaCl, 5 mM KCl, 2 mM EGTA, 25 mM Mes, pH 5.2) complemented with 0.6 M mannitol (720 mosmol/kg). Cells were suspended for 10 min in NIB plus 0.7 M mannitol (830 mosmol/kg). After centrifugation, cells were incubated for 10 min in a hypotonic solution (NIB plus 0.2 M mannitol plus 0.025% Triton X-100; 300 mosmol/kg). The resuspended protoplasts were passed vigorously through a 22-gauge needle five times to release nuclei. The lysate was filtered through a 15-μm nylon mesh and centrifuged. The pellet, containing nuclei and starch grains, was suspended in a small volume of NIB plus 0.2 M mannitol (300 mosmol/kg; pH 6.8) depending on the concentration of the nuclei preparation. All centrifugations were performed in NIB at 120g for 3 min at 4°C. The isolation of nuclei was performed at 4°C.

Preparation of Tubulin

Tubulin and associated proteins from pig brain were obtained according to the method of Shelanski et al. (1973). Neurotubulin was purified on a phosphocellulose column (Weingarten et al., 1975). Tubulin was prepared either with Piperazine-N,N’-bis (2-ethanesulfonic acid) (Pipes) or Mes buffers.

Nucleation Assays

Plant nuclei were incubated with tubulin in the presence of 1 mM GTP, 5% glycerol in the nucleation buffer (80 mM Pipes or 100 mM Mes,
with 1 mL of 10% glycerol in cold nucleation buffer. When needed, nuclei were preincubated with 10^-6 M oryzalin for 20 min at 4°C be-
fore being used for the assays. Nuclei were inactivated by incubation with urea (2 to 4 M in nucleation buffer) for 30 min at 4°C. Nucleation
assays using isolated calf thymus centrosomes (gift of V. Chevrier, Centre d'Etudes Nucleaires, Grenoble, France) were performed as
previously described (Mitchison and Kirschner, 1984; Bornens et al., 1987).

Microscopy

Samples were spun on coverslips (Klotz et al., 1990), post-fixed with cold methanol (~20°C. 5 min), and processed for immunocytochemistry. Preparations were incubated with an anti-β-tubulin antibody (N347; Amersham, Les Ulis, France; diluted 1:2000), washed, and incubated with a fluorescein isothiocyanate–conjugated goat anti–mouse IgG (diluted 1:20). Nuclei were stained with 4,6-diamidino-2-phenylindole (1 μg/mL). The double-labeling experiments using an anti-tubulin antigen and a monoclonal antibody directed against pericentriolar material were performed as described previously (Chevrier et al., 1992). Observations were made using either a Leitz Orthoplan (Wetzlar, Germany) microscope with epifluorescence or Nomarski differential interference contrast optics (Carl Zeiss, Oberkochen, Germany) (63x Numerical Aperture, 1.3 objective). The analysis of the preparations was performed with an image processing system (Crystal-Sapphire Quante, Montigny le Bretonneux, France) using a camera (model No. WV 1850G; Panasonic) and a high-resolution monitor (model No. PVM 122 CE; Sony, Tokyo, Japan), coupled to a video-enhanced light microscope system. DNA quantification of isolated nuclei was made using a MPM 200 microscope photometer (Carl Zeiss, Inc.). For electron microscopy, nuclei preparations were processed as described previously (Vantard et al., 1990) and observed in an electron microscope (model No. H600; Hitachi, Tokyo, Japan).

Electrophoresis and Immunoblotting

Plant nuclei were incubated for 30 min at 4°C with urea (2, 4, and 6 M) in TE buffer (10 mM Tris, 2 mM EDTA, pH 6.8). After centrifugation (100,000g for 15 min at 4°C) and dialysis against TE buffer, soluble and insoluble fractions were processed as described previously (Schellenbaum et al., 1993). Proteins were separated electrophoretically, transferred to Immobilon (Millipore Corp., Bedford, MA), and reacted with mAb6C6 (1:500). After rinsing, secondary antibodies were applied: horseradish-conjugated goat anti–mouse IgG (Amersham).

The reaction was developed using the ECL Western Blotting detection system (Amersham).

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