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Live-Cell Analysis of Mitotic Spindle Formation in Taxol-Treated Cells

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Abstract

Taxol functions to suppress the dynamic behavior of individual microtubules, and induces multipolar mitotic spindles. However, little is known about the mechanisms by which taxol disrupts normal bipolar spindle assembly in vivo. Using live imaging of GFP- α tubulin expressing cells, we examined spindle assembly after taxol treatment. We find that as taxol-treated cells enter mitosis, there is a dramatic redistribution of the microtubule network from the centrosomes to the cell cortex. As they align there, the cortical microtubules recruit NuMA to their embedded ends, followed by the kinesin motor HSET. These cortical microtubules then bud off to form cytasters, which fuse into multipolar spindles. Cytoplasmic dynein and dynactin do not re-localize to cortical microtubules, and disruption of dynein/dynactin interactions by over-expression of p50 “dynamitin” does not prevent cytaster formation. Taxol added well before spindle poles begin to form induces multipolarity, but taxol added after nascent spindle poles are visible—but before NEB is complete—results in bipolar spindles. Our results suggest that taxol prevents rapid transport of key components, such as NuMA, to the nascent spindle poles. The net result is loss of mitotic spindle pole cohesion, microtubule re-distribution, and cytaster formation.

Keywords

asters; microtubules; mitosis; spindle; taxol

INTRODUCTION

The cell cycle transition from interphase to mitosis is accompanied by a sweeping reorganization of the microtubule network, in which the mono-focused interphase microtubule array becomes transformed into a bipolar mitotic spindle [reviewed in Compton, 1998; Wadsworth and Khodjakov, 2004]. Coordinate with the morphological changes in microtubule organization is a profound alteration in the dynamic behavior of the microtubules themselves as cells enter mitosis. As somatic cells transition from G₂ into mitosis, the relatively long interphase microtubules switchover and become short and highly dynamic [Saxton et al., 1984; Cassimeris et al., 1988; Zhai et al., 1996; Rusan et al., 2002]. Also, there is a marked increase in the microtubule nucleating capacity of the centrosomes, driven at least in part by

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an increase in the amount of γ -tubulin associated with each mitotic centrosome [Dictenberg et al., 1998; Khodjakov and Rieder, 1999]. Finally, the re-modeling of the interphase microtubule network into a mitotic spindle involves the severing of long microtubules along their length by mitotically activated severing proteins like katanin [McNally and Vale, 1993]. The net result is that mitotic microtubules are shorter, more numerous and more dynamic, compared to their interphase predecessors [reviewed in Desai and Mitchison, 1997]. Current models suggest that the increase in both microtubule number and dynamic instability reflects the requirement for the mitotic cell to generate the numerous microtubule plus-ends necessary to perform rapid “search-capture” events [Schuyler and Pellman, 2001; Piehl and Cassimeris, 2003; Vaughan, 2004]. Plus-end mediated “search-capture” plays a major role in generating the microtubule-kinetochore attachments that connect the sister chromatids to the opposing spindle poles [Hayden et al., 1990]. The dynamic microtubules also search out key spindle pole components, such as NuMA, HSET, and dynein/dynactin, and allow them to become transported back to the minus-end of microtubules, thereby focusing them into functional spindle poles [Echeverri et al., 1996; Gaglio et al., 1997; Mountain et al., 1999; Merdes et al., 2000; reviewed in Vaughan, 2004]. Both of these dynamic microtubule mediated events are thought to be essential to establish normal bipolar mitotic spindle morphology [reviewed in Vaughan, 2004]. However, the effect of globally suppressing plus-end mediated search-capture on microtubule events during mitotic spindle assembly in general, and principally on generating two functional spindle poles, has not been systematically evaluated, particularly in living somatic cells.

One method that has been used to block microtubule plus-end dynamics is treatment of cells with taxol. Taxol is a potent chemotherapy agent, which has been shown to target microtubules [Schiff et al., 1979; Schiff and Horwitz, 1980; De Brabander et al., 1981]. Live-cell studies have revealed that taxol functions specifically to suppress the dynamic behavior of individual microtubule plus-ends [Yvon et al., 1999; Shannon et al., 2005]. Importantly, adding taxol to cells before they enter mitosis induces the formation of aberrant mitotic spindles [De Brabander et al., 1981; Jordan et al., 1996]. Static images of these cells have revealed that they contain multiple microtubule asters (cytasters), which presumably contribute to the multipolar mitotic phenotype observed [De Brabander et al., 1981]. In contrast, if taxol is added to somatic cultured cells after they have entered mitosis, the spindles remain bipolar, but the cell cycle becomes arrested/delayed prior to the onset of anaphase, presumably because the loss of plus-end microtubule dynamics leads to the activation of the kinetochore attachment checkpoint, which monitors microtubule attachment and tension at the kinetochore [Snyder and Mullins, 1993; Waters et al., 1998; Yvon et al., 1999; Shannon et al., 2005]. Addition of taxol to cells in anaphase also does not disrupt spindle bipolarity, but does inhibit anaphase B spindle elongation [Amin-Hanjani and Wadsworth, 1991].

Much of our current knowledge of taxol's function during mitosis has come from in vitro studies, where microtubule asters are assembled from cellular extracts derived from mitotic cells [Verde et al., 1991; Mountain et al., 1999]. The results of these studies have revealed several key cellular components necessary for cytaster formation, most notably the nuclear mitotic protein (NuMA) and a sub-set of microtubule motor proteins [reviewed in Fant et al., 2004]. However, as mentioned above, the morphology of mitotic cells is quite different if taxol is added before the G₂/M transition (resulting in multipolar spindles), compared to the morphology of cells in which taxol was added after entry into mitosis (which maintain bipolar spindles). This suggests that cytaster assembly and multipolar spindles formed in taxol cells are not solely due to microtubule stabilization and de novo microtubule assembly, but result from morphological changes to the microtubule cytoskeleton that occur during the G₂/M transition. Thus, we hypothesize that taxol's ability to suppress microtubule dynamics and/or microtubule structure could have a potent effect in vivo on the ability of the microtubule network to become re-modeled into a strictly bipolar array specifically at the G₂/M transition. Moreover, it is not clear what the effect of suppressing plus-end microtubule dynamics has on

the dynein/dynactin-mediated transport of key cargos, such as NuMA, on the formation and stability of the spindle poles. To address these questions, we have generated a cell line constitutively expressing GFP- α tubulin, and using long-term video microscopy, we have directly observed microtubule behavior in living taxol-treated cells as they transition from interphase into mitosis. This has allowed us to test—in living cells—the previously proposed models for taxol's function, which were derived from in vitro experiments.

MATERIALS AND METHODS

Cell Culture and Treatment

BSC-1 cells (ATCC, Manassas, VA) were cultured in DMEM containing 10% FBS (Gibco, Grand Island, NY) and 1 mg/ml pen-strep (Sigma-Aldrich, St. Louis, MO) in 10% CO₂. For taxol treatments, cells were treated with paclitaxel (taxol: Sigma) at a final concentration of 10 μ M, unless noted otherwise.

BSC-1 cells were transfected with the GFP- α -tubulin construct (Invitrogen, Carlsbad, CA) using Fugene 6 (Roche Applied Science, Indianapolis, IN) in antibiotic-free DMEM. Cells were left overnight at 37°C and then HEPES buffered DMEM medium containing G418 (2 mg/ml, Sigma) was added. After 4 days in G418, cells were passaged and colonies of cells were removed with a yellow pipette tip, and seeded into 24 well dishes. The concentration of G418 was lowered to 50 μ g/ml. After 2 days, cells in each well were passaged; with half the cells passaged into 24 well dishes and the other half passaged onto 18-mm coverslips in six-well dishes.

After 3 days, cells on coverslips were screened by live-cell fluorescence microscopy. Primary clones were identified by expression level of GFP-microtubules. Selected clones were passaged onto 10-cm dishes and grown for 3 days. Colonies of cells were then seeded onto 24 well dishes, grown and re-screened as described above. Secondary clones were selected with pipet tips, and diluted so that single cells were placed in 24 dishes containing a feeder layer of PtK2 cells (ATCC). These cells were grown for 4 days in G418 containing media (2 mg/ml), allowing the clone to grow, and selectively removing the feeder layer. The BSC-1 GFP- α -tubulin cells were passaged onto coverslips and 24 well dishes, and screened as described above. Tertiary clones were identified and stocks stored in LN₂. We selected six tertiary clones, of which one (BSC-1 atg) was used in this study.

Microscopy

Cells on coverslips were either fixed in cold methanol (−20°C), or with 1% glutaraldehyde [Wheatley and Wang, 1998]. For glutaraldehyde fixation, cells were first extracted at RT for 1 min in cytoskeleton buffer (137 mM NaCl, 5 mM KCl, 1.1 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 2 mM MgCl₂, 2 mM EGTA, 5 mM PIPES, 5.5 mM Glucose, pH 6.1) with 0.3% Triton-X 100 and 0.5% glutaraldehyde added, followed by fixation for 15 min in cytoskeleton buffer with 1% glutaraldehyde added. To reduce free aldehyde groups, fixation was followed by treatment with 0.5 mg/ml NaBH₄ in cytoskeleton buffer for 5 min.

Coverslips were labeled with anti-centrin 2 (1:1000; mouse monoclonal; gift of Dr. Jeff Salisbury, Mayo Medical School), anti- γ -tubulin (1:1000, rabbit polyclonal, Sigma), anti- α -tubulin (1:1000, mouse monoclonal clone DM1A, Sigma), anti-cytoplasmic dynein intermediate chain (V3 rabbit polyclonal); [Vaughan et al., 2001], anti-p150 dynactin (D'art, rabbit polyclonal) [Vaughan et al., 1999], anti-NuMA (rabbit polyclonal; gift of Dr. Duane Compton, Dartmouth Medical School) and/or anti-HSET (rabbit polyclonal; gift of Dr. Duane Compton, Dartmouth Medical School). The secondary antibodies used were Alexa-594 goat anti-rabbit (Invitrogen, Carlsbad, CA), Alexa-488 goat anti-mouse, and/or Alexa-660 goat anti-

rabbit in a 1:1000 dilution. Fixed slides were imaged with a 63×1.4 NA oil immersion Plan-Apo CS objective on a Leica DM RXA2 upright microscope (Leica Microsystems, Bannockburn, IL). Images were acquired with Simple PCI software (Compix, Sewickley, PA) using a cooled Hamamatsu ORCA-ER CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). Images were acquired as Z-stacks at 0.2- μ m intervals and compiled as maximum projections. Figures were assembled and final brightness and contrast of images was done using Photoshop 6.0 (Adobe, San Jose, CA). Some images were further subjected to 2D blind deconvolution using the Autoquant algorithm (licensed to Simple PCI, Compix).

For time-lapse imaging of living cells, BSC-1- α tg cells were plated onto bio-cleaned glass coverslips in Imaging Media: DMEM w/o phenol red, containing 12 mM Hepes, pH 7.2, and 10% fetal bovine serum, and assembled onto aluminum support slides, as described previously [Hinchcliffe et al., 2001; Hinchcliffe, 2005]. Time-lapse images were captured using a Leica DM RXA2 microscope stand, equipped with fluorescence and differential interference optics, using either a Leica $20\times/0.70$ NA Plan Apo or $40\times/0.70$ NA Plan Apo objective. The scope was enclosed in a custom-made Plexiglas box maintained at 37°C [Hinchcliffe, 2005]. Images were captured using Simple-PCI software.

High-resolution live-cell fluorescence images were captured using a Leica Plan Apo $63\times/1.3$ NA Plan Apo glycerol immersion objective (37°C optimized). Confocal images were captured using Simple PCI imaging software driving a Yokagawa CSU-10 spinning disk confocal head, as modified by McBain Industries (San Diego, CA). Illumination of the fluorescent images was done with a 488 nm, 200 mW continuous wave optically pumped solid state “lazer” (Sapphire, Coherent Inc, Santa Clara, CA). The laser was connected through a fiber optic cable into the excitation port of the spinning disk confocal head, and shuttered via a TTL pulse through a Ludl MAC5000 shutter controller (Ludl Electronic Products, Hawthorne, NY). The detector on the confocal microscope was a Hamamatsu C9100-12 BT EMCCD camera.

Over-Expression of p50-mCherry

The cDNA construct containing p50 (“dynamitin”) fused to mCherry red fluorescent protein was created by tagging p50 (H50A clone), [Echeverri et al., 1996] at COOH-terminus with the mCherry fluorescent protein [Shaner et al., 2004]. This construct was inserted into the pCI-neo mammalian expression plasmid (Promega, Madison, WI). Over-expression of the construct was induced by nucleofection using the Amaxa Nucleofector (Amaxa Biosystems, Koln, Germany). Cells were plated onto 18-mm coverslips for immunofluorescence or 22-mm coverslips for live cell imaging. After 24 h, cells were treated with 10 μ M Taxol for 4 h, then fixed for immunofluorescence as described above.

RESULTS

Taxol Causes Microtubules to Redistribute From the Centrosome to the Cortex as Cells Enter Mitosis

To image spindle assembly in vivo, we generated a line of African Green Monkey kidney (BSC 1) cells constitutively expressing GFP- α tubulin (termed BSC 1- α tg cells). GFP- α tubulin has been used successfully to fluorescently label microtubules in other mammalian somatic cell types [see Rusan et al., 2002]. We can image BSC 1- α tg cells over extended periods of time using fluorescent optics [Hinchcliffe, 2005]. Also, the expression of low levels of GFP- α tubulin - representing 17% of the endogenous α -tubulin in these cells - does not inhibit cell cycle timing or mitotic timing of these cells compared to un-transfected cells.

In order to examine the microtubule events of spindle assembly in the presence of taxol, BSC 1- α tg cells were loaded into imaging chambers, then paclitaxel (taxol: Sigma) was added to

the culture medium at a final concentration of 10 μ M. This concentration was used because previous work has shown that it results in suppression of plus-end microtubule dynamics within 5 min of addition, as judged by loss of EB-1-GFP tip-tracking in PtK2 cells [Shannon et al., 2005]. We note that intracellular concentrations of taxol dramatically increase over time; addition of nM concentrations of taxol can lead to intracellular concentrations several hundred fold over the extracellular concentrations after 20 h [Jordan et al., 1996; Jordan and Wilson, 1999]. Our goal was to rapidly saturate the cell with taxol, to ensure suppression of plus-end microtubule dynamics. To demonstrate plus-end suppression in our parental BSC-1 cells, we transfected EB-1-GFP, and imaged plus-end mediated microtubule tip tracking by time-lapse video microscopy. After addition of 10 μ M taxol, we observed complete suppression of plus-end microtubule tip-tracking within 5 min (Supplemental video 1). This suggests that we are rapidly reaching microtubule saturation with taxol.

To examine the effects of completely suppressing microtubule plus-end dynamics on mitotic spindle assembly, we added 10 μ M taxol to an asynchronous culture of BSC 1-*atg* cells. These were then continuously observed for a period of 20 h by time-lapse video microscopy with 20 $\times$ magnification and fluorescence optics (Fig. 1 and supplemental video 2). In these preparations, cells were observed transitioning into mitosis, assembling multiple asters and multipolar spindles. These cells eventually exited mitosis, with most cells failing cytokinesis and exiting as single multinucleate daughters. However, we observed some cells that did divide in two in the continued presence of taxol (Fig. 2H, arrowheads). BSC-1 *atg* cells in 10 μ M taxol spend on average 551 min in mitosis, compared to 56 min for control cells. Cells in 1–5 μ M taxol spend slightly less time in mitosis, but considerably longer than controls (see Table I).

Analysis of both low magnification and high magnification video sequences of cells entering into mitosis revealed that aster formation was not spontaneous or *de novo*, as had previously been observed *in vitro* [Verde et al., 1991]. Instead, the formation of cytasters resulted from pre-existing microtubules that became re-organized following entry into mitosis. High-magnification video sequences showed that as these taxol-treated cells transition into mitosis, the organized microtubules become relocalized to the cell periphery (Fig. 3, and supplemental video 3). These cortical microtubules shorten, and become aligned at the cell cortex (Fig. 3B). It is not clear if all of the cortically aligned microtubules move there as intact microtubules, which have been released from the centrosome, or whether some result from the severing of existing microtubules, followed by slow re-assembly at cortical sites. It is clear from the video recordings, that some intact microtubules do traffic to the cortical sites (see supplemental movie 3A), where they assemble into the developing cytasters.

As they become aligned, sheets of microtubules curve at their cortex-embedded ends, and form large, hollow asters, which bud off of the cell cortex (Figs. 3C–3H, arrow). Some of the forming asters fuse together as they bud from the cortex, forming large cytasters (Fig. 4, and supplemental video 4).

As the microtubule asters detach from the cortex, they migrate towards the chromosomes, and many fuse together to form the typical taxol-induced multipolar spindles. The final multipolar spindle configurations vary between cells, and some even adopt a roughly bipolar configuration, albeit with unaligned chromosomes and un-equal spindle poles (see Fig. 5D). We note, however, that some of the asters that form at the cortex do not persist, and instead, depolymerize before they can become incorporated into the multipolar spindle. Figure 2 shows frames from a high-magnification view of a taxol-treated cell entering mitosis. Initially, eleven asters assemble from cortically re-distributed microtubules, along with seven small asters that form around the disintegrating nuclear envelope. Six of the cortical asters migrate towards the region of the condensed chromosomes, fuse together, and remain stabilized. These asters become part of the multipolar spindle that surrounds the chromosomes. However, four of the

cortical asters fail to migrate towards the cell center, and remain in the distal cytoplasm (Fig. 2B, asterisks). Over ~20 min these cytasters depolymerize and disappear, the most prominent of which is shown in Figs. 2A–2D, arrow.

These observations reveal that taxol-induced cytasters are not absolutely stable. We find that asters that form and migrate into the region of the condensed chromosomes remain stabilized, and fuse together to form multipolar spindles. Those asters that form, but do not migrate into the region of the chromosomes are dynamic and eventually disappear. This suggests that the region near the chromatin influences taxol-induced cytaster stability. Previous studies have shown that during bipolar mitotic spindle assembly, a group of proteins referred to as “spindle assembly factors” (SAFs) are activated proximal to chromosomes [Gruss et al., 2001; Tsai et al., 2003]. The activation of these factors near the condensed chromatin is due in part because of an activation gradient of the small GTPase, RAN in its GTP bound state [Wilde and Zheng, 1999; Kalab et al., 2006]. RAN-GTP is activated by RCC-1, the RAN guanine exchange factor (GEF), which is bound to mitotic chromatin [reviewed in Moore, 2001]. When we examined RCC-1 localization during early prophase in taxol-treated cells, we found that it remains localized in the region of the chromosomes, and does not re-localize to the cell periphery or cortex (supplemental Fig. 1). In contrast, RAN-GAP and RAN-Bp1 (both factors needed to hydrolyze GTP and inactivate RAN) are localized throughout the cytoplasm (supplemental Fig. 1), where they are also found in non-taxol treated cells [Moore, 2001]. These observations suggest that the re-localization of microtubules to the cell cortex, and the formation of cytasters from cortical microtubules, is not driven by a mis-localization of active RAN from the chromosomes to the cell periphery. These observations also suggest that chromatin—or a region proximal to it—*does* influence the eventual stability of taxol-induced asters and multipolar spindle assembly.

Taxol Induces a Release of the Microtubule Network From the Centrosome Coordinate With Entry Into Mitosis

The pericentriolar material surrounding the centrosome anchors the γ -tubulin ring complexes (γ -TURCs) that serve to establish the microtubule-nucleating center (MTOC), thereby constraining the interphase microtubule network into a single array that emanates from the centrosome [Moritz et al., 1995; Zheng et al., 1995]. Much of the γ -tubulin in cells is soluble, and exchanges with the centrosomal population [Khodjakov and Rieder, 1999]. Therefore, it is a formal possibility that γ -tubulin could re-distribute to the cell cortex—along with the microtubules—in response to taxol treatment. To determine whether γ -tubulin is associated with the microtubules as they form cytasters around the nucleus and at the cell cortex, we used immunofluorescence microscopy to localize γ -tubulin in taxol-treated cells. In control BSC-1 cells, γ -tubulin localizes to two distinct foci, which correspond to the centrosomes, as judged by co-labeling with anti-centrin 2 that labels the centrioles (supplemental Figs. 2A–2A'''). Cells with a single, unduplicated centrosome (containing one pair of centrioles) contain two γ -tubulin foci, each of which surrounds a centriole. Those cells with duplicated centrosomes, containing two pairs of centrioles also have exactly two γ -tubulin foci (supplemental Fig. 2).

To determine if γ -tubulin is re-distributed following taxol treatment, cells were treated with 10 μ M taxol for 2 h, then fixed and labeled with antibodies to α -tubulin and γ -tubulin (Fig. 5). Most of the γ -tubulin remains associated with the two centrosomes (Figs. 5A–5A''', arrow). We always observed two γ -tubulin foci in taxol-treated cells. However, the cortically aligned microtubule ends are not labeled with γ -tubulin, nor do we observe extensive γ -tubulin labeling of the small asters that form near the nucleus. While it is a formal possibility that anti- γ tubulin cannot detect individual γ -TURCs attached to the ends of embedded microtubules, we do not find a concentration of γ -tubulin label at the center of the aster. Interestingly, as taxol-treated cells enter mitosis, the centrosomes do not always remain associated with the disintegrating

nuclear envelope, as they do in un-treated cells—where they assemble the two spindle poles. Instead, the centrosomes often drift away from the condensing chromosomes (Figs. 5A and 5B, arrows). Loss of centrosome association with the nuclear envelope occurs well before the cortical asters begin to form. In many cases, the two centrosomes have few microtubules associated with them, and in some cases no detectable microtubules at all (compare microtubule distribution in Figs. 5B and 5C, arrows; also see Table II). We note that BSC-1 cells do not undergo centrosome amplification (repeated centrosome duplication without cell cycle progression) when arrested in S-phase with DNA synthesis inhibitors, as is seen for other transformed cell types [Balczon et al., 1995; our unpublished data]. Thus, it appears that taxol is not inducing multiple centrosomes that could organize the multiple cytasters observed. Instead, we find that suppressing plus-end microtubule dynamics results in the centrosomes being unable to direct the position of the nascent spindle poles.

Spindle Pole Components Relocate to the Cortically Arrayed Microtubules and Cytasters in Response to Taxol Treatment

Because we observe microtubules being released from the centrosomes—the natural point where spindle poles form—and we see cytasters forming at the cell periphery following entry into mitosis in taxol-treated cells, we used immunofluorescence microscopy to examine the distribution of several key spindle pole components during taxol-induced aster formation. The microtubule cross-linking protein NuMA [Gaglio et al., 1996, 1997], the minus-end directed kinesin HSET [Mountain et al., 1999], and the minus-end directed motor protein dynein associated with its regulatory complex dynactin [Vaisberg et al., 1993; Echeverri et al., 1996; Gaglio et al., 1996] are all required to assemble spindle poles in control cells [reviewed in Fant et al., 2004; Wadsworth and Khodjakov, 2004]. These pole components have also been shown to be required for taxol-induced aster formation in mitotic extracts [Verde et al., 1991; Gaglio et al., 1996; Mountain et al., 1999].

In control cells, NuMA is nuclear during interphase, then rapidly re-distributes to the spindle poles as the nuclear envelope begins to break down [Compton and Cleveland, 1994; our Supplemental Fig. 2]. We find that in taxol-treated BSC-1 cells, NuMA re-localizes from the nucleus to the distal microtubule ends as they re-distribute to the cell cortex (Figs. 6A–6D). However, careful examination of these micrographs reveals that the microtubules re-distribute to the cortex before the NuMA localizes there (Fig. 6A' and Fig. 9C'). As the cortical microtubules begin to form into asters, the NuMA is localized to the embedded microtubule ends and at the center of the large, hollow asters (Figs. 6B'–6D'). Note that these micrographs reveal that cortically-derived cytasters begin as curved sheets of microtubules that then form open or c-shaped arrays (Fig. 6C', arrow). These open arrays then close and fuse together into large, hollow asters. In cortical regions that lack microtubules, anti-NuMA staining is lacking, suggesting that NuMA is targeted to the microtubule ends, not the cell cortex (Fig. 6C', arrowhead). NuMA remains associated with the cytasters as they assemble into the spindle. Previous work by Maekawa and colleagues and Kallajoki and colleagues also reported microtubule re-distribution to the cell cortex, and a concentration of the SP-H and SPN antigens (NuMA homologs) to these cortical sites [Maekawa et al., 1991; Kallajoki et al., 1992].

We find that the minus-end directed kinesin HSET also localizes to the center of the cytasters as they form and bud off of the cell cortex (Figs. 6E–6G). Anti-HSET also decorates the cytasters as they form around the nuclear region (Fig. 6E). However, HSET does not localize to the cortically embedded microtubule ends early in prophase (Fig. 6E). Instead, HSET localization to the cytasters begins only as they form off the cortex (Fig. 6F). Thus, it appears that HSET localization to taxol-induced cortical microtubules follows that of NuMA, because NuMA appears at the cortex prior to HSET (compare Figs. 6A'–6E'). This suggests that HSET is targeted to NuMA at the microtubule ends, where it may be driving the curving and budding

off of the cytasters. Our findings are consistent with previous work, which showed that NuMA and HSET are required for taxol aster formation in vitro [Gaglio et al., 1996; Mountain et al., 1999].

When the localization of cytoplasmic dynein, and its associated complex dynactin were examined in taxol-treated cells, we found that these did not re-distribute to the cortex or the cytasters until after these had fully formed (Fig. 7). In the case of antibodies against dynein intermediate chain (anti-IC), there is considerable localization to the kinetochores in early prophase taxol-cells (Fig. 7A', inset), but no detectable localization to the cortex or forming cytasters (Figs. 7A–7C). We did not observe any concentration of dynein at the cell cortex as the microtubules re-distribute (Fig. 7A'). We note that in control cells, anti-IC prominently labels spindle poles beginning in very early prophase [Echeverri et al., 1996].

Antibodies to the dynactin subunit p150 also labeled kinetochores in taxol-treated prophase cells, but did not decorate the embedded microtubule ends or assembling cytasters (Fig. 7D'). Localization of p150 to taxol cytasters only occurs once these have formed (Figs. 7E' and 7F'). We note that in control cells, both anti-IC and anti-p150 prominently label spindle poles beginning in very early prophase. Thus, when compared to NuMA and HSET localization, cytoplasmic dynein and dynactin do not appear to be associated with taxol-induced cytasters until well after these have formed (see Discussion).

Taxol-Induced Aster Formation In Vivo Does not Require Dynein/Dynactin Activity

Immunofluorescence microscopy of taxol-treated cells revealed that both cytoplasmic dynein and the dynactin complex do not localize to cytasters until after their formation (Fig. 7). These observations suggest that cytoplasmic dynein is not required for the initial events of taxol-induced cytaster formation in vivo, contrary to previous results derived from in vitro extract studies [Verde et al., 1991]. To test this in vivo, we transfected BSC-1 cells with the p50 subunit of dynactin. Over-expression of p50 “dynamitin” results in the disruption of the dynactin complex, and prevents dynein/dynactin-mediated transport, thereby disrupting spindle pole organization [Echeverri et al., 1996; Merdes et al., 2000]. Dynamitin over-expression also disrupts the interaction of cytoplasmic dynein and NuMA [Merdes et al., 2000]. Cells were transfected for 24 h with a p50-mCherry RFP construct, then treated with 10 μ M taxol for 6 h and fixed. Mitotic cells over-expressing p50-mCherry were identified and the microtubule configuration was imaged. We identified 25 early mitotic cells expressing p50-mCherry in three separate transfection experiments. In all cases, the microtubules had re-distributed to the cell cortex and cytasters had formed (Figs. 8A–8C). Transfected cells in the absence of taxol formed a variety of abnormal spindle conformations, confirming that the p50-mCherry construct is active in disrupting the dynactin complex (Fig. 8D). Therefore, we find that disrupting the dynein/dynactin interaction does not affect the ability of microtubules to re-distribute to the cell cortex and form asters in the presence of taxol.

Taxol-Induced Microtubule Reorganization to the Cortex

We next addressed the question of whether or not the effects observed by treatment with taxol were cell cycle specific. Previous work using taxol suggested that cytaster/multipolar spindle formation were due to taxol lowering the critical concentration necessary for tubulin to polymerize into microtubules [De Brabander et al., 1981; Verde et al., 1991]. The prevalent model based on these results is that the de novo assembly of microtubule asters out-strips the ability of the centrosome to organize the spindle, and that the de novo microtubule assembly in the cytoplasm gives rise to the cytasters and multipolar spindles observed. However, the results presented here suggest that cytaster formation is not driven by spontaneous polymerization of microtubules in the cytoplasm, but rather, by a re-distribution of pre-existing microtubules.

To test if adding taxol to cells before and after the initiation of spindle pole assembly results in re-distribution of microtubules to the cortex, we added 10 μ M taxol to cells for 5 and 20 min, then fixed the cells and labeled them with antibodies against either NuMA to detect the position of the spindle poles, or μ -tubulin to detect the position of the centrosomes. Again, this concentration of taxol blocks plus-end directed motility within 5 min, though the concentration of taxol is increasing over the 20-min timecourse.

Examination of early prophase cells treated with taxol for 5 min did not reveal extensive microtubule re-distribution to the cortex, nor cytaster formation (Fig. 9). Instead, these cells assembled two major microtubule asters (spindle poles) positioned near the disintegrating nuclear envelope (Fig. 9A). These two prominent spindle poles were both NuMA-positive, and also assembled coincident with the γ -tubulin containing centrosomes (Figs. 9A, 9B, 9D, and 9E).

Taxol treatment for 20 min resulted in prophase cells with microtubule re-distribution to the cortex (Figs. 9C and 9F). Under these conditions, the taxol-treated cells were morphologically indistinguishable from cells that had been incubated in taxol for several hours. We did not find prominent microtubule asters associated with the γ -tubulin foci, nor were there two major NuMA foci near the nucleus.

To further examine the timing of taxol's effect, we treated cells with 10 μ M taxol for either 5 or 20 min, then observed spindle formation *in vivo* by live-cell imaging. As noted above, treatment of BSC-1 cells with 10 μ M taxol for 5 min is sufficient to suppress plus-end mediated microtubule based tip tracking, as judged by live-cell observations with EB1-GFP expressing cells (supplemental video 1); [also see Shannon et al., 2005]. Thus, for the 5-min time-point, we began our observations at the time when plus-end microtubule dynamics had just ceased. Taxol was added to the imaging chamber, candidate cells entering mitosis were located using DIC optics, and then spindle assembly was imaged using fluorescence optics. The difficulty in these measurements is identifying cells that undergo the G₂/M transition exactly 5 min after taxol treatment. Cells were selected that had intact nuclear envelopes, but showed disintegration of the nucleoli, which is an indication that the cell is entering into mitosis. However, in 19/19 cases, cells entering mitosis after 5 min in taxol had already assembled two prominent microtubule-nucleating centers, even prior to nuclear envelope breakdown (Fig. 10A, arrows), and in all of these cases, a roughly bipolar spindle was formed. We did not observe significant re-distribution of microtubules to the cortex, or loss of centrosome-cohesion (Fig. 10). There were some asters that formed in the position of the condensing chromosomes, but the spindle maintained a rough bipolar symmetry (Fig. 10J, arrows).

When taxol was added to the cells for 20 min prior to observation, we found microtubule re-distribution to the cell cortex, and multipolar spindle formation (Figs. 10K–10R). In all 12 cases, the cells failed to form the prominent asters associated with the nucleus that are the hallmark of normal spindle pole assembly. Instead, these cells released their microtubules into the cytoplasm, which migrated to the cortex and assembled large cytasters (Figs. 10L and 10M, arrow).

DISCUSSION

Here we have used live-cell imaging of GFP- α tubulin expressing cells to examine the early events of mitotic spindle assembly in the presence of taxol. We find that treatment of mammalian somatic cells with high concentrations of taxol—prior to entry into M-phase—results in the re-distribution of the pre-existing microtubule network to the cell cortex, followed by the re-distribution of select spindle pole components to these cortical microtubule ends. This results in the formation of numerous cytasters, which migrate towards the cell center,

fuse, and drive multi-polar spindle formation. We do not observe a complete disassembly of the intact microtubule network, rather a re-distribution of exiting microtubules from the spindle poles to the cell cortex.

Here we have used 10 μM taxol to rapidly suppress microtubule dynamics, as previously reported [Shannon et al., 2005], and this high concentration of taxol induces mitotic arrest with multipolar spindles. However, previous work has shown that mitotic arrest—with multipolar spindles—can also occur at much lower concentrations, in the nanomolar range [Jordan et al., 1996; Yvon et al., 1999]. This raises the question of whether or not the effects we see are due to very high taxol concentrations. It is important to note that taxol loads into cells over time, leading to extraordinarily high intracellular concentrations of the drug. Indeed, Jordan and Wilson have shown that treatment of HeLa cells with 100 nM–1 μM extracellular taxol results in intracellular concentration of 70–240 μM after 20 h [Jordan et al., 1996]. Also, addition of either 10 nM or 100 nM extracellular taxol to HeLa cells results in intracellular concentrations in the μM range within 10 min, and half maximal concentrations within 25–100 min [Jordan and Wilson, 1999]. Thus, mitotic arrest in response to nM taxol treatment is really arrest in response to μM intracellular taxol. The major difference is how quickly we drive saturation of the microtubules. By adding 10 μM taxol, we are suppressing microtubule plus-end dynamics within 5 min (as judged by loss of EB-1 GFP “tip-tracking”).

Taxol perturbs microtubule dynamics [Jordan and Wilson, 1999], and has been shown to specifically suppress the plus-end dynamics of individual microtubules in living cells [Yvon et al., 1999]. Taxol does not appear to significantly reduce minus-end dynamics in mitotic cells [Waters et al., 1998]. Cells treated with taxol have been observed to contain multipolar spindles, and this is thought to inhibit cytokinesis, leading to polyploidy [De Brabander et al., 1981; Yvon et al., 1999]. However, if taxol is directly added to mitotic cells, bipolar spindle organization is not significantly perturbed, and cytasters do not assemble, but rather mitotic progression is inhibited, presumably because the loss of dynamic microtubules activates the cell cycle checkpoint that monitors kinetochore attachment and tension [Waters et al., 1998; Shannon et al., 2005].

While it is clear from previous work that taxol induces multipolarity, these studies have not revealed a mechanism that could explain how suppressing microtubule dynamics during spindle assembly leads to multipolar spindles, primarily because individual cells were not followed in vivo. What little is known has come from previous work in vitro, which suggested that taxol lowers the critical free concentration of tubulin needed for polymerization, and it is microtubule assembly de novo in the mitotic cytoplasm that induces microtubule aster formation in mitotic extracts [Verde et al., 1991; Gaglio et al., 1996].

Here we show that the formation of multipolar spindles in vivo is not merely a consequence of bundling microtubules, or extensive de novo microtubule assembly in the cytoplasm. Rather, cytaster formation requires the suppression of microtubule dynamics prior to the building of a nascent spindle pole. This induces the microtubules to re-distribute to the cortex. And once re-organized, these cortical microtubules recruit the spindle pole components NuMA and HSET to their cortically embedded ends. Interestingly, cytoplasmic dynein and the dynactin complex do not appear to play a role in the early events of cytaster formation in vivo. Antibodies to dynein IC and to the p150 subunit of dynactin do not localize to the cortex as the microtubule re-distribute. Both eventually recruit to the cytasters, suggesting that they target to the asters once they have formed. Functional data—using over-expression of p50 “dynamitin”—strongly suggests that dynein/dynactin are not required to form taxol asters in vivo. This further suggests that cytasters assembled in vivo are very different from those assembled from isolated mitotic extracts, because in vitro aster assembly requires cytoplasmic dynein and dynactin [Verde et al., 1991; Gaglio et al., 1996]. This difference may reflect the fact that cytasters assembled in

vitro first require de novo microtubule assembly, whereas those assembled in taxol-treated cells arise from a re-modeling of a pre-existing microtubule array. Further work will be needed to understand these different requirements.

Both dynein and dynactin are required to assemble normal spindle poles, in part by rapidly transporting NuMA to microtubule minus ends [Echeverri et al., 1996; Merdes et al., 2000]. However, in the presence of high taxol concentrations, NuMA/dynein and dynactin are not concentrated at spindle poles. Instead, NuMA appears at the embedded cortical microtubules ends, apparently without dynein-mediated motility. The simplest explanation for this observation is that NuMA has an affinity for microtubule ends, and as it diffuses from the nucleus it encounters microtubule ends (at the cortex) and assembles there. Our data suggest that during prophase a major role played by the dynein/dynactin complex is to rapidly transport key spindle pole components, such as NuMA, to the nascent poles. Previous work has shown that the loading of dynactin and dynein onto microtubules requires the presence of dynamic microtubule plus-ends [Vaughan et al., 1999]. For example, during minus-end directed transport of golgi membranes, dynamic microtubule plus-ends function to search out and capture the dynactin complex, followed by dynein bound to its vesicular cargo [Vaughan et al., 2002]. This tip-loading of dynactin to the growing microtubule plus-end also requires phosphorylation of the dynein intermediate chain in order to bind the dynactin [Vaughan et al., 2002; reviewed in Vaughan, 2004]. The requirement of dynamic microtubules may also be required in order for dynein/dynactin-mediated NuMA transport to the developing spindle poles. Indeed, the increase in centrosome-mediated microtubule nucleation at the onset of prophase could reflect the requirement for an increase in dynamic micro-tubule plus-ends necessary to facilitate search-capture of key mitotic cargos [Zhai et al., 1996; Piehl and Cassimeris, 2003]. However, there are also examples of increased dynein-mediated transport in cells with suppressed microtubule dynamics, as in the case for p53 transport to the nucleus in the presence of low doses of taxol [Giannakakou et al., 2000, 2002].

Previous work has also shown that loss of dynein activity or perturbation of dynactin-dynein interactions results in a loss of spindle pole cohesion, which may reflect a loss of minus-end directed transport of NuMA to the developing spindle poles, as well as the eventual cohesion [Vaisberg et al., 1993; Echeverri et al., 1996; Gaglio et al., 1997; Merdes et al., 2000]. Together, these studies suggest that the role of the centrosome in spindle assembly is to serve as a focus for microtubule nucleation (generating the so-called “spindle aster”); [Compton, 1998]. This serves to establish two points in the mitotic cell where NuMA and motor proteins can become concentrated, and in doing so, focus the microtubule minus-ends into a cohesive spindle pole. Once these two poles are established, they can direct the assembly of the spindle [reviewed in Wadsworth and Khodjakov, 2004]. The two nascent spindle poles also serve as a sphere of attraction, where disassembling cytoplasmic microtubules are rapidly drawn in towards the focused minus-ends [Rusan et al., 2002].

A Model for Spindle Pole Assembly and Cohesion

Based on our results, we propose a model for taxol's function in vivo, in which stabilizing microtubules by saturating them with taxol—prior to the assembly of the nascent spindle poles—results in a decrease in the accumulation of spindle pole components at the centrosomes. The net result is that centrosomes cannot maintain cohesion of the microtubule minus ends, and the microtubules themselves become released. These microtubules redistribute to a default position on either the nuclear envelope or cell cortex. Once there, NuMA and HSET (and other as yet unidentified factors) are recruited, and contribute to cytaster formation in vivo.

How microtubules become released from the nascent spindle poles remains unclear. One possibility is that at the G₂/M transition, rapid and sustaining dynein-mediated localization of NuMA is required to keep the microtubule minus ends embedded in the spindle poles.

Alternatively, as they become stabilized, taxol-microtubules could be susceptible to severing, and begin to disassemble at their minus ends. As they re-distribute to the cortex, they then slowly re-grow from the minus ends.

The re-distribution of microtubules from centrosomes that fail to accumulate NuMA suggests that during normal spindle assembly, microtubules are released from the centrosomes, but captured by the “focusing activity” of the developing spindle poles [Gaglio et al., 1997; Merdes et al., 2000]. Indeed, several lines of evidence suggest that the true spindle poles are a NuMA/motor driven focus of microtubule minus-ends that are physically detached from the centrosomes themselves [reviewed in Compton, 1998]. If suppressing microtubule dynamics blocks this “focusing activity”, then the release of the microtubule minus-ends from the centrosomes is not accompanied by the assembly of a spindle pole. It appears that one consequence of not coordinating microtubule release from the centrosome with recruitment of spindle pole “focusing complexes” is to liberate the pre-existing microtubule network out into the cytoplasm. Alternatively, the rapidly increasing concentrations of taxol could alter microtubule structure, thereby preventing the efficient transport of NuMA to the developing poles, irrespective of plus-end dynamics. Either way, the net result is loss of spindle pole cohesion, and re-distribution of the microtubules to the cell cortex.

Our present work reveals that setting up a nascent spindle pole—prior to NEB—is sufficient to prevent microtubule re-distribution and extensive cytaster formation, even in the face of rapidly increasing taxol concentrations. If spindle pole formation has begun at the time of taxol addition, then it persists, and the cell establishes a roughly bipolar spindle configuration, with the centrosomes dictating the position of the poles (Figs. 9 and 10). If microtubules are stabilized well before the nascent spindle pole begins to develop, then the centrosomes cannot influence spindle polarity, and they lose the ability to position the microtubule array.

Our results suggest that during the G₂/M transition, there is a period of taxol “sensitivity”, which begins just before the nuclear envelope starts to breakdown. During this period, spindle pole components—such as NuMA—reside in the nucleus, and are released as the nuclear envelope breaks down (see supplemental Fig. 2B). Once released from the nucleus, NuMA is normally transported to the developing poles in the absence of taxol [see Gaglio et al., 1997; Merdes et al., 2000]. The result is the establishment of two cohesive spindle poles adjacent to the separated centrosomes. Importantly, this is a rapid and dynamic process that is coordinate with NEB. If the cells are saturated with taxol before NuMA begins to be released from the nucleus, then spindle pole cohesion is never established, and the microtubules re-distribute to the cortex. If taxol is added after the nucleus has begun to “leak” NuMA into the cytoplasm, then spindle pole cohesion is maintained.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Time-lapse imaging of BSC-1 α tg cells in taxol. **A–O.** Frames from a low-magnification video microscopy sequence showing a field of BSC-1 α tg cells as they progress into mitosis over a period of 19 h (time stamp in h:min). The cell denoted by the arrow is the first to enter mitosis (beginning in frame B). A close-up view of this cell is shown in the lower panels 1–12. Notice that spindle assembly begins as the microtubules are released from the MTOC, and move to the cell periphery (frames 4–6). Microtubules at the periphery shorten and form into cytasters (frames 7–9). As the cell rounds up, the asters move towards the cell center, and fuse together into a multipolar spindle (Frames 10–12). Fluorescence optics, Bar in A–O = 100 μ m, Bar in 1–12 = 10 μ m. (Blow-up of spindle/cyaster assembly from cell (designated by arrow above). Also see supplemental video 2.

Fig. 2.

Cytaster assembly and disassembly following taxol treatment. **A–H.** Frames from a time-lapse video sequence showing a BSC-1 atg cell assembling asters from its cortex. Of the eleven cytasters that form from the cortex, seven migrate to the cell center and become part of the spindle, while four (marked with “*” in panel A) fail to migrate and depolymerized. The most prominent of the depolymerizing asters is denoted with an arrow (A–E). Despite forming a multipolar spindle, this cell cleaves into two distinct daughter cells when it eventually exits mitosis (arrowheads in H). Time stamp is h:min. Fluorescence optics. Bar = 20 μ m.

Fig. 3.

Microtubule re-modeling in taxol-treated prophase cell. **A–H.** Frames from a video sequence showing microtubule re-distribution to the cell cortex and cytaster formation *in vivo* following taxol treatment. Note the formation of a major aster (arrow) from a curving sheet of cortically arrayed microtubules. Time stamp is Hrs:Min. Fluorescence optics. Bar = 20 μ m. Also see supplemental video 3.

Fig. 4. Microtubule asters forming and fusing at the cortex. **A–H.** Frames from a high-magnification time-lapse sequence showing the formation of cytasters at the cell cortex following taxol treatment. In frame A, there are three asters forming (arrow and arrowheads) that eventually fuse together. In B–C, the two asters denoted by the arrowheads fuse together. This aster—denoted by an arrowhead—fuses with the large aster denoted by the arrow (E–G). Bar = 3 μm . Also see supplemental video 4.

Fig. 5.

Localization of γ -tubulin/centrosomes in taxol-treated cells. Maximal projections of immunofluorescence images showing the localization of α -tubulin (A–D), γ -tubulin (A'–D'), and DAPI (A''–D'') of taxol treated cells. The three color overlays (A'''–D''') depict microtubules in green, γ -tubulin in red, and dapi in blue. A–A'''. Early prophase taxol-treated cell. Note the two γ -tubulin positive centrosomes remain close together (A', inset), and have wandered towards the cell periphery (arrow in A'''–C. Centrosome localization in multipolar cells. In B''', the centrosomes do not have any detectable microtubules associated with them (arrows). In C''', the major microtubule asters are not assembled around the two centrosomes (arrows). D. The two centrosomes do not appear to be influencing the organization of the spindle, which has adopted a rough bipolar configuration, but one with sister chromatids surrounding both poles. Bar in A'''–C''' = 20 μ m, in D''' = 5 μ m.

Fig. 6.

Localization of NuMA and HSET in taxol-treated cells. Maximal projections of immunofluorescence images showing the localization of α -tubulin (**A–G**), NuMA (**A'–D'**), HSET (**E'–G'**), and DAPI (**A''–G''**) in taxol treated cells. **A–D**. In taxol-treated cells, NuMA translocates from the disintegrating nucleus to the cortically aligned microtubule ends (**A'**, arrows). Note that where there are no microtubules at the cortex, NuMA staining is lacking (**B–B'**, arrowhead). As the cortical microtubule sheets curve in and become asters, NuMA localizes to the inner surface of the forming asters (arrowhead in **C'**). NuMA decorates the inner surface of the large hollow asters that form (arrows in **D'–G'**). The minus-end directed kinesin HSET localizes to microtubules ends. **E–E'''** shows a prophase cells. HSET does not decorate the cortex except where a small aster is forming (**E'**, arrow). As the microtubules align and shorten at the cell cortex, HSET decorates the embedded ends (**F'**) and the asters as they assemble (**G'**). Bars in **D'''** and **G'''** = 20 μ m.

Fig. 7.

Localization of cytoplasmic dynein and dynactin in taxol-treated cells. Maximal projections of immunofluorescence images showing the localization of α -tubulin (**A–F**), cytoplasmic dynein intermediate chain (**A'–C'**), p150 subunit of dynactin (**D'–F'**), and DAPI (**A''–F''**) in taxol treated cells. **A–C**. Dynein localizes to the kinetochores (**A'**, inset), but does not decorate the cortical regions. Dynein does decorate the center of asters after they have formed (**C'**, arrows). **D–F**. p150 also decorates kinetochores, as well as cytasters once they have formed. p150 does not localize to the cortex (**A'**). Bar = 10 μ m.

Fig. 8. Over-expression of p50 dynamitin in taxol-treated cells. Maximal projections of immunofluorescence images showing the localization of α -tubulin (A–D), p50 “dynamitin” coupled to mCherry fluorescent protein (A’–D’), and DAPI (A’’–D’’) in BSC-1 atg cells. Over-expression of the p50 does not prevent microtubules from re-distributing to the cortex in response to taxol treatment (A), nor forming cytasters (B–C). Over-expression of p50 in untreated cells results in mitotic spindle pole defects (D–D’’). This cell has assembled a monopolar spindle. Bar = 10 μ m.

Fig. 9.

Spindle pole morphology in cells treated with taxol for 5 or 20 min. Maximal projection of immunofluorescent images showing spindle pole organization in cells treated with taxol for 5 min (**A–B**, and **D–E**) or 20 min (**C–F**). Cells were labeled with anti-NuMA to detect spindle poles (**A'–C'**), and anti-g tubulin to detect centrosomes (**D'–F'**). In those cells treated with taxol for 5 min, two spindle poles formed (**A'–B'**, arrows), and very little microtubule re-distribution to the cortex was observed. Such cells assembled the two microtubule foci around the centrosomes (**D'–E'**, arrows). In cells treated with taxol for 20 min, no major spindle poles were organized, and the centrosomes no longer organized the microtubule network (**B** and **D**). Bar in **A'''** = 20 μ m, in **B'''–D'''** = 10 μ m.

Fig. 10.

Live-cell analysis of spindle assembly in cells treated with taxol for 5 or 20 min. Frames from two time-lapse video sequences showing spindle assembly in a cell treated with taxol for 5 min (**A–J**) or 20 min (**K–R**). In the cell treated for 5 min, two spindle poles have formed, and these persist as the nuclear envelope breaks down (**A**, arrows). While the spindle does not have a normal appearance, it does remain bipolar (**J**, arrows). There are few microtubules re-distributing to the cortex (**B–I**, small arrow). In the cell treated for 20 min, the microtubules re-distribute to the cell cortex. The major cytaster formed is derived from cortically aligned microtubules (**L–R**, arrow). Time stamp is Min:Sec, and represents the time from taxol addition. Bar in **J** = 20 μm , in **R** = 10 μm .

TABLE IDuration of Mitosis in Taxol-Treated BSC-1 α tg Cells

Taxol concentration	Average time in mitosis (min)	Range		Sample size
		High	Low	
10 μ M	551	837	353	40 cells
5 μ M	474	931	260	40 cells
2.5 μ M	497	782	258	36 cells
1.0 μ M	471	810	175	38 cells
Control	56	99	24	40 cells

Duration of mitosis was measured directly by time-lapse video microscopy and mitotic duration represents time from nuclear envelope breakdown to nuclear envelope reformation.

TABLE II

Number of Centrosomes With an Associated Microtubule Aster in Taxol-Treated Cells

	Both centrosomes with an associated MT aster	One centrosome with an associated MT aster	Neither centrosome with an associated MT aster
DMSO Control	599	1	0
1 μ M Taxol	474	90	36
10 μ M Taxol	341	86	157
N5600 for each condition			