

A non-canonical mode of microtubule organization operates throughout pre-implantation development in mouse

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In dividing animal cells, the centrosome, comprising centrioles and surrounding pericentriolar-material (PCM), is the major interphase microtubule-organizing center (MTOC), arranging a polarized array of microtubules (MTs) that controls cellular architecture. The mouse embryo is a unique setting for investigating the role of centrosomes in MT organization, since the early embryo is acentrosomal, and centrosomes emerge *de novo* during early cleavages. Here we use embryos from a GFP::CETN2 transgenic mouse to observe the emergence of centrosomes and centrioles in embryos, and show that unfocused acentriolar centrosomes first form in morulae (~16–32-cell stage) and become focused at the blastocyst stage (~64–128 cells) concomitant with the emergence of centrioles. We then used high-resolution microscopy and dynamic tracking of MT growth events in live embryos to examine the impact of centrosome emergence upon interphase MT dynamics. We report that pre-implantation mouse embryos of all stages employ a non-canonical mode of MT organization that generates a complex array of randomly oriented MTs that are preferentially nucleated adjacent to nuclear and plasmalemmal membranes and cell-cell interfaces. Surprisingly, however, cells of the early embryo continue to employ this mode of interphase MT organization even after the emergence of centrosomes. Centrosomes are found at MT-sparse sites and have no detectable impact upon interphase MT dynamics. To our knowledge, the early embryo is unique among proliferating cells in adopting an acentrosomal mode of MT organization despite the presence of centrosomes, revealing that the transition to a canonical mode of interphase MT organization remains incomplete prior to implantation.

Introduction

The centrosome, consisting of a pair of perpendicular centrioles surrounded by a cloud of pericentriolar material (PCM), is the dominant microtubule organizing center (MTOC) in most animal cells. In interphase, the centrosome typically lies adjacent to the nucleus, organizing a polarized microtubule (MT) array with MT minus-ends proximal to the centrosome and plus-ends oriented toward the cell periphery. This MT network organizes the cytoplasmic architecture, controlling cell shape, polarity and motility.^{1–3}

Early development poses a challenge in terms of centrosome number. Inheritance of a complete centrosome from each gamete would cause a centrosome excess after fertilization, with potentially damaging consequences.^{4,5} To circumvent this, oocytes of many species degrade their centrioles and the fertilizing sperm contributes the centrioles for the embryo.^{6–8} For reasons that remain poorly understood,⁸ in mouse the sperm also lacks centrioles,^{8–10} and embryonic development begins without centrioles or centrosomes. Immunofluorescence studies revealed that centrosomes containing the MT-nucleator γ -tubulin are first evident in interphase at morula-stage (~16–32 cells).^{11,12} These centrosomes

are initially acentriolar, and classic electron micrographs first detect centrioles at the blastocyst stage (~64–128 cells).^{11,13,14} The absence of centrosomes and centrioles in early pre-implantation development suggests that the interphase MT network must be controlled in a centrosome-independent manner. How interphase MTs are organized in early embryos, and how embryos transition from acentrosomal to centrosomal MT organization is unknown.

Here we employ a GFP::CETN2-expressing transgenic mouse to observe the emergence of centrioles and centrosomes during early development and use high-resolution microscopy and tracking of MT growth events in live embryos to quantitatively assess the impact of centrosome emergence upon MT organization. Our experiments detail an acentrosomal mode of MT nucleation that functions in embryos and, unexpectedly, reveal that embryos continue to employ this pathway to govern MT layout in interphase despite the emergence of centrosomes in morulae and blastocysts.

Results

Centriole emergence occurs rapidly across both major cell lineages in late pre-implantation embryos. In mouse, centrioles are

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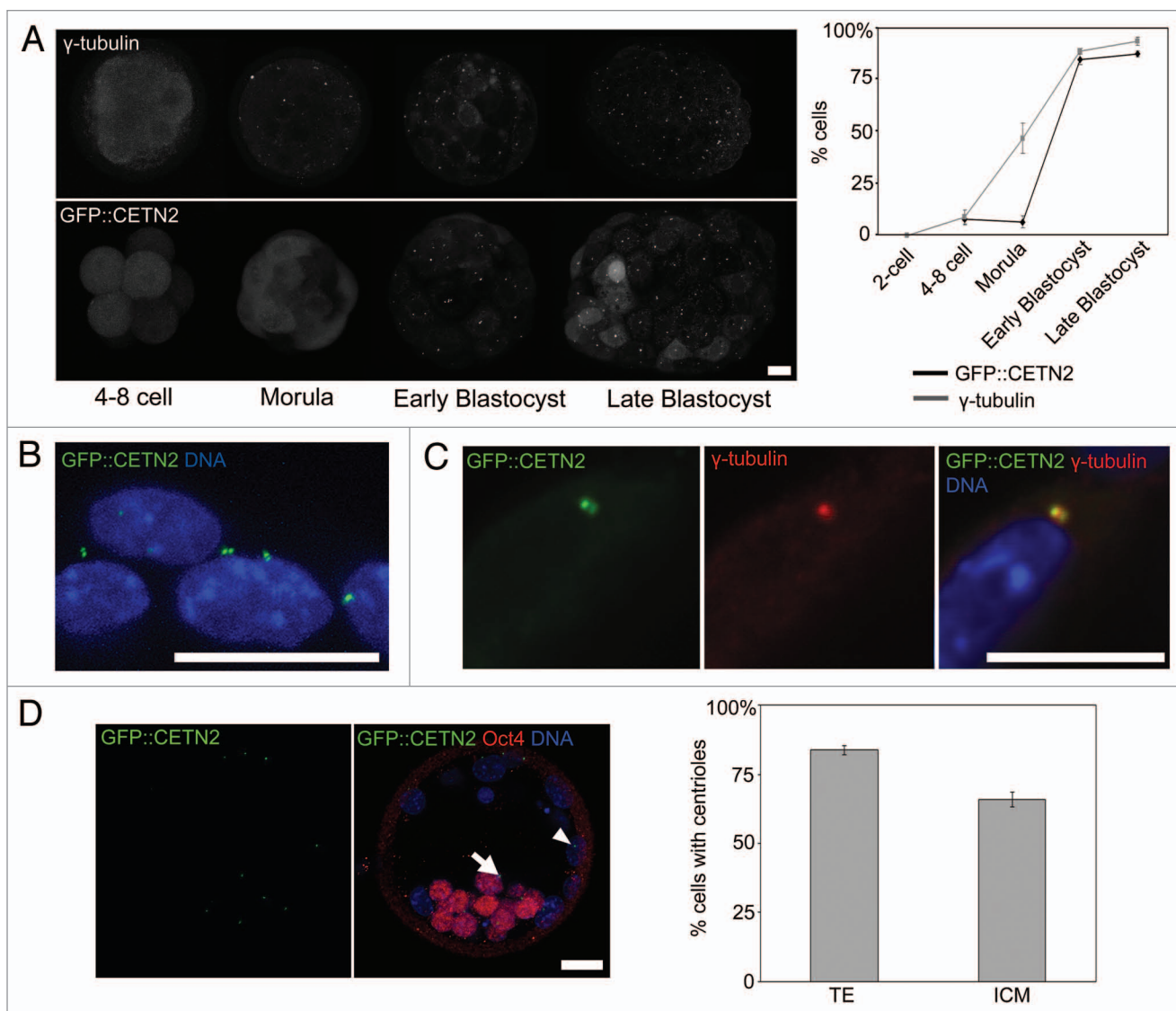


Figure 1. De novo emergence of centrosomes and centrioles in murine embryos. (A) Confocal Z-projections of GFP::CETN2-labeled and γ -tubulin immunolabeled embryos. Quantification of the proportion of nuclei with an associated GFP::CETN2 centriole pair (10–23 embryos/stage examined) or γ -tubulin focus (6–12/stage) is to the right. Note that the 2-cell embryo fluorescence pattern was identical to the 4-cells. (B) A zoomed image of a GFP::CETN2 blastocyst. Note pairs of GFP::CETN2-labeled centrioles associated with each nucleus. (C) Colocalization of γ -tubulin with GFP::CETN2-labeled centrioles. (D) Comparison of the proportion of Oct4-positive and Oct4-negative cells possessing centrioles in early cavitating blastocysts ($n = 18$). Note that although there is a greater proportion of centriole-containing cells in the trophoectoderm (arrowhead) compared with inner cell mass (arrow), the majority of cells possess centrioles in both lineages. Scale 20 μ m.

absent in sperm and oocytes and are first detectable by electron microscopy in blastocysts (~64–128 cells), revealing that centriole formation must occur de novo in late pre-implantation development.^{11,14} We first revisited this unusual scenario using a newly available transgenic mouse that constitutively expresses a GFP-tagged copy of the centriolar protein centrin-2 (GFP::CETN2). GFP::CETN2 mice display a pair of fluorescent centrioles in each somatic cell and have no distinguishable phenotype¹⁵ (and our unpublished data). GFP::CETN2 fluorescence was homogeneous in blastomeres of 4–8-cell stage embryos and in morulae (~16–32-cell stage, Fig. 1), confirming the absence of centrioles. In blastocysts, however, GFP::CETN2 was arranged into pairs of tightly focused spots associated with each nucleus in the majority

of cells (Fig. 1), similar to somatic cells. GFP::CETN2-labeled centrioles co-labeled with centrosome markers, including the key MT nucleation factor γ -tubulin (Fig. 1C; Fig. S1). Previous studies suggested that interphase centrosomes, identified as accumulations of centrosomal markers such as γ -tubulin, can be detected in morulae (16–32-cell stage) despite the absence of centrioles.^{11–14} Accordingly we found that prominent interphase γ -tubulin foci were frequently evident in morulae (Fig. 1A). Thus, as previously suggested,¹¹ centriole emergence occurs at the blastocyst stage of development, and is preceded by the formation of acentriolar centrosomes in ~16–32 cell morula stage embryos.

The morula-blastocyst transition involves the establishment of two distinct cell lineages—the inner cell mass (ICM)

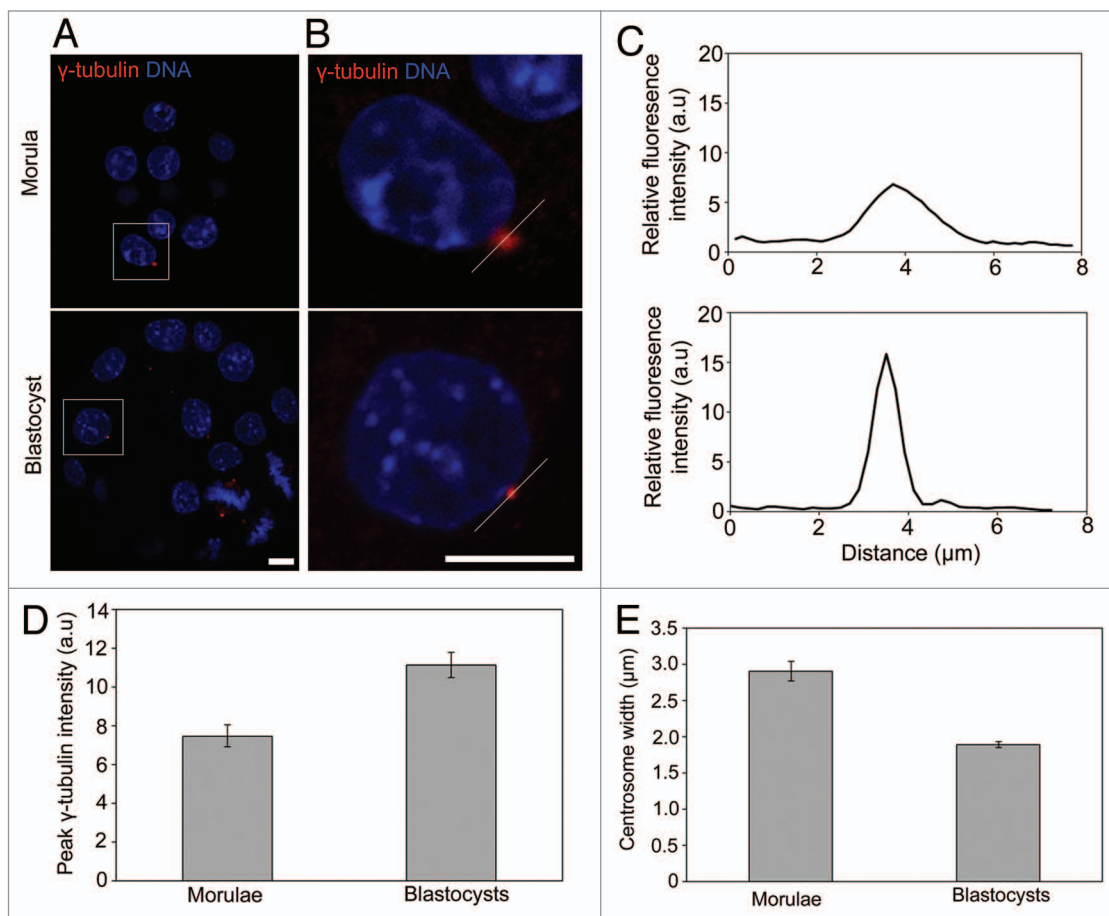


Figure 2. Centrosome focusing concomitant with centriole emergence. (A) Typical confocal sections of γ -tubulin foci in morulae and blastocysts. (B) Zoom of the area highlighted in (A). White lines indicate the linescan plotted in (C). (D) Quantification of peak γ -tubulin intensity and (E) centrosome width in morulae and blastocysts. 50–70 γ -tubulin foci examined from 9–10 embryos per stage. Scale 10 μm .

and trophoctoderm (TE). To determine whether centrioles appear de novo in one lineage before the other, we examined GFP::CETN2-labeled centrioles in blastocysts co-stained for the ICM marker Oct4. In order to differentiate the emergence of centrioles in the 2-cell lineages, we examined GFP::CETN2 mice from newly forming blastocysts. At this stage, there was a significantly greater proportion of centriole-containing cells in the TE compared with in the ICM ($p < 0.001$). Nonetheless the majority of cells in both lineages possessed centrioles at this stage (83.74 \pm 1.67% in the TE vs. 65.98 \pm 2.69% in the ICM; $p < 0.001$). Given that centrioles are almost never seen in morulae (16–32 cells), this indicates that centriole emergence at the morula-blastocyst transition is rapid and relatively synchronous across both cell types at the time of the morula-blastocyst transition (Fig. 1D). In summary, the early embryo lacks centrosomes, but acentriolar centrosomes are first detected in morulae (16–32 cells). Centrioles emerge 1–2 cell cycles later around the time of blastocyst formation (64–128 cells) within a relatively narrow window across both developing cell lineages.

Centrosome focusing at the morula-blastocyst transition. We next set out to examine the geometry of the γ -tubulin-containing centrosome in morulae and blastocysts. Given the key role of centrioles in centrosome organization,^{16,17} we wondered

whether centriole emergence in blastocysts might coincide with a change in centrosome shape. To address this, we performed a quantitative comparison of the geometry of γ -tubulin-labeled centrosomes in morulae and blastocysts (Fig. 2). Centrosomes were immunolabeled using γ -tubulin antibodies (Fig. 2A and B), and linescan fluorescence intensity analysis of confocal images used to quantify centrosome geometry (Fig. 2B and C). Normalized peak fluorescence was used as a measure of γ -tubulin density at the centrosome, and the width of the linescan curve was used to measure centrosome width (Fig. 2C). Peak fluorescence intensity was significantly greater ($p < 10^{-4}$, Fig. 2D) in blastocysts compared with morulae, and average centrosomal width was significantly smaller in blastocysts ($p < 10^{-12}$, Fig. 2E), suggesting a focusing of the centrosome at the morula-blastocyst transition. Thus, preliminary centrosomes possessing γ -tubulin first emerge in morula, but then become focused in blastocysts coincident with the emergence of centrioles.

Centrosome emergence does not affect MT organization. Having clarified the dynamics of centrosome and centriole emergence, we sought to explore the impact of centrosome emergence upon interphase MT organization. In typical dividing cells, the centrosome is juxta-nuclear and serves as a significant MTOC from which new MTs are nucleated.^{1,2,18,19} Accordingly, as is the

case in many cultured cells, we found in HeLa cells that the centrosome is at the center of a significant thickening of MTs that radiate outwards toward the cortex (see Fig. 3A). To examine MT organization in embryos, we generated high-quality immunofluorescence images of interphase MTs. In 4–8-cell stage embryos, we observed an intricate network of MTs extending throughout the cytoplasm that was enriched adjacent to the nuclear membrane and plasmalemma, as reported for isolated early blastomeres.^{20,21} Consistent with the absence of centrosomes, we found no evidence of a thickening of MTs that would indicate a dominant site of MT nucleation (Fig. 3B).

We next examined MTs in morulae and blastocysts. Notably, we were also unable to find a major thickening of MTs in these later embryos (Fig. 3B). This was unexpected, since these cells possess centrosomes. Examination of late blastocysts (day 6 post-hCG) expressing GFP::CETN2 or labeled with γ -tubulin antibodies revealed that centrioles and centrosomes were usually found at MT-sparse sites (Fig. 3C; Fig. S2A). Centrosomes were only occasionally found at sites of relative MT enrichment (< 15% of centrosomes; Fig. S2), and we never observed a radial array of MTs emanating from the centrosome. This suggested that centrosomes of the late pre-implantation embryo fail to serve as an interphase MTOC.

Examination of MT dynamics in interphase embryos. To better understand MT organization in embryos, we set out to perform more detailed analyses of interphase MT dynamics. For this we employed EB1::GFP, which labels growing MT plus-ends in live cells without affecting cellular function and so allows direct visualization of MT growth events and measurement of growth velocities and trajectories in live cells.^{18,19,22,23} EB1::GFP-expressing somatic cells are characterized by a major accumulation of EB1::GFP at the centrosome, with EB1::GFP comets radiating outwards from this central site toward the cell periphery^{18,19} (Fig. S3).

We expressed EB1::GFP in embryos by microinjection of mRNA at the 2-cell stage. Importantly, this approach had no effect upon embryo health as assessed by development to blastocyst *in vitro*. We first examined successive Z-sections of EB1::GFP-expressing embryos and found no evidence of a focal accumulation of EB1::GFP that might indicate a major MTOC at any stage, including morula and blastocysts which have centrosomes (Fig. S4). To obtain more detailed information, we generated rapid-acquisition (~2 sec intervals) movies of EB1::GFP-expressing embryos. At all developmental stages the movies revealed numerous comet-like structures moving in approximately linear trajectories, similar to those observed in other systems (Fig. 4A and B). To quantitatively assess the movement of the EB1::GFP comets, we used plusTipTracker software,^{24,25} which identifies EB1::GFP comets for automated assessment of location, velocity and directionality of MT growth events (Fig. 4A and B). We first analyzed EB1::GFP movie data sets made in 4–8-cell embryos. Consistent with our immunofluorescence observations of MT enrichment in the cortex and peri-nuclear region, EB1::GFP movies and automated analysis established that MT growth events are more frequent adjacent to nuclear and plasmalemmal membranes compared with the

remainder of the cytoplasm (Fig. 4C). Analysis of trajectories of MT growth events in the remainder of the cytoplasm revealed them to be randomly oriented (Fig. 4D). In contrast to somatic cells, but consistent with the absence of centrosomes in 4–8-cell embryos, there was no single subcellular location acting as a source of EB1::GFP comets.

We next examined the MT growth events in morulae and blastocysts. Similar to earlier developmental stages, growth events were frequent in the perinuclear region and the sub-plasmalemmal region (Fig. 4C). In addition, MT growth events were enriched at sites of cell-cell contact (Fig. 4B and C). Experimentally reversing embryo compaction by removing extracellular Ca^{2+} reduced the density of MT growth events at the plasmalemmal region, confirming that cell-cell interfaces act as major sites of nucleation (Fig. 4E), as has been seen in epithelial cells.^{26,27} Strikingly, however, there was no evidence of any single subcellular location serving as a major source of MT nucleation in morulae and blastocysts. Similar to earlier developmental stages, cytoplasmic comets had random trajectories (Fig. 4B and D). Although there was a slight decrease in MT growth velocity (Fig. S5), analysis of MT growth trajectories revealed no change in MT orientation between 4-cell and blastocyst stages (CHI^2 $p = 0.64$, Fig. 4D). The early embryo therefore employs a non-canonical acentrosomal mode of interphase MT organization consisting of apparently randomly oriented MTs enriched at nuclear and plasmalemmal surfaces and cell-cell boundaries. Unexpectedly, this persists as the major MT assembly pathway despite the presence of γ -tubulin-rich centrosomes at later developmental stages.

Discussion

Here we have examined MT dynamics in an unusual setting in which dividing cells transition from acentriolar to centriolar divisions within a relatively narrow developmental window. Our data detail an acentrosomal mechanism of interphase MT organization that operates throughout pre-implantation mouse development to generate a random non-radial cytoplasmic MT array enriched in the juxta-nuclear and plasmalemmal regions. Unexpectedly, however, centrosome formation and focusing has little or no effect upon the MT network, and embryo blastomeres continue to employ the acentrosomal MT assembly pathway despite the presence of centrosomes. The contrasting patterns of MT nucleation in the early embryo compared with the “text-book” dividing cell are illustrated by the cartoon in Figure 5. The discussion that follows will address the unusual scenario of *de novo* centrosome emergence in the mouse embryo, the mechanism of MT organization in embryos, and the physiological relevance of non-canonical MT dynamics in the early embryo.

De novo centriole and centrosome formation in mouse embryos. Centrioles and centrosomes are absent in oocytes of many species, and the absence of centrioles in mouse sperm has been known for some time, classic studies revealing that centrioles are first detectable by electron microscopy in 64–128-cell blastocysts.^{11,13} The availability of a transgenic mouse expressing GFP::CETN2 allowed us to revisit this question, examining the

presence of centrioles within each cell in relatively large numbers of embryos. We confirm that centriole emergence occurs in blastocysts, and show that this happens within a relatively narrow time-course across both major cell lineages and corresponds with a focusing of the previously acentriolar centrosomal material. The mechanism by which centrosomes and centrioles emerge in the embryo remains to be addressed. In somatic cells, a network of proteins has been identified that control centriole replication during mitotic S-phase.^{28,29} Whether the same network of proteins is involved in de novo assembly in mouse embryos remains to be seen. It is intriguing, however, that some members of this pathway including SAS6 and PLK4 can induce centriole-like structures when overexpressed in acentriolar *Drosophila* eggs,³⁰⁻³² indicating that the same pathway may operate in centriole replication and de novo formation.

An interesting feature of centrosome emergence in the mouse embryo is that centriole formation is preceded by the emergence of an acentriolar accumulation of PCM in morulae. It might seem counterintuitive that acentriolar centrosomes should precede the emergence of bona fide centrioles, since centrioles are commonly viewed as the platform upon which the centrosome is assembled, and experimental disruption of the centrioles causes disassembly of the centrosome.^{16,17} A clue as to the role of the acentriolar centrosome in morulae may come from experiments in which overexpression of a core PCM protein generates extra sites for the assembly of extra centrioles.³³ Similarly, the accumulation of PCM in morulae might be a prerequisite for the assembly of centrioles in blastocysts. The molecular underpinnings of centriole and centrosome emergence in the mouse embryo remain to be dissected.

Acentrosomal MT nucleation in mouse embryos. A focus of the current study was to use the mouse embryo to study how MTs are organized in dividing cells acquiring centrosomes and centrioles for the first time. We first detailed the mechanism of MT organization in acentriolar embryos, finding that MTs are randomly oriented and enriched adjacent to the nucleus and plasmalemma. We then examined MT dynamics in morulae and blastocysts, expecting the emergence of centrosomes

and centrioles to cause a shift toward a canonical centrosome-dictated polarized MT array commonly seen in other cell types. Unexpectedly, however, two independent approaches failed to uncover a substantial impact of centrosome emergence. First, immunofluorescence images showed no evidence of a substantial thickening of MTs in blastocysts that might be expected to

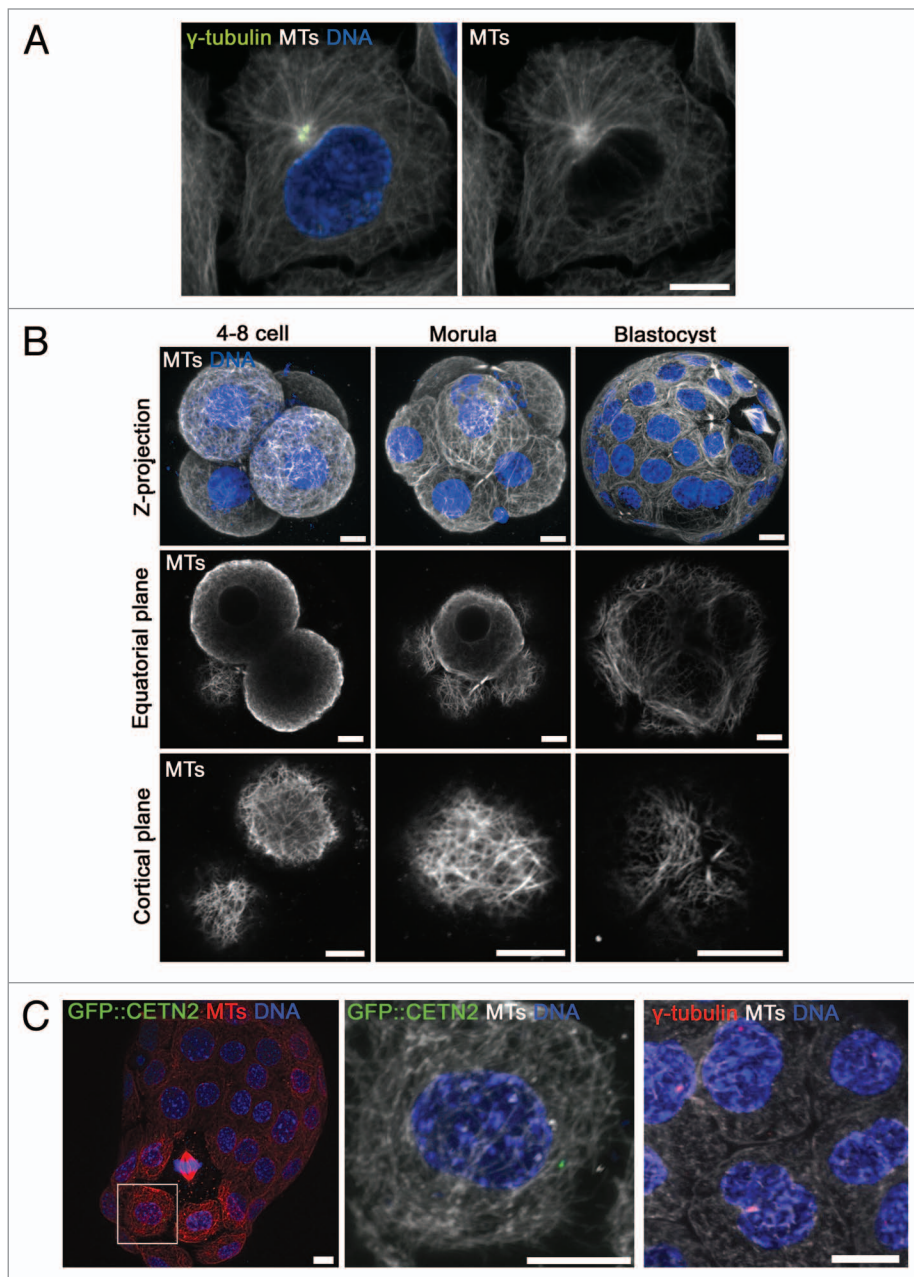


Figure 3. Centrosomes are not major MTOCs in interphase embryos. (A) An interphase HeLa cell immunolabeled for MTs and γ -tubulin. (B) MT immunofluorescence in embryos. For each stage, a Z-projection is shown along with the equatorial and cortical plane from the same embryo. Note the intricate network of MTs observed in the cell cortex. However, no single major focus of MT nucleation was observed at any developmental stage. (C) Zoomed images of MT immunofluorescence in late (hatching; 6 d post-hCG) blastocysts from GFP::CETN2 mice (left) or labeled with γ -tubulin antibodies (right). Note that centrioles and centrosomes are not at sites of MT enrichment in the majority of blastomeres. 433 centrosomes were examined from 29 blastocysts. See also Figure S2. Scale 10 μ m.

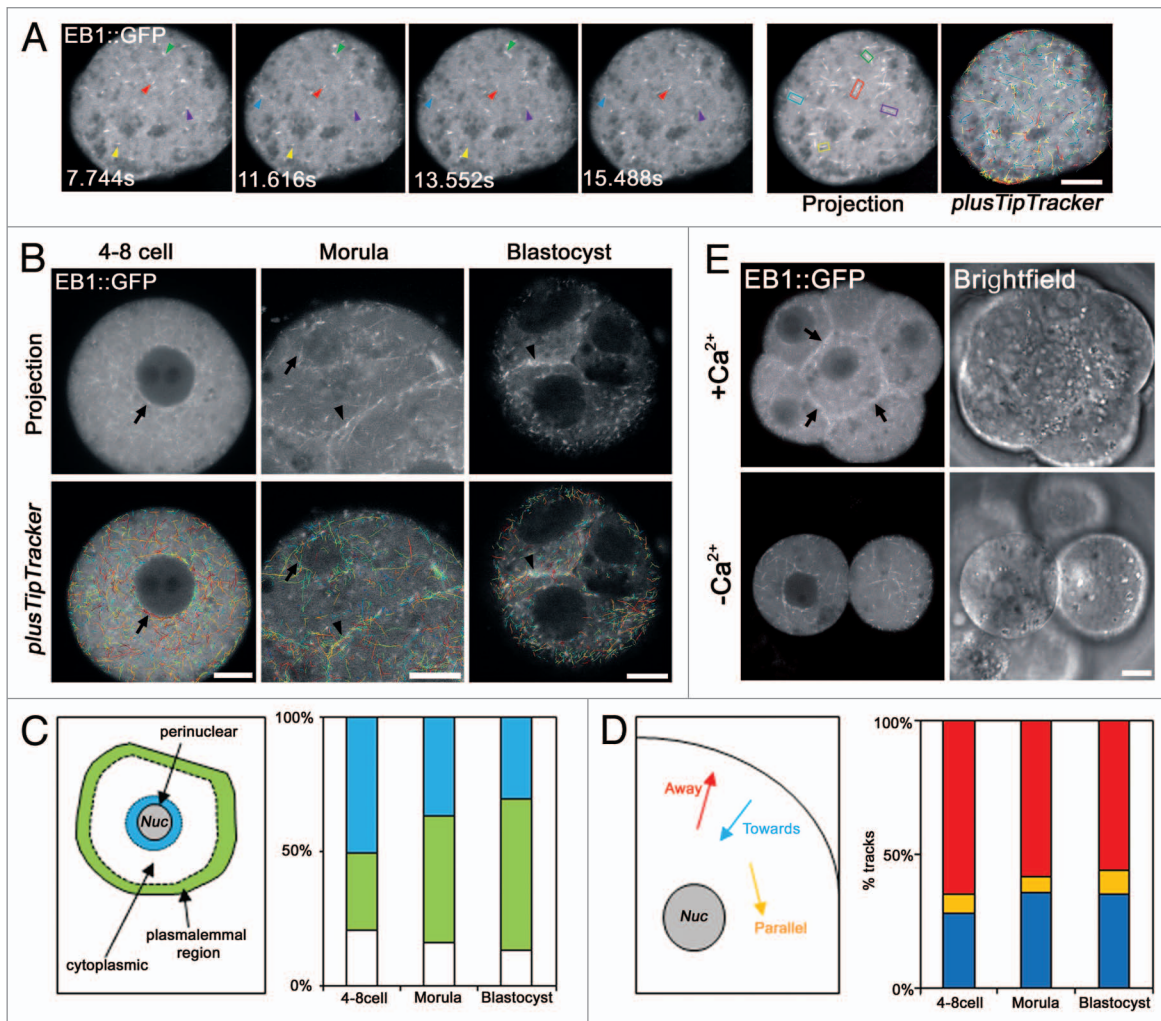


Figure 4. Dynamic tracking of MT growth events in early embryos. **(A)** Representative confocal time-series of EB1::GFP comets in a 4–8-cell stage blastomere. Each colored arrowhead tracks the movement of a single EB1::GFP comet. A maximum intensity projection of the entire time series is shown (right). Each MT growth event appears as a line of fluorescence. MT growth events identified by plusTipTracker software are shown far right. **(B)** Typical examples of MT growth events during development. **(C)** Analysis of relative density of MT growth events corrected for area in perinuclear, plasmalemmal and cytoplasmic regions. 1016–8309 tracks from 5–12 movies examined per developmental stage. **(D)** Analysis of cytoplasmic MT growth directionality in developing embryos. 49–125 tracks from 5–11 movies. Tracks were considered parallel to the nucleus within a $\sim 10^\circ$ range of the nuclear tangent. **(E)** Projections of morula-stage embryos experimentally de-compacted by removal of extracellular Ca^{2+} . Note loss of cell-cell adhesion reduces EB1::GFP track abundance at cell-cell interfaces (arrows; $n = 20$). Scale 10 μm .

accompany the centrosome, and the centrosome was typically found at MT-sparse sites. Second, detailed analyses of MTs using EB1::GFP uncovered no shift in growth trajectories during morula and blastocyst formation when the centrosomes are forming. The centrosome-independence of MT dynamics is apparently specific to interphase, as GFP::CETN-2 and γ -tubulin are located at spindle poles in mitosis in blastocysts (refs. 11, 12 and 34; see Fig. S6), suggesting that centrosomes influence M-phase MT dynamics. Nonetheless, centrosomes in morulae and blastocysts apparently fail to dictate MT layout in interphase, and the acentrosomal mode persists as the dominant means of organizing MTs.

The absence of centrosomes in oocytes and (in some species) embryos is well known, and the mechanism of spindle assembly during M-phase in these settings has been intensely studied.³⁵

However, less is known about how MTs can be organized in the absence of centrosomes in interphase. MTs can self-organize in experimentally generated acentrosomal cell fragments,^{36–39} or following laser ablation of centrosomes.⁴⁰ Acentrosomal MT organization occurs normally in some terminally differentiated cells, where formation of linear MT arrays can accompany cellular specialization.⁴¹ However, examples of acentrosomal MT organization in normal dividing cells are rare. One clear example is dividing *Drosophila* cells, in which centrosomes disassemble in interphase,⁴² such that PCM dissociates from the centrioles. In this setting the MTs organize into a non-radial acentrosomal MT array morphologically similar to that which we see in mouse embryos,⁴² and the birth of live flies following centriole disruption reveals the functional sufficiency of the acentrosomal interphase array.^{43,44} The scenario in the mouse embryo is mechanically

distinct from *Drosophila*, however, in that centrosomes do not disassemble in interphase mouse embryos, as indicated by the continued presence of γ -tubulin at the centrioles in interphase. What nucleates MTs in interphase mouse embryos, and why the cytoplasm elects to persist with this pathway despite the presence of γ -tubulin-rich centrosomes in later pre-implantation embryos remain to be unravelled. To our knowledge, cells of the pre-implantation mouse embryo are unique among non-differentiated cells in employing an acentriolar mode of MT assembly to organize the cytoplasm despite the presence of γ -tubulin-rich centrosomes.

Transition toward canonical microtubule dynamics in the early embryo. Understanding the mechanisms of MT dynamics is of particular interest in the early mammalian embryo, since the early embryo is extraordinarily susceptible to chromosome segregation errors, which may be a contributing factor to infertility.^{45,46} Recent studies revealed that mitotic spindles in blastocyst are morphologically similar to somatic cell spindles, suggesting that mitotic MT organization transitions from an oocyte-mode to a somatic-mode by the end of pre-implantation development.^{34,47} In contrast, our detailed analysis of interphase MT dynamics provides clear evidence of non-canonical MT behavior throughout pre-implantation development. Further detailed analyses of spindle MT dynamics in blastocysts may yet uncover idiosyncrasies of mitotic MT dynamics that might underpin the apparent lack of chromosome segregation quality control in these cells. Interestingly, aneuploidy is also common in cultured human embryonic stem cells,⁴⁸⁻⁵⁰ which originate from the inner cell mass. We speculate that the completion of the journey toward canonical MT organization might coincide with a tightening of chromosome segregation fidelity.

Materials and Methods

Oocyte and embryo handling. To obtain embryos, MF1 female mice were administered with PMSG (7IU; Intervet) and hCG (5IU; Intervet) at 48 h intervals and mated with male mice at the time of hCG administration. Two-cell embryos were collected 48 h later. GFP::CETN2-expressing embryos were obtained by mating MF1 females with hemizygous transgenic males¹⁵ purchased from Jax Mice. Embryo handling was performed in HEPES-KSOM,⁵¹ and long-term embryo culture was performed in KSOM media (Millipore). Microinjection was performed on a Leica DMI4000 microscope using Narishige micromanipulators, as previously described.⁵²

Fluorescent proteins and imaging. EB1::GFP¹⁹ was subcloned from pEGFP-N1 (Clontech) into pcDNA 3.1/myc-His(-) using *NheI* and *NotI* (NEB). Polyadenylated mRNA was manufactured using mMessage mMachine Ultra (Ambion), as previously.⁵² Oocyte and embryo processing for immunofluorescence was performed as previously.⁵³ Antibodies used: mouse anti- γ -tubulin (Sigma-T5326; 1:1,000), mouse anti- β -tubulin (Sigma-T4026; 1:1,000), rat anti- α -tubulin (YL1/2 abcam ab6160), with fluorescent secondary antibodies as appropriate (Invitrogen). Chromatin was labeled using 5 μ g/ml Hoechst 33343 for 5 min for fixed samples, and 5 sec for live samples.

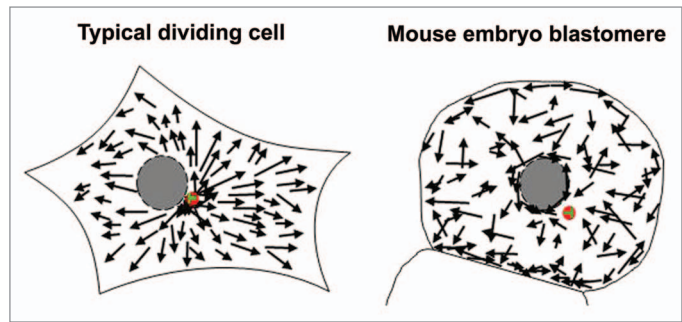


Figure 5. Comparison of MT nucleation in embryos and the typical dividing cell. Cartoon comparing MT nucleation in embryos and the typical dividing cell. In dividing cells the centrosome (red) nucleates MTs, which radiate toward the cell periphery. In embryo blastomeres, in contrast, the centrosome sits at MT sparse sites, with apparently random directionality of cytoplasmic MT growth events.

Imaging was performed on a Zeiss-710 confocal microscope. Imaging of live embryos was performed at 37°C.

Image analysis. Analysis of centrosome focusing was performed using the “plot profile” function in ImageJ and normalized for each image.

Confocal images of live EB1::GFP-expressing embryos were obtained at 1.936 sec intervals. EB1::GFP movies were analyzed using plusTipTracker software, which identifies and quantitates comet motion. We first used plusTipParamSweepGUI to identify optimal tracking parameters for analysis of MT growth events. We tested a range of values for the following parameters: search radius, maximum gap length, maximum forward angle and fluctuation radius. Variations of each of these had a minimal effect on the number, speed and lifetime of growth tracks identified. We therefore used the standard parameters described in Applegate et al. (2011),²⁴ which have previously been used to track MT growth events in mammalian cells, adjusted for pixel size and frame rate. Tracks that start in the first frame or finish in the last frame were automatically excluded from growth speed analysis. ROIs were defined using plusTipSeeTracks, which allowed identification of tracks in each of the three regions. Growth events were categorized as perinuclear or cortical if more than 50% of their lifetime was spent within 2 μ m of the nuclear/plasma membrane, respectively. Analysis of cytoplasmic MT growth directionality was performed manually on ~20 plusTipTracker-identified tracks per movie.

Disclosure of Potential Conflicts

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/cc/article/24755

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