Neuronal migration and process formation require cytoskeletal organization and remodeling. Recent studies suggest that centrosome translocation is involved in initial axon outgrowth, while the role of centrosomal positioning is not clear. Here, we examine relations between centrosomal positioning, axonogenesis, and microtubule (MT) polarization in multipolar and bipolar neocortical neurons. We monitored dynamic movements of centrosomes and MT plus ends in migratory neurons in embryonic mouse cerebral slices. In locomoting bipolar neurons, the centrosome oriented toward the pia-directed leading process. Bipolar neurons displayed dense MT plus end dynamics in leading processes, while trailing processes showed clear bidirectional MTs. In migrating multipolar neurons, new processes emerged irrespective of centrosome localization, followed by centrosome reorientations toward the dominant process. Anterograde movements of MT plus ends occurred in growing processes and retrograde movements were observed after retraction of the distal tip. In multipolar neurons, axon formed by tangential extension of a dominant process and the centrosome oriented toward the growing axon, while in locomoting neurons, an axon formed opposite to the direction of migration and the centrosome localized to the base of the leading process. Our data suggest that MT organization may alter centrosomal localization and that centrosomal positioning does not necessarily direct process formation.

Keywords: axon, centrosome, microtubules, migration, polarization

Introduction

Microtubule (MT) organization is essential to neuronal migration and process formation (Tsai and Gleeson 2005; Reiner and Sapir 2009; Marín et al. 2010; Kuipers and Hoogenraad 2011; Stiess and Bradke 2011). Migrating cells typically are polarized in the direction of migration and exhibit asymmetrically organized MTs (Ridley et al. 2003). Capturing MT plus ends at the leading edge is thought to localize the centrosome in front of the nucleus (Kirschner and Mitchison 1986; Gundersen 2002; Vallee and Stelman 2005; Watanabe et al. 2005). The relative positioning of the leading centrosome and the trailing nucleus plays a key role in dynin-dependent nucleokinesis (Tsai and Gleeson 2005; Vallee et al. 2009; Marín et al. 2010).

Neuronal process formation, including axonogenesis and dendritogenesis, relies on MTs (Solomon 1980). Studies in cultured neurons suggest that the position of centrosome is a determinant of axon specification (de Anda et al. 2005). Although in vitro analysis of dissociated cells has provided valuable information, it is important to study centrosome positioning in situ for proper understanding of MT function in different types of axonogenesis (de Anda et al. 2010; Distel et al. 2010). The complexity of cytoplasmic MT organization in neurons has also precluded a complete understanding of how MTs function in axon and dendrite formation (Baas et al. 1989; Kuipers and Hoogenraad 2011).

Cells exhibit multiple types of migration and process formation during cortical development. Young excitatory neurons born in the ventricular zone migrate toward the brain surface through the intermediate zone (IZ) and cortical plate (CP) (Fig. 1; Nadarajah et al. 2003; Marín et al. 2010). Migrating neurons in the IZ exhibit multiple processes (Stensaas 1967; Shoukimas and Hinds 1978) and frequently alter their direction during migration (multipolar [MP] migration; Tabata and Nakajima 2003; Noctor et al. 2004). Radially migrating bipolar (BP) neurons in the CP display a pia-directed thick leading process and a thin trailing process (locomotion; Rakic 1972; Nadarajah et al. 2001), which is thought to form the future axon (Schwartz et al. 1991; Hatanaka and Murakami 2002; de Anda et al. 2008; Hatanaka and Yamauchi 2013).

A previous study suggested that centrosome positioning plays an instructive role in the specifying where an axon will form in embryonic mouse neocortical neurons (de Anda et al. 2010). However, a recent study of neurons in zebradish telecephalic hindbrain nuclei showed that axonogenesis could be initiated in a direction opposite to that of centrosome location (Distel et al. 2010). Others also report that once a nascent axon has formed, further extension of that axon can continue without a functional centrosome (Stiess et al. 2010). Thus, further experiments are needed to determine how centrosome positioning governs axonogenesis.

In this study, we monitored movement of centrosomes in migrating neocortical neurons by using a slice culture of embryonic mouse cerebral. We first found that processes of MP neurons exhibit variable protrusive activity. Centrosome moves toward the most dominant growing process and the dominant process can be defined prior to the reorientation of the centrosome to its base. In MP neurons, axons appeared to form by extension of a dominant process with the centrosome oriented toward the growing axons. By contrast, in locomoting bipolar neurons, axons formed opposite the leading process where the centrosome was located. These observations suggest that centrosome positioning in neocortical neurons may reflect protrusive activities of multiple processes rather than determine the site of new process formation.

Materials and Methods

Plasmids

The neuron-specific α-tubulin promoter (Tα), its high specificity enables us to prevent undesired expression in neural progenitors, was used in all expression plasmids constructed in this study (Gloster
with 95% O2/5% CO2 gas by bubbling just before use. Slices were from electroporated cerebral hemispheres for imaging analysis. Slice Culture and Live Imaging

50 ms at 450-ms intervals. All animal experiments were performed in 200 T1302 Microtubule Dynamics in Polarizing Neocortical Neurons In Situ

Locomoting neurons in the CP are known to have an axon-like trailing process to change their shape from MP to BP when they enter the CP (MP neurons exhibit unidirectional migration toward the pia (locomotion) and are thought of neuronal migration, axonogenesis, and dendritogenesis in the developing mouse neocortex. Young pyramidal neurons born in the ventricular zone (VZ)—the deepest embryonic cortical layer facing the lateral ventricle—migrate radially toward the brain surface through the intermediate zone (IZ) and cortical plate (CP). Neurons migrating in the IZ have a multipolar shape (MP) and frequently change the direction of migration (multipolar migration). Neurons in the CP have a bipolar shape (BP) with a major leading process oriented toward the pia and a thin trailing process. These neurons exhibit unidirectional migration toward the pia (locomotion) and are thought to change their shape from MP to BP when they enter the CP (MP-BP transition). Locomoting neurons in the CP are known to have an axon-like trailing process (axonogenesis).

et al. 1999). This reduces background fluorescence due to overlapping basol processes of electroporated neuronal progenitors and facilitates to analyze neuronal morphology during in situ imaging. To achieve "sporadic" low-density cell imaging with enough high expression level of fluorescent proteins, we used a Cre-loxP system (Matsuda and Cepko 2007; Morin et al. 2007; Konno et al. 2008). The pTα-LPL (loxP-polyA-loxP) expression plasmid was designed with an insertion containing a polyanadenylation signal flanked by 2 loxP sites between the promoter and open reading frame. pTα-Cre was used for neuron-restricted excision of the floxed polyadenylation signal in the pTα-LPL plasmids. The following plasmids were used for electroporation experiments: pTα-LPL-LynN-EGFP (MGC1KSKRDK-EGFP; Lyn N-terminal membrane-targeted signal-fused EGFP) to assess neuron morphology; pTα-LPL-PACT-mKO1 for live monitoring of the centro-some (Konno et al. 2008); pTα-LPL-EB3-EGFP to analyze the motility of MT plus ends (Stepanova et al. 2003).

In Utero Electroporation

To introduce expression plasmids into cortical excitatory neurons, in utero electroporation was performed on E12.5 or E13.0 embryos in timed pregnant ICR mice as described previously (Saito and Nakatsuji 2001; Tabata and Nakajima 2001). For sporadic expression from Tcα-LPL plasmids, ~1 μL of plasmid mixture containing 0.0005 μg/μL Tc-Cre and 0.3–0.5 μg/μL Tc-LPL plasmids was injected into the lateral ventricle, and embryos were pulsed 5 times with 32 V for 50 ms at 450-ms intervals. All animal experiments were performed in compliance with relevant laws and regulations for animal experiments at Nagoya University.

Slice Culture and Live Imaging

Two or 3 days after electroporation, coronal slices were prepared from electroporated cerebral hemispheres for imaging analysis. Slice cultures were carried out as described (Miyata et al. 2001). Briefly, 200–300-μm-thick coronal slices were prepared with a V-Lance microsurgery knife (Alcon) in ice-cold DMEM/F-12 (1:1) medium saturated with 95% O2/5% CO2 gas by bubbling just before use. Slices were mounted in a glass bottom 35-mm culture dish with 300 μL of collagen gel (mixture of 150 μL of DMEM/F-12 and 150 μL of 2x collagen gel solution 60% 3 mg/mL Atelocollagen (Koken), 20% 5x DMEM/F-12, 10% water, and 10% neutralization buffer composed of 2.2% sodium bicarbonate and 200 mM HEPES/NaOH, pH 7.4). The dish was kept at room temperature for 10 min and then incubated at 37 °C for 10 min to solidify the gel. Samples were then cultured at 37 °C in 40% O2/5% CO2/55% N2 gas, after adding 700 μL of phenol red-free DMEM/F12 medium supplemented with 25 μg/mL insulin, 100 μg/mL transferrin, 20 nM progesterone, 30 nM sodium selenate, 60 μM putrescine, 10 ng/mL epidermal growth factor, 10 ng/mL basic fibroblast growth factor, 5% horse serum, and 5% fetal bovine serum. For imaging, slices in a glass bottom dish were kept at 37 °C in an ONICS humidified incubator chamber (Tokai Hit) on the stage of an Olympus IX-81 inverted microscope, with a continuous supply of 45% O2/5% CO2/50% N2 gas. Cross-sectional images through the glass bottom were acquired with a ×40/0.75 UPLFLN objective lens (Olympus) using a FY-1000 confocal laser scanning microscope system (Olympus).

Three-dimensional time-lapse images (image size: 512 × 512; up to 15 Z-stacks at 2.5–3.0-μm intervals) were collected every 20 min for up to 38 h. Images were analyzed using Volocity Visualization software (Improvision). To analyze MT plus end motility, time-lapse images were acquired using a CV-1000 spinning disc confocal scanner system (Yokogawa) and a x60/1.35 UPLSAPO objective lens (Olympus). Three-dimensional time-lapse images (image size: 512 × 512; up to 11 Z-stacks at 1.4–1.5-μm intervals) were collected every 3 s for 5 min. As EB3-EGFP puncta moved rapidly, a cycle of Z-stacked image acquisitions was completed within 3 s to analyze puncta trajectory in kymographs. To avoid photobleaching, we acquired sequential images every 3 s for 5 min with 25-min intermissions. Kymographs were created using Metamorph software (Molecular Devices). The mean speed values of EB3-EGFP movement are provided with standard deviation.

Results

Centrosomes Move to the Base of Dominant Growing Processes in MP Neurons

Centrosomes in MP neurons are not consistently positioned relative to the nucleus (Shoukimas and Hinds 1978) nor is it known how the position of centrosome changes in these cells. To assess the role of MT organization in neuronal migration and process formation in situ, we first focused on centrosome dynamics in migrating MP neurons, which form and retract multiple processes in the IZ. To monitor centrosome position in MP neurons in the embryonic mouse cerebral slice, centrosome was labeled with a centrosome-targeted orange fluorescent protein, PACT-mKO1. The morphology of neurons and their processes were simultaneously visualized using the plasma membrane marker LynN-EGFP. A time-lapse movie shows a representative neuron with multiple processes in the IZ (Fig. 2A, see Supplementary Movie 1). Although it exhibits multiple processes, the protrusive activity of processes varies. Some processes extended and others retracted. In most cases, a “dominant” process can be defined by its most consistent protrusive activity; it was not necessarily the largest process. The dominant process at each time point (magenta arrows) was determined by observing the growth rate in a time-lapse sequence of images. For the first 6 h of the migration, the cell migrated toward the right and the centrosome was located at the base of the rightward growing process. Before the direction of migration switched from right to left, a new process formed to the left (8–12 h). When this new process began to form (8 h), the centrosome was still located on the right of the nucleus (Fig. 2C, orange cell). During the same period, the right-side process began to retract while the centrosome remained localized at its base.
Centrosomes of MP Neurons Localize to the Base of Axons Formed by Tangential Extension of Dominant Processes

We next analyzed cell behavior when migrating neurons changed their morphology from multipolar to bipolar (the MP–BP transition) at the border between the IZ and CP. We analyzed 15 time-lapse movies covering the entire MP–BP transition and carefully analyze the manner of morphological change and axonogenesis in these neurons.

In the first category, an axon formed by tangential extension of the dominant process in MP neurons prior to the MP–BP transition (MP-stage axonogenesis; 6 of 15 cases). Figure 3A (see Supplementary Movie 2) shows a representative MP cell. This cell formed active protrusions toward the left at the beginning of observation (0 h). Then, a leftward dominant process extended tangentially and formed a thin axon-like fiber (1–4 h, magenta arrow). The centrosome located left of the nucleus at the base of tangentially growing axon (1–4 h). A new dominant process then formed toward the pia (8 h; aqua arrow), while maintaining the leftward thin axon. During the formation of the pia-directed new dominant process, the centrosome moved to the base of that process (8 h). The pia-directed process then became the leading process and nucleus translocated into it (14–20 h). Finally, the cell shows typical BP shape and the tangentially extended axon-like fiber becomes an L-shaped trailing process (20 h).

Figure 3B (see Supplementary Movie 3) shows another representative cell with multiple processes at 0–1 h. First one process extended tangentially to the right (2–6 h; magenta arrow), followed by formation of a pia-directed dominant process (8 h; aqua arrow) into which the nucleus moves (12–16 h). The centrosome was located to the right of the nucleus, while the rightward process extends (2–6 h) and then moved to the pia-side of nucleus during the formation of the pia-directed leading process (8–16 h).

In all 6 cases of MP-stage axonogenesis observed in 15 MP–BP transitions, we found that an axon formed by extension of dominant process and that the centrosome localized at the base of that axon (Supplementary Fig. S2). In these cases, we observed both dorsomedial (n = 2) and ventrolateral (n = 4) axonal extension.

Centrosomes Locate at the Base of Leading Process Opposite to the Initiating Axon During BP-Stage Axonogenesis

In 7 cases of MP–BP transitions we observed, cells entered the CP and began locomotion in prior to axonogenesis.
(BP-stage axonogenesis; 7 of 15 cases), as shown in Figure 4A (see Supplementary Movie 4). During the first 16 h, this representative neuron exhibits multiple processes and the centrosome remained on the pial side of the nucleus as the neuron grew and retracted processes. Then the cell formed a thick leading process toward the pia and migrated into the CP, assuming a bipolar shape and short tail (16–28 h). During locomotion, the centrosome basically localized at the base of the
Figure 4. The centrosome localizes to the base of the leading process when an axon forms opposite to the leading process in BP neurons. (A) The centrosome marker PACT-mKO1 (centrosome; red) was electroporated along with the plasma membrane marker LynN-EGFP (plasma membrane; green) into VZ progenitors in utero at E12.5 and the cerebral hemisphere sliced at E14.5. Fluorescent images were captured every 20 min for 38 h as an MP neuron made the transition from MP migration to locomotion in the upper IZ and subsequent backward axonogenesis in the CP (Supplementary Movie 4). Images obtained every 2 h are shown (top panels; note that processes from a neighboring neuron are visible in upper right of frames from 26 to 38 h). In Supplementary Figure S1, a broad view image of the last frame shows axon trajectory. Cell somata are shown at higher magnification in the bottom panels. Dorsomedial is left, ventrolateral is right, pia is toward the top, and the ventricle is toward the bottom. Also shown are levels of the pial surface, CP, and IZ. This MP neuron forms and retracts processes in the IZ (0–16 h). The centrosome position is indicated by white arrowheads. Prior to entry into the CP, the cell shape changed from MP to BP with a major leading process (aqua arrows) and a short tail (18–28 h). After the MP–BP transition, backward extension of a long axon fiber (yellow arrows) was initiated from the short tail (30 h). At the onset of axonogenesis, the centrosome remained in front of the nucleus and was not localized to the site of axonogenesis (28–30 h). The elongated axon, with collateral processes, persisted until the end of the observation period (38 h). Scale bar, 10 μm. (B) Time-lapse images of another representative MP–BP transition are shown. Fluorescent images were captured every 20 min for 12 h (Supplementary Movie 5). Critical images were chosen from the movie and are shown as a montage (top panels). A broad view image of the last frame is provided in Supplementary Figure S1. Higher magnification images of cell somata are shown in bottom panels. Dorsomedial is right, ventrolateral is left, pia is toward the top, and the ventricle is toward the bottom. The level of the pial surface, CP, and IZ is also shown. At the beginning of the movie, a neuron at the IZ/CP border forms multiple processes (0–2 h). Then the pia-directed leading process (aqua arrows) grows and nucleokinesis occurs in that process (3–5 h). Then a thin process (yellow arrows) opposing the leading process extends and forms an axon (6 h). Finally, the cell shows a typical bipolar shape with a leading process and an axon (7–12 h). During initial formation (1 h) and extension (6 h) of the axon, the centrosome localizes to the base of the pia-directed process opposite the axon (1–6 h). Scale bar, 10 μm.
pia-directed leading process, ahead of the nucleus. However, by 18–26 h, the translocating nucleus transiently overtook the centrosome in migrating neurons, similar to activities reported in cerebellar granule neurons (Unemishina et al. 2007). After a few cycles of pia-directed nucleokinesis, the locomoting neuron began a backward axonogenesis (30 h). Prior to forming the process, the centrosome remained in front of the nucleus and did not move to the rear, suggesting that close proximity of the centrosome to the process is not essential for axon formation. Once the axon formed, however, the centrosome slightly moved toward the rear of the cell (30–36 h). The centrosome later relocated to the pial-side of the nucleus (38 h).

Another representative case of BP-stage axonogenesis is shown in Figure 4B (see Supplementary Movie 5). Among active protrusions of this cell, a pia-directed process extended into the CP and became the leading process (1–4 h, aqua arrows). During that period, a thin process was formed and retracted toward the IZ (yellow arrows). After translocation of the nucleus into the leading process (4–5 h), cells showed bipolar morphology with a thin trailing process, which then grew an axon over a 6 h period (6–12 h). The centrosome remained opposite to the trailing process during initiation of growth of a short tail (1 h) and extension of a long axon (6 h).

We also observed 2 cases of unusual axonogenesis (Supplementary Fig. S3). In these cells, formation of the leading process and axon occurred within a relatively short period. An axon extended opposite to the direction of leading process as in BP-stage axonogenesis, but the axon initiation site was near the base of leading process on the same side of the nucleus. At axon initiation, the centrosome was located between the leading process and axon (Supplementary Fig. S3A, 2–3 h; Supplementary Fig. S3B, 10 h) and then moved toward the leading process (Supplementary Fig. S3A, 6–9 h; Supplementary Fig. S3B, 12–16 h).

**At the MP–BP Transition Distal Movements of MT Plus Ends are Enriched in the Leading Process**

To analyze movements of growing MT plus ends in neocortical neurons, we employed the MT plus end-binding protein EB3-EGFP to observe them in the MP–BP transition over 6 h (Fig. 5A, see Supplementary Movies 6–11). Intracellular distribution of EB3-EGFP as represented by a fluorescence intensity profile is shown in different colors in Figure 5B. Initially, the cell showed a typical MP shape (0 h). Anterograde movements of MT plus ends occurred regularly in processes (Fig. 5C–F). The average speed of anterograde movements in nonaxonal processes was 0.26 ± 0.042 μm/s (n = 107), while that speed tended to decrease near the distal tip of processes (Fig. 5C, green arrowheads). Retrograde movements (0.27 ± 0.039 μm/s, n = 40) were irregularly observed after retraction of process tips (Fig. 5C, orange arrowheads). Speeds of anterograde and retrograde movements in axons were 0.25 ± 0.033 μm/s (n = 33) and 0.28 ± 0.049 μm/s (n = 14), respectively.

Once the cell began the MP–BP transition (2–3 h), a pia-directed process grew thicker (Fig. 5A, 2 h, aqua arrow) and became the leading process, as other thin processes retracted and the cell assumed BP morphology (5 h). During this period, the intensity of EB3-EGFP fluorescence detected in the pia-directed thick process increased (Fig. 5B, 2–3 h).

Although the orientation of MTs in the thick leading process was not clear, this raises a possibility that the targeting of the centrosome toward the leading process (Figs. 3 and 4) may be influenced by the leading process-enriched cytoplasmic MTs.

**Bidirectional Movements of MT Plus Ends in the Axons of BP Neurons**

We also analyzed the direction of MT plus end movements in BP-shaped neurons of the CP (Fig. 6, Supplementary Movie 12). A greater number of EB3-EGFP puncta were detected in pia-directed leading processes (n1, n3, n4) than in trailing axons (n2, n5). Bidirectional movements of EB3-EGFP puncta were clearly detected in the axons. Average speeds of axonal anterograde (0.29 ± 0.036 μm/s, n = 35) and retrograde (0.30 ± 0.053 μm/s, n = 21) movements of MT plus ends were similar.

**Discussion**

In the present study, we examined centrosome positioning in neocortical excitatory neurons during migration and axon formation. We observed that the centrosome moves toward the dominant growing process in MP neurons (Fig. 7, bottom). During the MP–BP transition of neocortical neurons, we observed 2 distinct modes of axonogenesis. When an axon was formed by tangential extension of the dominant process in MP neurons, the centrosome was oriented toward the initiating axon (Fig. 7, MP-stage axonogenesis). However, our data suggests that centrosome proximity to the axon-emerging site is not needed for axonogenesis opposite to the leading process in BP-shaped neurons (Fig. 7, BP-stage axonogenesis). Our analysis of MT plus end motility suggests that the relative number of growing MTs in processes may alter centrosome positioning in these cells.

We observed that the location of centrosome in MP neurons in the IZ occasionally changes, and that centrosomes move to the base of an active growing process (Fig. 2). Moreover, formation of a new dominant process precedes the centrosome reorientation. Thus, new process formation likely does not require a nearby centrosome. These observations also suggest that centrosome positioning can be affected by relative growth activities among processes; most dominant growing process attracts the centrosome (Fig. 7). Of note, we often observed formation and retraction of multiple processes without marked changes in centrosome position (Fig. 4A, 0–14 h; 4B, 0–3 h). Since changes in centrosome localization follow formation of new dominant process with some delay, rapid changes in dominant process location may not always be reflected in subsequent centrosome positioning.

Previous studies reported that MT plus ends are aligned toward the tip of the leading process in cortical neurons and cerebellar granule cells (Rakic et al. 1996; Tsai et al. 2007). These observations are consistent with our findings that distal tip-directed movements of MT plus ends are enriched in the dominant process of MP neurons and the leading process of BP neurons (Figs. 5 and 6). Although the mechanism by which the centrosome moves toward the dominant/leading process remains unclear, it has been suggested that a MT/plasma membrane interaction requiring the dynein system generates pulling force needed for centrosome movement (Kirschner and Mitchison 1986; Paschal and Vallee 1987).
Figure 5. MP neurons processes exhibit distal tip-directed movements of MT plus ends, which are enriched in the leading process at the MP–BP transition. (A) EB3-EGFP, a marker of MT plus ends, was introduced into VZ progenitors at E12.5 by in utero electroporation, and the cerebral hemisphere was sliced at E14.5. Fluorescent images of a MP neuron in the upper IZ were captured every 3 s for 5 min (Supplementary Movies 6–11). Movies of 5-min duration were made every 30 min with 25-min intermissions. Beginning images of the movies are shown as a montage (0–6 h). In this montage, dorsomedial is right, ventrolateral is left, pia is toward the top, and the ventricle is toward the bottom. At the MP–BP transition, the density of EB3-EGFP puncta markedly increases in a pia-directed process (aqua arrow, 2 h). The thickness of the pia-directed process increases, and the MP cell shape gradually changes to BP (2–3 h). Scale bar, 10 μm. (B) Intensity profiles of fluorescent images in A are shown with different colors. A marked increase in EB3-EGFP fluorescence in the nascent leading process (white arrowhead) is observed at the onset of the MP–BP transition (2:00). (C–F) The maximum EB3-EGFP fluorescence signal along the proximodistal axis of processes was analyzed every 3 s for 5 min (100 time points) by making kymographs from movies shown in (A) during the indicated time periods (C: 0:00–0:05 Supplementary Movie 6, D: 1:00–1:05 Supplementary Movie 7, E: 2:00–2:05 Supplementary Movie 8, F: 2:30–2:35 Supplementary Movie 9). Positional changes along the axon (#1: yellow lines) and other processes (#2–#7: aqua lines) shown in the left panel were plotted in the kymographs (right panels). In the kymographs, proximal is toward the bottom and distal is toward the top. Anterograde movements of EB3-EGFP puncta toward the distal tip of processes were observed in growing processes. The average speed of anterogradely moving puncta in the proximal segment of nonaxonal processes was 0.26 ± 0.042 μm/s (n = 107), while the speed gradually decreased as puncta neared the tip of processes (green arrowheads). Retrogradely moving EB3-EGFP puncta (orange asterisks) were irregularly observed after process tips retraction (orange arrowheads). The average speed of retrograde movements in nonaxonal processes was 0.27 ± 0.039 μm/s (n = 40). Average speeds of EB3-EGFP movements in axons were 0.25 ± 0.033 μm/s (n = 33) for anterograde and 0.28 ± 0.049 μm/s (n = 14) for retrograde. Scale bars, 10 μm.
Thus, a large number of cytoplasmic MTs polarized toward the tip of growing process may influence MT capture in the growing tip and target the centrosome to the dominant/leading process.

Support for the hypothesis that the centrosome targets the dominant growing process, which contains enriched cytoplasmic MTs, explains how centrosome positioning occurs during different modes of axonogenesis. During MP-stage...
axonogenesis (Fig. 3), the centrosome orients toward the initiating axon, most likely because that axon is the dominant process. This type of axonogenesis resembles hippocampal polarization in which one selected process is transformed into an axon (Dotti et al. 1988) and the centrosome reportedly orients toward the growing axon (de Anda et al. 2005). In contrast, during BP-stage axonogenesis, the centrosome was not localized at the base of an initiating axon (Fig. 4). Typically, BP cells exhibited 2 major growing processes: a thick leading process in which the cytoplasmic MTs are enriched and a thin trailing axon (Fig. 6). Thus, leading processes contain a greater number of MTs than do axons. Taken together, it is tempting to speculate that abundant MT organization in the thick leading process may have a more dominant effect on centrosome positioning than compactly aligned MTs in the initiating axon during the BP-stage axonogenesis.

A recent article reported that centrosome position determines the axon initiation site as axonogenesis begins (de Anda et al. 2010). However, our data indicate that close proximity to the centrosome is not needed for the BP-stage axonogenesis during which an axon forms opposite to the leading process, whereas the centrosome orients toward the growing axon during the MP-stage axonogenesis (Fig. 7). These different contexts can be explained by our simple hypothesis that the centrosome targets dominant growing process in which MT plus ends are enriched. Thus, the centrosome orients toward the initiating axon when the axon is the dominant process, however, centrosome does not localize at the base of initiating axon when the dominant process is other than the axon.

In cultured hippocampal neurons, MT plus ends are oriented distal to the cell soma in short nascent processes and extending axons, whereas MTs are nonuniformly aligned in mature dendrites (Baas et al. 1988, 1989). In our observation of MT polarity in neocortical neurons in slice culture, clear bidirectional movements of MT plus ends were detected in axons (Fig. 6). Interestingly, we also observed that retraction of the process tip might initiate retrograde movements (Fig. 5). Thus, the observation of bidirectional alignment of MTs in an axon suggests that occasional retractions can occur at the distal end of a growing axon. It is also possible that active remodeling of collaterals (see Figs. 3B and 4A) increases retrograde movement of MT plus ends. In contrast, during polarization of hippocampal neurons, only one axon consistently extended while other dendritic processes maintained a short length (Dotti et al. 1988). Taken together, based on differences in MT polarity, we conclude that axonal growth in neocortical neurons of the developing brain is likely more complicated than that seen in hippocampal neurons cultured in vitro.

In summary, our findings that the centrosome moves toward the dominant process in migrating neurons and that the initiating axon is not necessarily the dominant process enables understanding of MT function in different modes of axonogenesis. Further observations in various models of axonogenesis should reveal the role of cytoplasmic MTs in neuronal polarization in situ.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding
KAKENHI 21800906, 23113507, and 23500410 (to A. S.), and KAKENHI 20021016 and 22111006 (to T. M.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Notes
We thank A. Shitamukai, F. Matsuzaki, N. Galjart, and F. Miller for plasmids; and P. Spear, T. Kawauchi, S. Nakamuta, T. Watanabe, M. Sato and Y. Hatanaka for valuable comments. Conflict of Interest: None declared.

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Cerebral Cortex May 2014, V 24 N 5 1309