Mechanism of Centrosome Positioning during the Wound Response in BSC-1 Cells

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Abstract. Locomoting cells are characterized by a pronounced external and internal anterior-posterior polarity. One of the events associated with cell polarization at the onset of locomotion is a shift of the centrosome, or MTOC, ahead of the nucleus. This position is believed to be of strategic importance for directional cell movement and cell polarity. We have used BSC-1 cells at the edge of an in vitro wound to clarify the causal relationship between MTOC position and the initiation of cell polarization. We find that pronounced cell polarization (the extension of a lamellipod) can take place in the absence of MTOC repositioning or microtubules. Conversely, MTOCs will reposition even after lamellar extension and cell polarization have occurred. Repositioning requires microtubules that extend to the cell periphery and is independent of selective deetyrosination of microtubules extending towards the cell front. Significantly, MTOCs maintain, or at least attempt to maintain, a position at the cell's centroid. This is most clearly demonstrated in wounded monolayers of enucleated cells where the MTOC closely follows the centroid position. We suggest that the primary response to the wound is the biased extension of a lamellipod, which can occur in the absence of microtubules and MTOC repositioning. Lamellipod extension leads to a shift of the cell's centroid towards the wound. The MTOC, in an attempt to maintain a position near the cell center, will follow. This will automatically put the MTOC ahead of the nucleus in the vast majority of cells. The nucleus as a reference for MTOC position may not be as meaningful as previously thought.

The establishment and maintenance of an anterior-posterior polarity is a structural and functional characteristic of locomoting cells (for reviews see Bornens and Karsenti, 1984; Singer and Kupfer, 1986; Vorobjev and Nadezhdina, 1987). If stimulated to commence directional locomotion, many cell types express an internal and external polarization of various cytoplasmic components. Among these is a shift of the centrosome, the cell's microtubule-organizing center (MTOC), into a position between the nucleus and the cell anterior. This position is believed to be of strategic importance for directional cell movement and indeed may help establish the polarized morphology seen in many locomoting cells (Malech et al., 1977; Albrecht-Buehler, 1981; Gotlieb et al., 1981; Koonce et al., 1984; Wong and Gotlieb, 1988). However, not all observations on the behavior of MTOCs in locomoting cells are easily reconciled with this view. In several cell types or under certain experimental conditions directional locomotion is observed in the absence of a distinct position of the MTOC or indeed a microtubule system (e.g., Anderson et al., 1982; Malawista and de Boisfleury-Chevance, 1982; Euteneuer and Schliwa, 1984; Sameshima et al., 1988; Gudima et al., 1988; Schütze et al., 1991). In addition, neither the roots of this apparent causality nor the mechanisms of MTOC repositioning during the initiation of locomotion have been addressed experimentally.

One of the best model systems used to study MTOC involvement in cell motility and cell shape changes is that of experimental wounds in vitro, where a strip of cells is removed from a monolayer by scraping and the behavior of cells at the wound edge is monitored thereafter. Cells at the wound edge will reposition the MTOC and the Golgi apparatus toward the open wound within 30 min to several hours, depending on the cell type (Gotlieb et al., 1981, 1983; Kupfer et al., 1982, 1983; Bergman et al., 1983; Gundersen and Bulinski, 1988). The fascinating observation that the MTOC will change its position and move in front of the nucleus has lent support to the idea that the MTOC has a major role in initiating the events that lead to wound closure. However, even though the phenomenon of MTOC repositioning in cells at the wound edge is well documented, its mechanism is largely unknown.

Here we use the in vitro-wound assay in an attempt to answer some of the questions concerning MTOC repositioning. What is the role of the MTOC in cell polarization? What is the role of microtubules in MTOC repositioning? Our observations help clarify the causal relationship between the initiation of cell polarization and MTOC repositioning. They suggest that MTOC relocation is the result of lamellipod for-
motion and extension. It is the ensuing change in cell shape to which microtubule organization adapts dynamically, leading the MTOC to adopt a position ahead of the nucleus.

**Materials and Methods**

**Cells**

African green monkey kidney cells, strain BSC-1, were obtained from American Type Culture Collection (Rockville, MD). They were maintained in DMEM supplemented with 5% NuSerum (Collaborative Research, Bedford, MA), 5% calf serum, and antibiotics (PSN; Gibco Laboratories, Grand Island, NY). Cells were plated on 18-mm coverslips and allowed to grow to confluency (2-3 d). Wounds were made by removing a strip of cells ~400–800-μm wide with a sterile pipette tip or a rubber policeman.

Primary chicken embryo fibroblast cultures were obtained from Dr. S. R. Martin (University of California, Berkeley, CA) and prepared as described elsewhere (Hanafusa, 1969). They were subcultured onto coverslips for the experiments.

**Preparation of Cytoplasts**

Cytoplasts were prepared from BSC-1 cells grown on 15-mm round coverslips and enucleated by centrifugation in the presence of 10 μg/ml cytochalasin B (Sigma Chemical Co., St. Louis, MO) according to the procedure described by Karsenti et al. (1984). Briefly, coverslips, cell-side facing down, were transferred to 50-ml Sorvall centrifuge tubes (Sorvall Instruments, Newton, CT) containing 4 ml medium with cytochalasin B and returned to the incubator for 30 min. Cells were enucleated in a centrifuge (J2-21M; Beckman Instruments, Palo Alto, CA) using a JS-13.1 rotor at 8.9,000 rpm for 50 min at 34°C. They were rinsed three times with fresh, warm, cytochalasin-free medium, and placed in the incubator for recovery. They were used after 3.5 h.

**Immunofluorescence Microscopy**

Cells were rinsed with PBS, lysed in PHEM buffer (60 mM Pipes, 20 mM Hepes, 2 mM MgCl₂, 4 mM EGTA) containing 0.5% Triton X-100 for 60 s and then fixed with 1% glutaraldehyde in PHEM buffer for 10 min (all solutions at 37°C). Alternatively, cells were rinsed in PBS and fixed in cold (-20°C) methanol. Cells were processed according to standard immunofluorescence procedures (Schliwa et al., 1981). We visualized microtubules with a mAb to beta tubulin; detyrosinated microtubules with a rabbit peptide antibody against Glu-tubulin (Gundersen et al., 1984); centrosomes (MTOCs) with a human autoimmune serum to centrosomal material (S051 serum, generously provided by T. Mitchison and M. Kirschner, University of California, San Francisco, CA) and actin filaments with rhodamine-phalloidin (a generous gift of T. Wieland, Max-Planck-Institut, Heidelberg, Germany). Secondary antibodies were obtained from Sigma Chemical Co. or Organon Teknika-Cappel (Cappell Laboratories, Cochranville, PA). Preparations were examined in a Zeiss Photomicroscope III (Carl Zeiss, Oberkochen, Germany) and photographed with Kodak Plus-X film (Eastman Kodak Co., Rochester, NY).

**Drug Treatments**

Nocodazole (Sigma Chemical Co.), cytochalasin B (Sigma Chemical Co.), and taxol (generous gift of Dr. M. Suffness, National Institutes of Health) were dissolved in DMSO and used at the concentrations indicated in Results.

**Data Collection and Morphometric Analysis**

Centrosome or microtubule staining was used to identify the location of the MTOC. MTOC position with respect to the wound edge was scored according to the scheme shown in Fig. 1a. The random, or baseline, distribution of MTOCs is 30.5% front, 39% side, and 30.5% back. Under all experimental and control conditions, ~20% of the cells did not allow the determination of MTOC positions based on the microtubule staining pattern. These cells were not included in the data. For each time point and/or experimental condition, at least 200 and up to 2,000 cells from 2-14 different experiments were scored. The centroid of the cell's two-dimensional projection was determined with the MorphoSys morphometric program (Meacham, C. A., and T. Duncan, University of California, Berkeley, CA). The parameters for this determination (cell outline and MTOC position) were traced onto drafting paper at a magnification of 800× and fed into an IBM AT computer via a CCD video camera. In all cells the MTOC location was represented as a circle of ~5 μm in diameter, rather than a point. This is based on the observation that microtubules are emanating from a centrosomal region, rather than from one centriole. The distance between the MTOC and the centroid was determined and categorized. An example of the graphic representation of the results is shown in Fig. 1b.
Results

Control Wound Response

The response to wounding in monolayer cultures has been described before and need not be iterated here. Like NRK or aortic endothelial cells (Gotlieb et al., 1981; Kupfer et al., 1982), BSC-1 cells show a pronounced response to in vitro wounds. Within 15 min, cells facing the wound have begun to extend a small lamellipod and/or microspikes towards the open substrate. The lamellipod continues to extend for at least 2 h and grows to a considerable size. It represents a new cell extension although in some cells it may initially only compensate for the area that was retracted in response to the wound. Fig. 2 illustrates the time course of this behavior during the first 2 h.

As in other cell types (Gotlieb et al., 1981; Gundersen and Bulinski, 1988), MTOC reorientation is a gradual process that starts 10–15 min after wounding. The percentage of cells with MTOCs in front of the nucleus increases from a basal level of ~30.5% (see legend to Fig. 1 a) to 43.5, 66, 69, and 82.5% after 30, 60, 120, and 180 min, respectively (Fig. 3). An example of cells at the wound edge processed for immunofluorescence with 5051 antiserum 2 h after wounding is shown in Fig. 4. We have followed the process of MTOC reorientation for up to 7 h at which time the position of the MTOC becomes more random again.

Orientation of Glu Microtubules

For 3T3 cells it has been reported that the polarization of the
Figure 4. Distribution of centrosomes visualized with 5051 antiserum at the wound edge 2 h after wounding. The position of the wound (W) is marked with a double arrow. The micrograph was slightly underdeveloped to reveal the position of the nuclei. Bar, 10 μm.

Figure 5. Distribution of detyrosinated (Glu−) microtubules in BSC cells within a confluent monolayer (a) and at the wound edge (w) two h after wounding (b). The wound is at the top of the micrograph. There is a relative sparsity of Glu-microtubules facing the wound. Bar, 10 μm.
MTOC involves selective orientation of detyrosinated (Glu) microtubules towards the wound (Gundersen and Bulinski, 1988). BSC-1 cells also possess a subset of Glu-microtubules with frequently sinuous morphology that generally do not extend to the cell periphery. Both within confluent sheets and at the wound edge, these microtubules are distributed more or less evenly around the MTOC (Fig. 5). Many cells at the wound edge even are characterized by a relative sparsity of Glu-microtubules in the anterior half of the cell facing the wound, whereas the distribution of Glu microtubules in the posterior resembles that of cells within monolayers. Thus selective orientation of Glu-microtubules towards the wound does not occur in BSC-1 cells.

**Experimental Manipulations of the Repositioning Process**

As reported by others (Gotlieb et al., 1983), most compounds that interfere with the observed repositioning of the MTOC fall into the category of microtubule poisons. Nocodazole at 0.2–1 µg/ml inhibits MTOC relocation effectively. The percentage of cells at the wound edge with MTOCs in front of the nucleus is ~30%, indicating a random distribution of the MTOC about the nucleus (Fig. 1a). However, activities of the cell periphery, such as ruffling, spike formation, and lamellar extension are not inhibited at all (Fig. 6). Drug-treated cells extend a lamellipod of the same dimensions and with the same time course as untreated cells. This finding suggests that microtubules and MTOC repositioning are not required for the development of a pronounced anterior–posterior polarity of cells at the wound edge.

We next determined whether a wound stimulus still exists after nocodazole-treated cells have completed the development of an external polarity and whether the MTOC will reposition if the drug is removed at this time. Confluent monolayers were pretreated with nocodazole (1 µg/ml) for 1 h to disassemble the majority of microtubules, kept in the drug during the first 2 h after wounding, and then allowed to recover drug free for another 2 h. After the removal of the drug, MTOCs repositioned with a time course similar to that seen in control experiments even though the wound already

*Figure 6. Cell behavior at the wound edge in the presence of 1 µg/ml nocodazole during the first 2 h after wounding. The time course of lamellipod extension is comparable to that of untreated cells (see Fig. 2). The time (in min) is indicated in the lower right. Arrowhead marks a reference point. Bar, 10 µm.*
Figure 7. Relocation of the MTOC after the establishment of a polarized cell morphology in the absence of microtubules. A1 = 1-h pretreatment of monolayer, wounding, and 2 h after wounding all in the presence of 1 µg/ml nocodazole. A2 = same as A1, followed by 2 h of recovery from nocodazole treatment. A3 = no pretreatment, nocodazole applied at the time of wounding for 2 h, followed by 2 h of recovery from nocodazole treatment. Under all experimental conditions the cells at the wound edge extended lamellipods into the open area in a manner indistinguishable from control cells (see Fig. 5). During recovery from nocodazole treatment (in experiments A2 and A3), the MTOCs reorient in 70-80% of the cells. The time course of MTOC reorientation is comparable to that of control cells (not shown).

existed for 2 h and a lamellipod had formed (Fig. 7). Thus, repositioning of the MTOC still occurs after completion of the initial wound response, namely, external cell polarization and the extension of a large lamellipod. In these experiments the position of the MTOC was visualized with the anticientrosomal serum 5051 (Tuffanelli et al., 1983; Calarco-Gillam et al., 1983) because the microtubule complex appeared to be less focused under these conditions.

The nature of a possible interaction between microtubules and the cell cortex during the development of a lamellipod is not known. We surmised that an aster of short (<10 µm) microtubules would not support interactions with the cell periphery but might enable the MTOC to change its position, e.g., by an interaction of microtubules with other components of the cytoskeleton and the cytomatrix. Attempts to produce short asters using a combination of the drugs taxol and nocodazole (de Brabander et al., 1981) were not successful. However, we were able to "adjust" the length of MTOC-associated microtubules by lowering the nocodazole concentration. The effect of the treatment is easily monitored at the end of the experiment by tubulin immunofluorescence, which also serves as the assay for MTOC location. These experiments were done on chicken embryo fibroblasts as well as BSC-1 cells to eliminate any cell-specific idiosyncrasies caused by the drug. The results are surprisingly clear and show a nice correlation between the dose of nocodazole used, the pattern of microtubules, and MTOC repositioning (Fig. 8). Short microtubules are not sufficient to mediate MTOC repositioning in response to wounding.

Correlation of MTOC Position with the Cell's Centroid

To assess MTOC position in cells undergoing a directional response, the nucleus is always used as a reference point (e.g., van Beneden, 1883; Malech et al., 1977; Albrecht-Buehler and Bushnell, 1979; Gottlieb et al., 1981; Kupfer et al., 1982). The importance of the finding that, in many cells, the MTOC is "ahead of the nucleus" relative to the direction of migration is unclear, however, within the framework of the cell as a whole. For example, does it imply that the MTOC is in the anterior half of the cell, closer to the front than the rear? We wished to obtain a nucleus-independent measure of MTOC position that uses the cell outline as a reference. We determined the centroid of two-dimensional projections of cells within confluent monolayers and of cells at the wound edge, and compared it to the position of the MTOC (defined as an area ~5 µm in diameter; see Materials and Methods). Within monolayers, the MTOC is located at the centroid in >80% of the cells and separated by no more than

Figure 8. MTOC reorientation in the presence of "low" concentrations of nocodazole. 1 h pretreatment followed by wounding and 2 h after wounding in the presence of the drug. (a) BSC-1 cells. 0.2 (B1), 0.1 (B2), 0.04 (B3), and 0.02 (B4) µg/ml of nocodazole. (b) Chicken embryo fibroblasts (CEF). Control (no nocodazole) (C1), 0.3 (C2), 0.2 (C3), and 0.04 (C4) µg/ml of nocodazole. (c) Distribution of microtubules in CEF cells at the edge of an in vitro wound (top of micrograph) in the presence of 0.3 µg/ml nocodazole (condition C2 in b). Cells possess an aster of short microtubules most of which do not extend to the cell margin. Bar, 10 µm.
Figure 9. Comparison of the position of the centroid and the MTOC in confluent monolayers (c) and at the wound edge (w) 2 h after wounding. Intact cells (cells). Enucleated cells (cytoplasts). In cells with an incongruency of centroid and MTOC (o, o), the MTOC is behind the centroid relative to the wound edge in 82% of the intact cells and 90% of the cytoplasts.

5 \mu m from it in the rest of the cells (Fig. 9). In cells at the wound edge, the corresponding percentages are 56 and 33%; an additional 11% shows a distance of 5-10 \mu m between the MTOC and the centroid. It is important to note that in cells at the wound edge that show an incongruency of MTOC and centroid position, the two are found in a preferred orientation to each other: in >80%, the MTOC “lags” behind the centroid with respect to the wound edge (the cell anterior). Thus in the majority of cells the position of the MTOC is congruent with the cell’s centroid, or closely follows—in both space and time—the shift of the centroid towards the wound.

The nucleus is located near the cell center in confluent monolayers and in the cell posterior in cells at the wound edge two hours after wounding. However, this does not imply that the nucleus has moved. In cells at the wound edge, nuclei merely remain in the position they occupied at the time of wounding (which then was near the cell center; see Fig. 2). The extension of the lamellipod now puts them in a posterior location.

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Discussion

Development of Cell Polarity

MTOC repositioning towards the cell side facing an in vitro wound is a well-documented phenomenon (Gotlieb et al., 1981; Kupfer et al., 1982; Gundersen and Bulinski, 1988). The close correlation between cell polarization and MTOC position established in these studies has been interpreted to reflect a determinative role of the MTOC in setting up cell polarity, where MTOC positioning precedes cell migration (Wong and Gotlieb, 1988). Using BSC-1 cells, we have found that (a) pronounced cell polarization, i.e., the extension of a lamellipod, takes place in the absence of MTOC repositioning and microtubules; (b) MTOCs will reposition even after lamellar extension and cell polarization have occurred; (c) MTOC repositioning requires microtubules that extend to the cell periphery; (d) MTOCs maintain, or at least attempt to maintain, a position at the cell’s centroid; and (e) MTOC repositioning does not require selective detyrosination of microtubules extending towards the wound.

One could envisage that certain features of the microtubules themselves contribute to the generation of cell polarity. Gundersen and Bulinski (1988) suggest a role for the selective stabilization of a subset of microtubules within a larger array of dynamic microtubules. They find that 3T3 cells extending into an in vitro wound generate an asymmetric microtubule array, with stable (Glu-) microtubules oriented...
preferentially toward the wound. This finding would lend support to the hypothesis that stabilization of a subset of microtubules contributes to the process of cellular morphogenesis (Kirschner and Mitchison, 1986). We find no evidence for a preferred orientation of Glu-microtubules towards the wound; they are arranged more or less symmetrically around the MTOC. The same is true for fibroblasts (data not shown; K. Schütze and M. Schliwa, unpublished observations). In Vero and MDCK cells, Bré and co-workers (1991) also did not see a clear reorientation of Glu-microtubules towards the wound. They further suggest that microtubules are generally less detyrosinated in the absence of cell contacts, leading to a preferred orientation of more dynamic microtubules towards the newly formed lamellipod. Theirs as well as our observations do not support the suggestion that cell polarization is determined by selective stabilization of microtubules in the direction of the wound.

Establishing cause and effect in a complex event such as MTOC positioning during the wound response is difficult because of the tight temporal and spatial coupling of several processes. Perhaps the most informative experiment would be to block lamellar extension and ask whether MTOCs still reorient. We have attempted these experiments using a variety of agents that affect lamellar extension and cortical actin function, including cytochalasin D (Schliwa, 1982), calcium channel blockers (Cooper and Schliwa, 1985), inhibitors of the phosphoinositide pathway (Lassing and Lindberg, 1985; Jannney and Stossel, 1987), the tumor promoter TPA (Schliwa et al., 1984), and EHNA (Schliwa et al., 1984). However, like cytochalasin B (Gotlieb et al., 1983), none of these treatments completely block lamellipod extension; they still allow some MTOC repositioning unless used at concentrations that cause gross cell distortion. These experiments are, therefore, inconclusive. We have taken the alternative approach of eliminating microtubules and find that cell polarization and the initial extension of the cell sheet into the wound occur with the same time course as in untreated cells. Thus MTOC relocation is not required for the initiation of polarity and migration; these are triggered by the asymmetric loss of cell contacts and are executed by the cortical actin filament system (Stossel, 1989). MTOC repositioning is secondary to these activities and appears to be due to other stimuli.

Centrosomes Maintain a Centroid Position

What stimuli might this be? The incentive to re-orient the MTOC persists long after wounding. It is so powerful, in fact, that after nocodazole treatment, MTOCs reposition despite a fully expressed external cell polarity (Fig. 6). We suggest that the driving force for MTOC repositioning is their attempt to remain in the cell center. We observe a congruence of MTOC and centroid position not only in monolayers, but also in the majority of cells at the wound edge at all times during the wound response. The tendency of the MTOC to be in the centroid is independent of the presence of a nucleus and even improves in its absence (as shown by the experiments with cytoplasts), and it depends on the presence of microtubules. Significantly, the MTOC is almost never found in the anterior half of the polarized cell.

The importance of a centroidal location of microtubule asters is born out by observations in many cell types. For example, in fertilized eggs the aster associated with the male pronucleus forms in the cell periphery and moves to the center of the embryo as astral rays increase in length (Wilson, 1928). Asymmetric positions of symmetrical asters and spindles can only be produced by experimental manipulations (Rappaport, 1981). In severed melanophore processes, a new MTOC is formed, and this MTOC migrates from the site of the cut to precisely the centroid position of the cell process (McNiven and Porter, 1988). Perhaps the best experimental demonstration comes from the work of Hamaguchi and Hiramoto (1986) on colcemid-treated sea urchin eggs where microtubule asters always migrate to the center of a colcemid-free window produced by UV irradiation. Asymmetric positioning of MTOCs, on the other hand, is caused by asymmetric deployment of factors determining microtubule organization: cortical sites specifying MTOC position (e.g., Mogensen and Tucker, 1987; Lutz et al., 1988; Hyman, 1989), or asymmetric MTOCs (e.g., Dan and Inoue, 1987; Holy and Schatten, 1990). It is plausible that the centroid location is favored because it represents an equilibrium position for microtubule asters. The nature of the forces that lead to this positioning has yet to be determined. We show that microtubules extending to the cell periphery are required. Conceivably, it is the dynamic instability behavior of the microtubule population that balances the microtubule aster in a centered position within the cell (Mitchison and Kirschner, 1984a,b; Sammak et al., 1988). The existence of such a "balancing force" was strongly suggested in experiments on flat, adherent leukocytes treated with cytochalasin D (Euteneuer and Schliwa, 1985). Here the MTOC with its microtubule aster assumes the most stable position at which all microtubules are minimally bent and of more or less equal length. This position is precisely in the geometric center of the cell. The nature of the forces and interactions that execute and achieve this balancing act remain to be determined. For example, microtubules might "push" against the cell cortex (Hill and Kirschner, 1982), thereby centering the MTOC in the centroid, or move the MTOC via interactions with other cytoskeletal components. These are vague descriptive terms, however, that need to be translated into molecular mechanisms in future experiments.

On the basis of our findings, we suggest that the primary response to the wound is the biased extension of a lamellipodium, which can occur in the absence of microtubules, MTOC repositioning, and a nucleus. The unidirectional extension of a lamellipod by δL will lead to an equally polarized shift of the centroid by 1/2 δL. The MTOC, in attempting to maintain a position in the cell center, will follow the centroid’s shift towards the wound. This will put the MTOC ahead of the nucleus in the majority of cells. Since lamellipod formation and the ensuing shift in the position of the centroid presumably is faster than the centrosomal shift (which requires length adjustments of microtubules and remodeling of the entire cytoplasmic microtubule complex), the MTOC will lag behind the centroid.

One can envision that this scenario may be of relevance also for other motile cells such as granulocytes where the MTOC is located in front of the nucleus during directional migration in the majority of cells (e.g., Schliwa et al., 1982; Koonce et al., 1984). In these cells the formation of protrusions is controlled by instructions originating at the plasma membrane, leading to MTOC repositioning. The microtub-
bule apparatus then essentially follows the continually extending lamellipodium. Under certain experimental conditions the two events are separable, as in the case of the "runaway" lamellipods of heat-treated leukocytes (Malawista and de Boisfleury Chevance, 1982) or amputated keratocytes (Euteneuer and Schliwa, 1984). This scenario does not imply that the MTOC is merely a functionless baggage dragged along by the lamellipod. As demonstrated by numerous studies involving microtubule depolymerization, the cytoplasmic microtubule complex is essential for motility (see Bellairs et al., 1982; Lackie, 1986).

Our observations suggest that the initiation of cell polarization is independent of MTOC repositioning. The nucleus as a reference for MTOC repositioning, although intuitively appropriate, may not be meaningful, as demonstrated by the cytoplasm experiments. Rather, the MTOC-microtubule complex merely attempts to maintain its position in the cell's centrosome where it helps support a polarized cytoplasmic organization. In that sense its position is strategic, and in that sense the MTOC serves a strategic function, not as the prime mover for reorientation, but as the cell's true central body (=centro-some).

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