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Selective stabilization of microtubules oriented toward the direction of cell migration

(experimental wounding/detyrosinated tubulin/microtubule organizing center)

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ABSTRACT **A small subset of the microtubule (MT) array in many cultured cells does not exhibit the rapid turnover ($t_{1/2}$ ≈ 10 min) shown by most cellular MTs. The function of the stable class of MTs is unknown and has been confounded by the apparent lack of organization of stable MTs within cells. Using an antibody against detyrosinated tubulin, a post-translationally modified form of tubulin that accumulates in stable MTs, we localized the stable MTs in mouse 3T3 cells induced to initiate directional migration by experimental wounding of confluent monolayers. Immediately after monolayer wounding, the distribution of stable MTs in cells at the wound edge resembled that in cells in the monolayer interior; most cells either contained randomly distributed stable MTs or lacked them entirely. However, by 20 min after wounding, cells at the wound margin began to generate an asymmetric MT array, with virtually all stable MTs oriented toward the cell edge in contact with the wound. Two hours after monolayer wounding, ≥80% of cells at the wound margin had generated this polarized array of stable MTs, and the array was maintained for at least 12 hr. MTs in the polarized array showed enhanced resistance to depolymerization by nocodazole, thus providing an independent test of their stability. Formation of the polar array of stable MTs appeared to precede onset of cell migration and closely paralleled reorientation of the MT-organizing center. These results show that cultured cells can remodel their MT array rapidly in response to an extracellular signal and suggest that selective stabilization of MTs is an early event in the generation of cellular asymmetry.**

Microtubules (MTs) are conspicuous elements of the cytoskeleton of nearly all eukaryotic cells. Composed of the subunit protein tubulin, they grow (or shrink) by the addition (or loss) of soluble tubulin at their ends. Besides their essential role in the spindle of mitotic and meiotic cells, MTs perform numerous functions in proliferating interphase cells and in terminally differentiated cells; e.g., MTs serve as tracks along which organelles move (1, 2) and as structural elements that contribute to the maintenance of cellular asymmetry (for review, see ref. 3). Because these functions are intimately coupled to the location of MTs within cells, much effort has been expended studying the factors that govern MT distribution within cells.

Cultured fibroblasts have often been used for studies of MT behavior *in vivo* because of the relative facility with which individual MTs can be visualized by immunofluorescence (4). In fibroblasts, location of one end of the MT is specified by its attachment to the centrosome, a MT organizing center (MTOC) near the nucleus; this attachment causes the typical stellate organization of interphase MTs. Although the centrosome anchors one end of each MT and may limit the number of MTs by limiting the number of attachment sites (5, 7), whether other factors, acting along the length of the MT or at the end distal to the centrosome, also contribute to specification of the MT array is not known.

The dynamics of MT polymerization and depolymerization also affect the distribution of MTs within cells. Recent studies have shown that the static picture of MTs portrayed in immunofluorescent images belies an amazing degree of dynamic behavior. In cultured fibroblasts, most MTs exist only transiently, with a half-life averaging only 5–10 min (6, 32), meaning that the entire interphase array of MTs is replaced approximately every hour. The function of this rapid turnover of MTs is unknown. Kirschner and Mitchison (8), however, have hypothesized that a dynamic array of MTs is a source of potential morphogenetic determinants; by the selective stabilization of individual MTs, a MT-based cellular asymmetry could be generated.

Considerable evidence exists, both from drug studies (9–11) and from the direct measurement of MT turnover (6, 12, 13), for a small population of MTs in cultured fibroblasts that is more stable than the bulk of MTs. These stable MTs frequently exhibit a sinuous morphology and are typically comprised of tubulin that has been post-translationally modified (10–13). Such stable MTs are prime candidates for a specialized cytoskeletal role—e.g., in organelle transport or in the maintenance of cellular polarity. However, reported distributions of stable MTs seem at odds with such a role; stable MTs appear randomly located and often do not extend to the cell periphery (9–14, 32).

In this study we examine the distribution of stable MTs in mouse 3T3 fibroblasts after applying a specific directional signal to the cells. We find that 3T3 cells can rapidly generate a new array of stable MTs and that these stable MTs are specifically associated with the developing asymmetry of the cell as it begins to migrate.

MATERIALS AND METHODS

Cell Culture and Wounding. NIH 3T3 cells (passage 125–133) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone, Logan, UT) at 37°C in 5% CO_2. For wounding experiments, cells were seeded onto glass coverslips and grown to confluency (≈3 days). Confluent monolayers were wounded by cutting several swaths (≈1–2 mm wide) through the monolayer with a disposable, sterile cell scraper (Costar, Cambridge, MA). The medium was removed, and the wounded monolayer was washed once before incubating the cells in fresh medium. Cells were treated with the MT-depolymerizing drug, nocodazole (Aldrich) as described (15), including the extraction step to remove monomeric tubulin before fixation.

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Abbreviations: MT, microtubule; MTOC, microtubule organizing center; [Glu]MT, detyrosinated MT; [Tyr]MT, tyrosinated MT; [Glu]-tubulin, detyrosinated tubulin; [Tyr]tubulin, tyrosinated tubulin.

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Immunofluorescence. Cells were fixed in −20°C methanol and stained by double indirect immunofluorescence as described (15), using a rabbit peptide antibody specific for detyrosinated tubulin ([Glu]tubulin) (14) and a rat monoclonal antibody specific for tyrosinated tubulin ([Tyr]tubulin) (16, 17). The rat monoclonal antibody was the gift of J. V. Kilmartin (Medical Research Council, Cambridge, U.K.).

RESULTS

Stable and Dynamic MTs in 3T3 Cells. We have previously shown that in proliferating cultured cells a small subset of MTs is enriched in post-translationally detyrosinated tubulin ([Glu]tubulin) (14) and these MTs (called [Glu]MTs) arise by the detyrosination of preexisting MTs comprised of [Tyr]tubulin (15). This postpolymerization detyrosination is a consequence of two factors: a restriction of the detyrosinating enzyme, tubulin carboxypeptidase (18), to the pool of polymeric tubulin and the maintenance of the tubulin monomer pool at ≥98% [Tyr]tubulin (15). Thus, MTs that can be immunolabeled with antibodies specific for [Glu]tubulin are more stable (long-lived) than their dynamic counterparts that label only with a Tyr-specific antibody (the latter are termed [Tyr]MTs).

In interphase 3T3 cells in confluent monolayers, the distribution of [Tyr]- and [Glu]tubulin resembles that reported for many other cultured cells (12–14, 19, 20): the more dynamic [Tyr]MTs predominate, whereas the long-lived [Glu]MTs compose a small subset (e.g., see Fig. 1 *a* and *b*). Although individual [Glu]MTs are distinctive in their frequently sinuous morphology, the distribution of [Glu]MTs within most cells seems largely unorganized. In addition, much cell-to-cell heterogeneity exists in both number and distribution of [Glu]MTs (see Fig. 1*b*). In confluent monolayers of 3T3 cells from five separate experiments, no [Glu]MTs were detectable in 39 ± 10% (n = 200) of the cells, [Glu]MTs arrayed without any particular orientation were found in 45 ± 11% of the cells, and 16 ± 3% of the cells had [Glu]MTs that were oriented predominantly in a single direction. [Glu]MTs in the latter category were frequently seen along the long axis or in a long extension of the cell.

Accumulation of [Glu]Tubulin in MTs Oriented Toward an Experimental Wound. The above results seem to argue that formation of stable [Glu]MTs in cells is not spatially regulated. But could an underlying organization of the [Glu]MT array be obscured by the random orientation of cells in the culture? We examined the distribution of [Tyr]- and [Glu]tubulin after the application of a specific and oriented stimulus to a 3T3 cell monolayer. As the stimulus we wounded the confluent monolayer by scraping off a narrow row of cells. Cells adjacent to the wound are relieved from contact inhibition and undergo a characteristic response: they rapidly reorient their MTOC and Golgi apparatus to a position between the nucleus and the leading edge of the cell (21, 22) and begin migrating into the wound.

Distributions of [Tyr]- and [Glu]tubulin in wounded monolayers of 3T3 cells are shown in Fig. 1 (wounds are located at the right). Shortly after wounding (10 min), we found no overall change in the [Tyr]- and [Glu]MT arrays in cells adjacent to the wound when compared with those in cells distant from the wound (Fig. 1 *a* and *b*). With increased time after wounding, however, the proportion of cells at the wound edge that contained distinct [Glu]MTs increased dramatically (see Fig. 3). More significantly, the array of [Glu]MTs became uniformly polarized; as shown in Fig. 1*d* for cells 4 hr after wounding, [Glu]MTs were specifically localized between the nucleus and the leading edge of virtually all cells adjacent to the wound. In contrast, the pattern of [Glu]MTs (both number and distribution) in cells removed even one cell layer from the wound was indistinguishable in appearance from that in unwounded monolayers.

The specific location of stable [Glu]MTs in cells at the wound margin is shown more clearly at higher magnification in Fig. 2. The array of [Tyr]MTs typifies the MT pattern found in interphase cells: MTs radiate from the MTOC and appear to fill the cell (Fig. 2*a*). More [Tyr]MTs are oriented toward the leading edge of the cell than away from it; yet, other regions of the cell contain many [Tyr]MTs. In contrast, [Glu]MTs are only found oriented toward the leading edge of the cell (Fig. 2*b*). Note that although many MTs oriented toward the leading edge were the detyrosinated type, not all of them were. Both [Tyr]- and [Glu]MTs closely approached the leading edge of the cell and sometimes appeared to bend and then run parallel to the edge (Fig. 2).

We deliberately chose the cell in Fig. 2 to show two additional aspects of the staining seen in wounded cells. (*i*) Reorientation of the MTOC to the leading edge (refs. 21 and 22; see also below) was not a prerequisite for the formation of oriented [Glu]MTs; in the cell in Fig. 2, the MTOC (arrowhead) is above the nucleus and toward the back of the cell (i.e., away from the leading edge or "front" of the cell). We also observed oriented [Glu]MTs in cells in which the MTOC was behind or beside the nucleus. (*ii*) Formation of oriented [Glu]MTs did not appear to require active protrusion of the leading edge. We observed oriented [Glu]MTs in many cells with concave leading edges (such as in Fig. 2), a morphology opposite to that expected for actively migrating cells.

Relationship Between MTOC Reorientation and Generation of Oriented [Glu]MTs. Previous studies have shown that the MTOC in cells adjacent to the wound reorients to a position between the nucleus and the leading edge before active migration of cells into the wound (21, 22). We determined the relationship between MTOC reorientation and generation of oriented [Glu]MTs in cells fixed at various times after wounding. The MTOC was identified as one or two spots brightly stained with the antibody against [Glu]tubulin (19) at the focus of the MT array. An oriented MTOC was one within the wedge defined by the leading edge and the shortest lines drawn from each side of the leading edge (where it contacted neighboring cells) to the nucleus. [Glu]MTs were counted as oriented when the great majority (usually >80%) of the [Glu]MTs in the cell radiated from the MTOC toward the leading edge (as in Figs. 1*d* and 2*b*).

We detected a small, but consistent, increase in the number of cells with oriented [Glu]MTs by 20 min after wounding; by 2 hr, 80% of cells had an oriented array of [Glu]MTs (Fig. 3). Polarized [Glu]MTs were maintained in >85% of cells for at least 12 hr. During the period in which the proportion of cells with oriented [Glu]MTs increased rapidly (20 min–2 hr), we observed two characteristics of the [Glu]tubulin staining pattern: (*i*) cells with only a few oriented [Glu]MTs were rarely seen (cells either had many or none); and (*ii*) the staining intensity of the [Glu]MTs appeared to increase with time after wounding. The former observation suggests that initial generation of polarized [Glu]MTs involves a relatively synchronous stabilization of many MTs, whereas the latter suggests that after initial formation of polarized [Glu]MTs, additional oriented [Glu]MTs are generated or the level of [Glu]tubulin within individual oriented MTs increases.

Reorientation of the MTOC after wounding closely paralleled the appearance of a polarized array of [Glu]MTs (Fig. 3). The proportion of cells with oriented [Glu]MTs initially lagged behind that with reoriented MTOCs, which would suggest that MTOC reorientation preceded polarized [Glu]MT formation; however, approximately one-third of the cells began the experiment with oriented MTOCs (as expected from random distribution of MTOCs in monolayer cells) and for these cells "reorientation" of the MTOC has already "occurred." Thus,

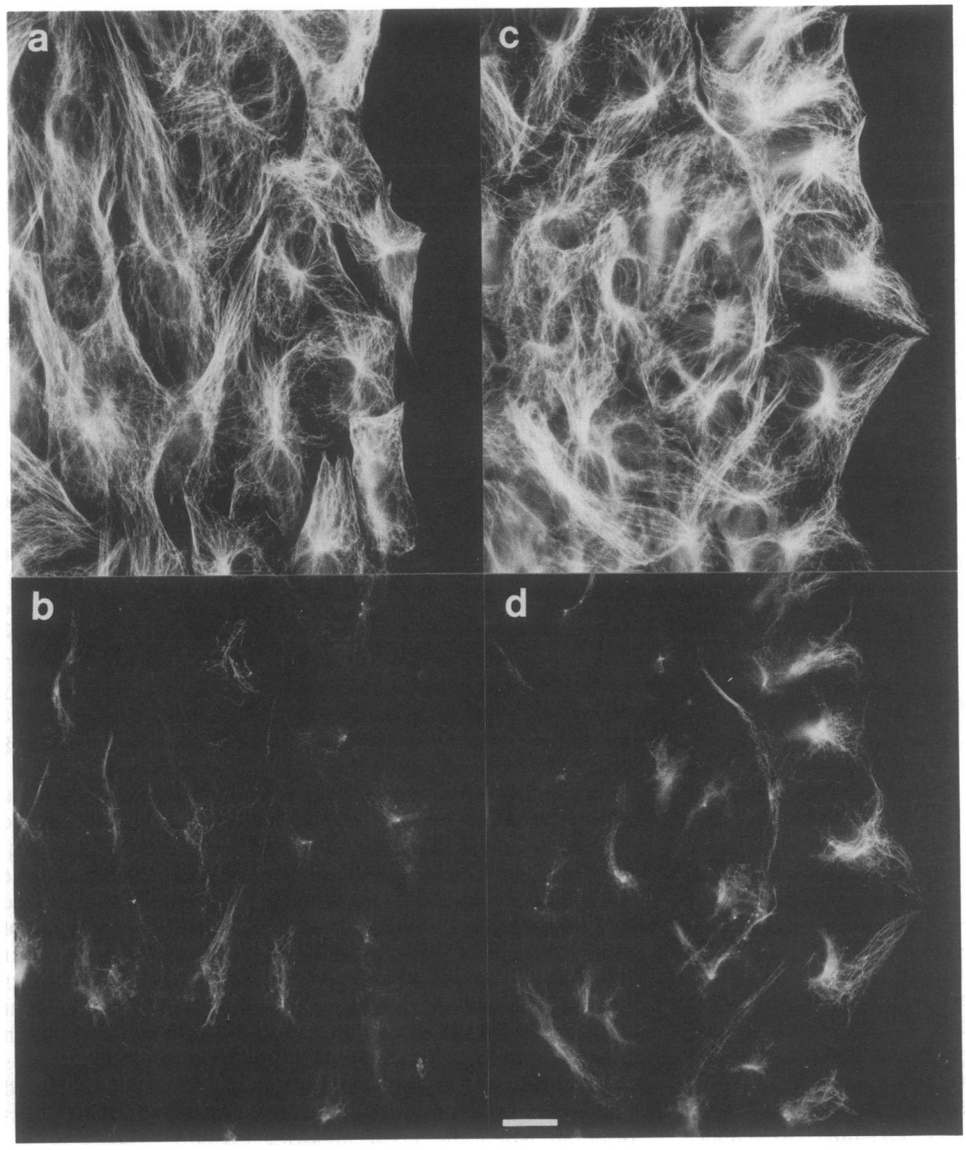

FIG. 1. Double immunofluorescence staining of wounded monolayers of 3T3 cells. Distribution of [Tyr]tubulin (*a* and *c*) and [Glu-]tubulin (*b* and *d*) is shown for cells 10 min (*a* and *b*) and 4 hr (*c* and *d*) after wounding. Wound is at right in each panel. Bar, 20 μm.

we cannot unambiguously establish the temporal order of the two events. We did see a small proportion of cells (≈1–10%), beginning with the 20-min time point, that had oriented [Glu]MTs but nonoriented MTOCs (as in Fig. 2). This observation suggests that MTOC reorientation was not necessary for generation of a polarized array of [Glu]MTs and that MT stabilization might precede MTOC reorientation.

Nocodazole Stability of Oriented [Glu]MTs. To test independently the stability of the oriented [Glu]MTs, we treated wounded monolayers with nocodazole to depolymerize MTs. Prolonged treatment (≥2 hr) of 3T3 cells with 5 μM nocodazole caused almost complete breakdown of MTs in all cells, including those at the wound margin. However, with shorter treatments (30–60 min) a population of resistant MTs was evident (Fig. 4). In cells at the margin of 4-hr wounds, the resistant MTs were almost exclusively oriented toward the leading edge and, as expected, were of the [Glu]MT type (i.e., stained with the antibody against [Glu]tubulin (Fig. 4*b*). Interestingly, the nocodazole-resistant MTs were also stained with the antibody against [Tyr]tubulin (Fig. 4*a*); this result shows that the stable MTs had not persisted long enough to become completely detyrosinated. This finding

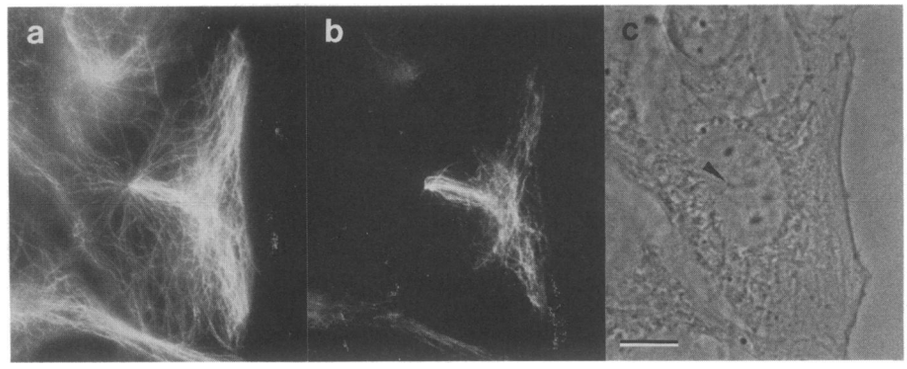

FIG. 2. High magnification of a single cell at the edge of a 4-hr wound. The distributions of [Tyr]tubulin (*a*) and [Glu]-tubulin (*b*) stained by double immunofluorescence are shown. In the phase image *c* of the cell in *a* and *b*, the MTOC (arrowhead) can be seen to lie over the nucleus. Bar, 10 μm.

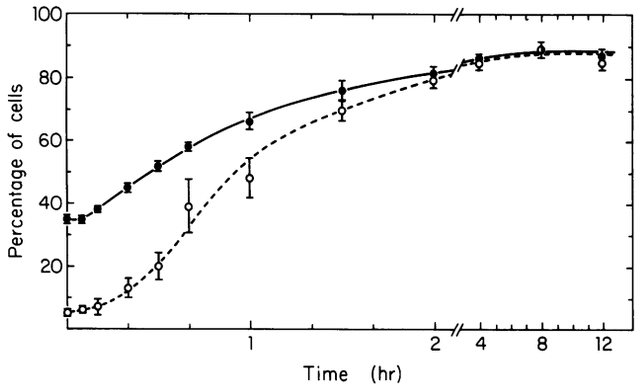

FIG. 3. Time course of MTOC orientation and oriented [Glu]MT appearance in wounded 3T3 monolayers. Percentage of cells exhibiting oriented MTOCs (●) and oriented [Glu]MTs (○), as defined in text, are plotted versus time after wounding. For each time point, 200 cells were examined. Bars represent the SEM from four independent experiments.

may also indicate that these MTs had been recently stabilized; however, other factors may contribute to the final level of [Glu]tubulin attained in stable MTs (15). The oriented distribution of nocodazole-stable MTs was seen in 84 ± 6% (n = 200 in each of four experiments) of cells at the edge of 4-hr wounds after 30-min nocodazole treatment. In contrast, when cells at the wound edge were treated with nocodazole (5 μM, 30 min) immediately after wounding, (i.e., when only ≈5% had an oriented array of [Glu]MTs), only 26 ± 3% (n = 200 in each of three experiments) of cells had a polarized array of resistant MTs. The change from a random to a polarized array of nocodazole-resistant MTs in most cells as they respond to wounding is consistent with the appearance of stable, increasingly detyrosinated MTs during wound recovery.

DISCUSSION

We have shown that [Glu]MTs accumulate in an oriented fashion in 3T3 cells stimulated to migrate into a wound.

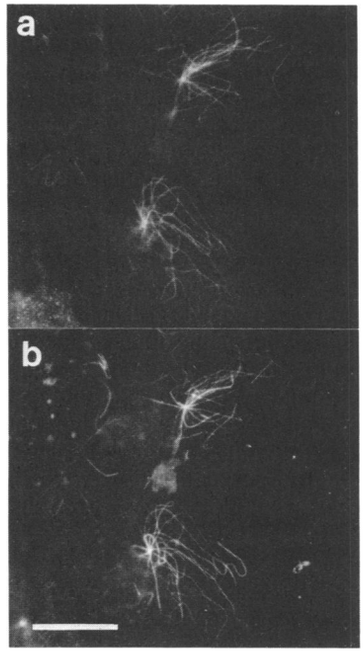

FIG. 4. Nocodazole treatment of wounded monolayers. Monolayers, allowed to recover from wounding for 4 hr, were subsequently treated with 5 μM nocodazole for 30 min before extraction, fixation, and double immunofluorescent staining for [Tyr]tubulin (a) and [Glu]tubulin (b). Wound is at right in each panel. Bar, 20 μm.

Coupled with earlier work demonstrating the time-dependent generation of [Glu]MTs by enzymatic detyrosination of preexisting [Tyr]MTs (16), we conclude that migrating 3T3 cells create stable MTs specifically oriented toward the direction of movement. The notion that [Glu]MTs are more stable than [Tyr]MTs *in vivo* has recently been demonstrated directly: Schulze *et al.* (12) and Webster *et al.* (13) showed that [Glu]MTs persist much longer than [Tyr]MTs—in some cases for almost a complete cell cycle (13), and Khawaja *et al.* (11) found that [Glu]MTs are quantitatively more stable than [Tyr]MTs to depolymerizing treatments. Although these and other experiments (e.g., ref. 19) have firmly correlated elevated levels of [Glu]tubulin with MT stability *in vivo*, increased [Glu]tubulin level does not appear to cause this stability (11). Nonetheless, the presence of immunologically detectable [Glu]tubulin in a MT is a useful marker of MT stability *in vivo*.

Although most experiments that established enhanced stability of [Glu]MTs used a different cell line (TC-7 monkey kidney cells), several lines of evidence show that in the wounded 3T3 fibroblasts, [Glu]MTs behave analogously and are, thus, stable MTs. (*i*) We showed that the oriented MTs exhibit greater resistance to nocodazole depolymerization than their nonoriented counterparts (see Fig. 4). This experiment demonstrates a stability independent of previous studies. (*ii*) We found that artificial stabilization of 3T3 MTs with the drug taxol causes rapid detyrosination of all cellular MTs, regardless of orientation (G.G.G., unpublished observations). Thus, [Glu]MTs are the presumptive product of MT stabilization in 3T3 cells. (*iii*) We confirmed that, as expected, in 3T3 cells [Glu]MTs are formed subsequent to [Tyr]MTs during *in vivo* regrowth of MTs from nocodazole treatment (G.G.G., unpublished observations). This was a hallmark of postpolymerization generation of [Glu]MTs in another cell line (15). Although [Glu]MTs are more stable than [Tyr]MTs in 3T3 cells, we still have little information about the extent of their stability: the oriented [Glu]MTs in 3T3 cells may exhibit the cell-cycle-long stability seen for [Glu]MTs in other cells (13), or they may be stabilized to a lesser degree. Nonetheless, our data show selective stabilization of MTs oriented toward the direction of cell migration in wounded monolayers of 3T3 cells.

Existence of a polarized array of less dynamic MTs has implications for the control of MT dynamics in cells. Because virtually all cells (>85%) at the wound edge respond by generating an oriented array of [Glu]MTs and 39% of cells did not contain [Glu]MTs at the time of wounding, 3T3 cells clearly can form *de novo* a stable array of MTs in response to an external stimulus. What is unusual about this response is that only a localized subset of the MTs is involved. We hypothesize that the stimulus (presumably lack of cell contact on one side of the cell) causes a transmembrane signal that alters the concentration of ions and/or small diffusible molecules that, in turn, alter activity of a MT-stabilizing factor. The direct effect of a diffusible second-messenger-type molecule on the MTs themselves seems unlikely because it would affect all MTs in the leading-edge region, and we consistently observed dynamic [Tyr]MTs among stable [Glu]MTs. However, if the second messenger activated a limited number of MT-stabilizing molecules or served to localize such factors, a regional and selective stabilization of MTs could be achieved. How the putative MT-stabilizing factor stabilizes MTs is unknown; however, one clue may come from the observation that [Glu]MTs in other cells add neither endogenous tubulin subunits (24) nor microinjected tubulin subunits labeled with reporter groups (13) to their distal ends. These results suggest that the growing ends of [Glu]MTs are blocked from further subunit addition and that the MT stabilizing factor may be a capping protein.

What role do MTs fulfill in cellular migration? Many studies have shown that pharmacological disruption of MTs neither inhibits the active protrusion of a leading lamella nor blocks random movement of cells (25–27). These functions are blocked by microfilament inhibitors. Instead, MT disruption leads to loss in *directionality* of movement. Our results suggest that selective stabilization of MTs in the direction of migration is important in maintaining this directionality. Significantly, we saw the stabilization event before any change in cell behavior was evident, suggesting that the alteration in MT dynamics is a *primum mobile* for morphological change. Thus, our data provide direct experimental evidence for the hypothesis that stabilization of a subset of MTs within a dynamic array is a mechanism by which morphogenetic events are evoked (8).

How the stable array of MTs is established and also how the array is used by the cell remain to be determined. Possibly the stable MT array serves as structural buttressing. In migrating fibroblasts, Rinnerthaler *et al.* (28) have seen a few MTs that apparently terminate in adhesion plaques near the leading edge. But recent evidence for organelle transport along MTs (1, 2) and for distinct MT-based motor proteins (29, 30) makes it more likely that the stabilization ensures or activates directed transport of membrane vesicles or organelles in the cell. In at least one instance, polarized insertion of a membrane protein into the leading edge of wounded cells has been shown (31). Stabilization of MTs may also be involved in the repositioning of the MTOC and Golgi apparatus that occurs during wound healing. For example, if the stable MTs function to tether these organelles within the cell, movement or shortening of stable MTs in wounded cells could move the MTOC and Golgi apparatus in tandem. Certainly, probing the mechanism by which MTs are stabilized and subsequently used will further our understanding of the molecular events in cell motility and morphogenesis.

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1. Allen, R. D., Weiss, D. G., Hayden, J. H., Brown, D. T., Fujiwake, H. & Simpson, M. (1985) *J. Cell Biol.* **100,** 1736–1752.
2. Schnapp, B. J., Vale, R. D., Sheetz, M. P. & Reese, T. S. (1985) *Cell* **40,** 455–462.
3. Dustin, P. (1984) *Microtubules* (Springer, New York), 2nd Ed.
4. Osborn, M., Webster, R. E. & Weber, K. (1978) *J. Cell Biol.* **77,** R27–R34.
5. Brinkley, B. R., Cox, S. M., Pepper, D. A., Wible, L., Brenner, S. L. & Pardue, R. L. (1981) *J. Cell Biol.* **90,** 554–562.
6. Schulze, E. & Kirschner, M. (1986) *J. Cell Biol.* **102,** 1020–1031.
7. Mitchison, T. & Kirschner, M. (1984) *Nature (London)* **312,** 232–237.
8. Kirschner, M. & Mitchison, T. (1986) *Cell* **45,** 329–342.
9. Thompson, W. C., Asai, D. J. & Carney, D. H. (1984) *J. Cell Biol.* **98,** 1017–1025.
10. Piperno, G., LeDizet, M. L. & Chang, X. (1987) *J. Cell Biol.* **104,** 289–302.
11. Khawaja, S., Gundersen, G. G. & Bulinski, J. C. (1988) *J. Cell Biol.* **106,** 141–150.
12. Schulze, E., Asai, D. J., Bulinski, J. C. & Kirschner, M. (1987) *J. Cell Biol.* **105,** 2167–2178.
13. Webster, D. R., Gundersen, G. G., Bulinski, J. C. & Borisy, G. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9040–9044.
14. Gundersen, G. G., Kalnoski, M. H. & Bulinski, J. C. (1984) *Cell* **38,** 779–789.
15. Gundersen, G. G., Khawaja, S. & Bulinski, J. C. (1987) *J. Cell Biol.* **105,** 251–264.
16. Kilmartin, J. V., Wright, B. & Milstein, C. (1982) *J. Cell Biol.* **93,** 576–582.
17. Wehland, J., Willingham, M. C. & Sandoval, I. V. (1983) *J. Cell Biol.* **97,** 1467–1475.
18. Argarana, C. E., Barra, H. S. & Caputto, R. (1978) *Mol. Cell. Biochem.* **19,** 17–22.
19. Gundersen, G. G. & Bulinski, J. C. (1986) *Eur. J. Cell Biol.* **42,** 288–294.
20. Geuens, G., Gundersen, G. G., Nuydens, R., Cornelissen, F., Bulinski, J. C. & DeBrabander, M. (1986) *J. Cell Biol.* **103,** 1883–1893.
21. Gotlieb, A. I., McBurnie May, L., Subrahmanyan, L. & Kalnins, V. I. (1981) *J. Cell Biol.* **91,** 589–594.
22. Kupfer, A., Louvard, D. & Singer, S. J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2603–2607.
23. Kumar, N. & Flavin, M. (1982) *Eur. J. Biochem.* **128,** 215–222.
24. Gundersen, G. G., Khawaja, S. & Bulinski, J. C. (1987) in *The Cytoskeleton in Cell Differentiation and Development*, eds. Maccioni, R. B. & Arechaga, J. (IRL, Oxford), pp. 75–81.
25. Vasiliev, J. M., Gelfand, I. M., Domnina, L., Ivanova, O. Y., Komm, S. G. & Olshevskaja, L. V. (1970) *J. Embryol. Exp. Morphol.* **24,** 625–640.
26. Goldman, R. D. (1971) *J. Cell Biol.* **51,** 752–762.
27. Malech, H. L., Root, R. K. & Gallin, J. I. (1977) *J. Cell Biol.* **75,** 666–693.
28. Rinnerthaler, G., Geiger, B. & Small, J. V. (1988) *J. Cell Biol.* **106,** 747–760.
29. Vale, R. D., Reese, T. S. & Sheetz, M. P. (1985) *Cell* **42,** 39–50.
30. Paschal, B. M. & Vallee, R. B. (1987) *Nature (London)* **330,** 181–183.
31. Bergmann, J. E., Kupfer, A. & Singer, S. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1367–1371.
32. Schulze, E. & Kirschner, M. (1987) *J. Cell Biol.* **104,** 277–288.