# Microtubule Polarity Confers Direction to Pigment Transport in Chromatophores

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*Abstract.* The cellular mechanisms used to direct translocating organelles are poorly understood. It is believed that the intrinsic structural polarity of microtubules may play a role in this process. We have examined the effects that differently oriented microtubules have upon the direction of pigment transport in surgically severed melanophore arms. In a previous paper (McNiven, M. A., M. Wang, and K. R. Porter, 1984, *Cell*, 37:753–765) we reported that after isolation, arms repolarized and reoriented their microtubules outward from their centers as if to form new "microcells." Pigment aggregation in these arms was toward a new focal point located at the arm centers. In this study we monitored pigment movement in isolated arms containing taxol-stabilized microtubules to test if the reversal in direction of pigment transport is dependent upon the repolarization of microtubules. We report that taxol delays both the microtubule reorientation and reversal in transport direction in a concentration-dependent manner. These and other presented data suggest that the polarity of the microtubule population within a melanophore confers direction on pigment transport.

THE directed translocation of organelles and trophic substances within cells is an important physiological process. Although a few types of intracellular particle movement have been termed "random" or "saltatory-like" (9, 31, 32), there are many instances in which cells specifically transport organelles from one location to another. This function is most graphically displayed within axons that rapidly transport neurosecretory products orthograde while degradation products such as multivesicular bodies and lamellar bodies are transported retrograde. Highly organized organelle transport is also seen during anaphase A and B of mitosis. Dividing cells are able to direct a motive force and ensure the accurate poleward transport of chromosomes and, at the same time, the elongation–segregation of their spindles.

These motility events demonstrate that force-generating mechanisms are designed to transport specific types of organelles in a directed manner. It is believed that microtubules and actin filaments are involved in this directing process since both these cytoskeletal filaments are polar in design. This polarity, as defined by in vitro studies, is the differential in subunit addition to one end of the filament vs. the other (4, 17, 21, 40). The "plus" end possesses a more rapid subunit exchange than the slower "minus" end. It seems likely that these filaments provide a structural template with which an ATPase interacts to produce a homogeneous unidirectional force used to translocate organelles. For actin-based motility, this concept is reinforced by the fact that inert latex beads, when coated with rabbit skeletal myosin, translocate unidirectionally along a population of uniformly oriented actin filaments (38, 39). The ability of a microtubule to confer direction on transport is less defined. Several models have implicated microtubule polarity in directing mitotic events (6, 22, 23) and axoplasmic transport (27); however, the influence of microtubule polarity on orienting organelle transport has not been directly tested. The fact that particles translocate in both retrograde and orthograde directions along a single microtubule clouds the issue still further (2, 10, 12, 18, 41, 42). The most provocative evidence to date has been the demonstration that kinesin-coated particles translocate orthograde exclusively along in vitro-nucleated microtubules of a defined polarity (43).

Recently, we have examined the effects of altered microtubule polarities upon pigment translocation, in vivo, using teleost melanophores (24, 25). These cells are useful for such studies since they transport thousands of pigment granules either retrograde or orthograde in a temporally distinct manner (5, 7, 11, 26). (For reviews see references 24 and 35). These movements are conveniently controlled by the addition of specific drugs to the culture medium. Epinephrine will stimulate a retrograde pigment aggregation into the cell center whereas caffeine induces an orthograde dispersion of pigment toward the cell margins (Fig. 1, *a–c*). Pigment transport appears to be microtubule dependent since exposing cells to microtubule-destabilizing drugs inhibits pigment movement (3, 37). Actin antagonists such as DNAse I and cytochalasins have, on the other hand, little or no effect (3).

The purpose of our previous experiments was to test whether microtubules within surgically isolated melanophore arms would maintain their original disposition and

polarity over time and whether any changes would alter the direction of pigment transport during aggregation and dispersion. We monitored the polarity of the arms' microtubule populations via a "polarity hook assay" developed by Heidemann and McIntosh which has been described in detail elsewhere (13). We found that severed arms, immediately exposed to epinephrine, aggregated pigment unidirectionally to their cut ends at a rate equal to that observed in intact control cells (Fig. 1, *d–f*), and as expected, the polarity of the microtubules in these arms was unchanged. A microtubule polarity assay revealed that microtubules remained polarized as in intact cells with their minus ends at the cut and their plus ends extending outward to the distal arm tip (Fig. 2 *a*). However, pigment transport in arms that were incubated 3 h after severing was dramatically different. Epinephrine treatment of the severed arm induced pigment aggregation bidirectionally into the arm center away from both proximal and distal arm ends (Fig. 1, *g–h*). We found that these arms (minicells) possessed two populations of microtubules oriented with their minus ends at the arm center and plus ends directed toward both proximal and distal tips (Fig. 2 *b*). Thus during the incubation period the microtubules within the arms had reorganized outward from the arm center, apparently without the presence of a centrosome. How this

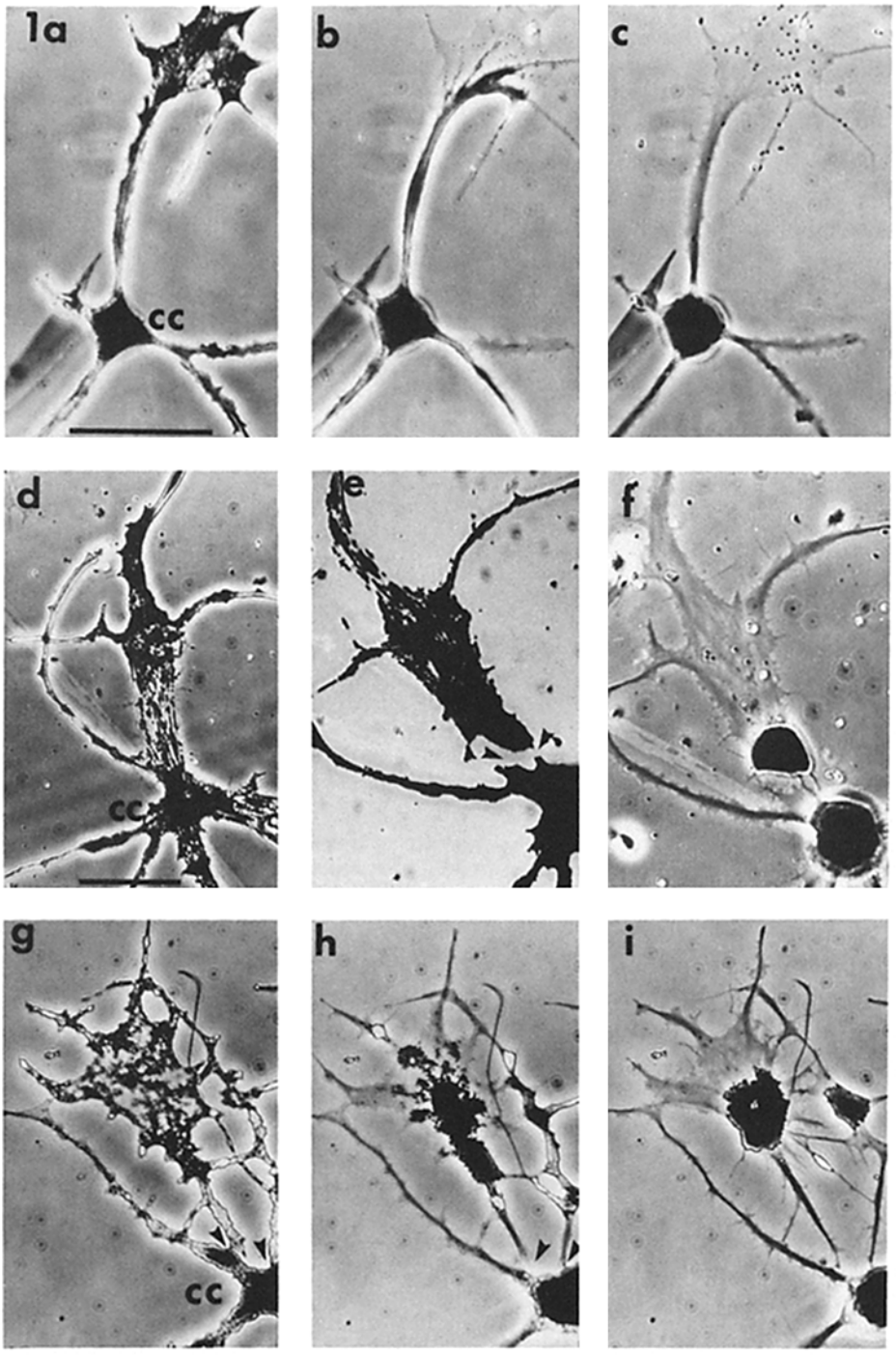



*Figure 1.* Composite figure showing the different types of pigment transport in either intact or severed melanophore arms. A normal epinephrine-induced pigment aggregation in a whole cell (*a–c*). Pigment is transported retrograde out of the arm and into the cell center. A long pigment-filled melanophore arm was severed from the cell (*d* and *e*) and immediately exposed to epinephrine, which induced an aggregation of pigment into the cut or "proximal" end (*f*). (*g–i*) Three pigment-filled melanophore arms, two of which have been severed. After surgical manipulation, the cell was returned to the culture incubator for 4 h and then stimulated with epinephrine. Pigment was transported away from the proximal and distal arm ends into the centers. Bar, 10 μm.

microtubule reorganization occurs and whether a nascent centrosome structure is formed are under investigation. It appeared then that concomitantly with this microtubule repolarization there was a reversal in the direction of pigment transport. From this observation we postulated that the direction of a translocating melanosome is oriented or controlled by the polarity of the microtubules with which it interacts. Aggregating pigment is directed, retrograde, into a cell or arm center along a plus-to-minus microtubule polarity whereas dispersing pigment moves orthograde, outward, along a minus-to-plus microtubule polarity (Fig. 2) (25).

Although the changes in microtubule polarity and direction of transport in severed melanophore arms appear to occur simultaneously, we did not know if these changes represented a cause and affect sequence, or a simple coincidence. We decided to test the dependence of one reversal event upon the other by stabilizing the microtubules within isolated arms with the microtubule-stabilizing drug taxol. If the polarity of microtubules does indeed play a role in directing transport of pigment then one would predict that a severed arm will continue to aggregate pigment unidirectionally retrograde to its cut end while in the presence of taxol. In this paper, we report that taxol does in fact inhibit the reversal of pigment aggregation into an arm center for up to 7 h in a concentration-dependent manner. This inhibition is rapidly reversed upon removal of the taxol from the culture medium. We have also found that over extended time periods, arms do eventually reverse the direction of their pigment transport, even in the presence of saturating concentrations of taxol. Microtubule polarity assays of these minicells show that a repolarization of microtubules occurs.

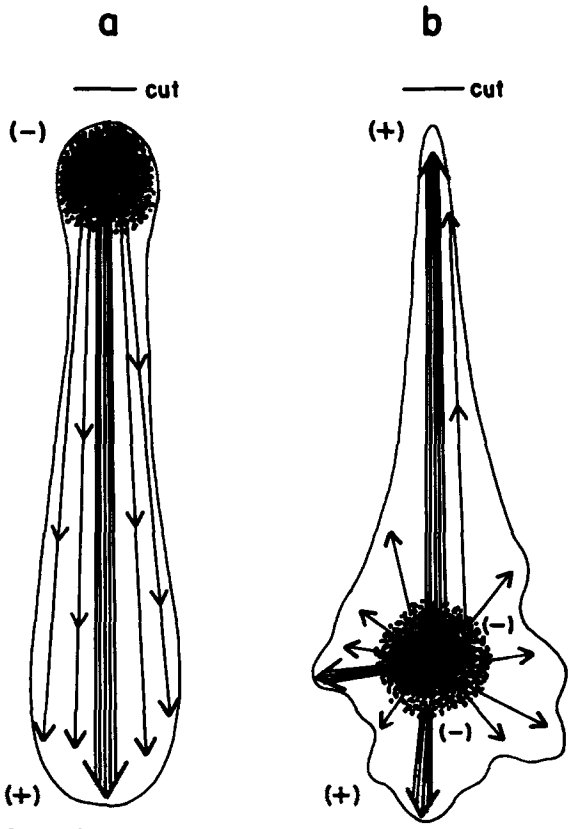



*Figure 2.* Diagram illustrates the effects of microtubule polarity on the destination of pigment within arms which were fixed immediately after severing (*a*), and 4 h after severing (6).

## Materials and Methods

Chromatophore culturing, cell microsurgery, growth of microtubule hooks, and electron microscopy have been described, in detail, in a previous paper (25).

### Taxol Treatment

Taxol in powder form was obtained through the courtesy of The Division of Cancer Treatment, Drug Synthesis and Chemical Branch, National Institutes of Health, Bethesda, MD. It was solubilized in dimethyl sulfoxide to a stock solution concentration of 10 mM and frozen at −30°C until needed. Working taxol solutions of $10^{-8}$ M through $10^{-4}$ M in culture media were made less than 1 h before use. Melanophore cultures were exposed to taxol-media solutions 1 h before severing. Melanophores arms were severed in the presence of taxol then placed in fresh taxol solutions for the subsequent incubation period. For extended incubation periods, cells were placed in freshly made taxol solutions every 2 h.

## Results

### Isolated Melanophore Arms Reorganize Their Microtubules before Pigment Aggregation

The fact that isolated melanophore arms appear to reorganize their microtubules while changing the direction of their pigment transport suggests that the intrinsic polarity of the microtubule population within an arm lends direction to pigment movement during aggregation. To test this concept it is necessary to establish the time point at which both events occur. For example, severed arms might aggregate pigment into their centers before a microtubule reorganization. It has been reported that >50% of the microtubules within a melanophore are dissassembled during pigment aggregation (36). Thus, it is possible that, upon aggregation, pigment is transported into the severed arm center concomitantly with a massive microtubule depolymerization; followed by a repolymerization and reorientation of arm microtubules. Therefore, the initial aggregation of pigment into the arm center would use unaltered arm microtubules and would not be dependent upon a repolarization of microtubules, indicating that other undetermined factors are responsible. We tested this possibility by examining the polarity of microtubules within arms which were severed, incubated for 3 h, but never aggregated. We found that in the two arms assayed, over 85% of the microtubule populations had reoriented and were polarized with their minus ends in the arm center and plus ends extending out toward the distal and proximal arm tips (Table I). This result indicates that the rearrangement of microtubules observed after arm severing is not dependent upon the process of pigment aggregation itself but takes place during the 3-h incubation period after severing. Thus microtubule reorganization precedes the reversal in direction of transport.

### Taxol Reversibly Delays Both the Reversal in Direction of Pigment Transport and the Reorganization of Microtubules

We have established that the microtubule reorganization in severed arms occurs before reversal of pigment transport. We next tested the dependence of each event upon the other. Can they be temporally separated or are they truly concomitant

*Table I. Severed Melanophore Arms Repolarize Their Microtubules before Pigment Aggregation*

| | | Clockwise microtubules | Counterclockwise microtubules | Clockwise | Counterclockwise |
|---|---|---|---|---|---|
| | | | | % | % |
| **Cell A* | Prox | 11 | 50 | 18 | 82 |
| | Dist | 62 | 6 | 91 | 9 |
| **Cell B* | Prox | 13 | 60 | 18 | 82 |
| | Dist | 52 | 4 | 93 | 7 |

* Polarities of microtubules in severed arms, never aggregated. *Prox*, Proximal to arm's center. *Dist*, Distal to arm's center.

and interdependent? To examine this question, we treated severed melanophore arms with the microtubule-stabilizing drug taxol and looked to see whether melanosomes persist in reversing the direction of their aggregation over time, even along a taxol-stabilized microtubule population. If so, it would demonstrate that the two events are independent of each other. First, it should be noted that the treatment of intact melanophores with high concentrations of taxol has little overall effect upon the rate of pigment transport although the drug does prevent a small amount of pigment from translocating into the cell center during aggregation. Taxol induces the formation of numerous, well-ordered microtubule bundles which course through the cytoplasm displacing the pigment into linear files (Fig. 3 *a*). The polarity of these bundles approaches homogeneity (plus ends extend outward toward the distal cortices), although there is a small population of microtubules with a reverse polarity. We assayed the polarities of microtubules in two intact, taxol-treated melanophores and found that 7 and 14% of the arm microtubules were of the opposite polarity and had their minus ends oriented toward the distal cortices and plus ends toward the cell center (Table II).

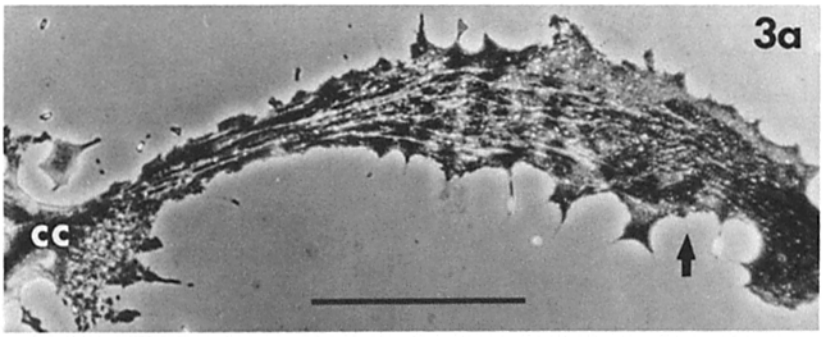
We next tested the effects of taxol on pigment transport within severed arms and found that the drug delays the aggregation of pigment into the arm center in a reversible, concentration-dependent fashion. These effects are illustrated in Fig. 4, which shows a melanophore arm treated with $10^{-5}$ M taxol before and after surgical isolation. When the taxol-treated arm was stimulated with epinephrine to aggregate its pigment 5 h after severing, the pigment did not move bidirectionally into the arm center, as in untreated arms at the same time point, but instead aggregated unidirectionally to the cut (Fig. 4 *b*). When the pigment was redispersed and the arm rinsed in drug-free medium for 30 min (Fig. 4 *c*), then stimulated to aggregate a second time, pigment aggregated away from both the cut and distal tip into the arm center (Fig. 4 *d*). Nine other severed arms treated the same way all responded in an identical fashion.

We have also observed the effects of varying taxol concentrations on severed arms over extended time periods and have found that, at saturating drug concentrations, arms continue to aggregate pigment unidirectionally to their cut ends for up to 8 h but will eventually reverse the direction of their pigment transport. Fig. 5 depicts, in a qualitative way, the delay in the reversal of pigment transport into the centers of severed arms by taxol. For each drug concentration examined, 10 melanophore arms were severed and stimulated to aggregate pigment every 30 min until a reversal in the direction of pigment transport took place. As expected, high concentrations of taxol prevented pigment from reversing its direction of transport for a longer time period than did low concentrations.

The fact that severed arms eventually aggregated pigment toward their centers while in $10^{-4}$ M taxol was surprising. We considered two explanations for such a response. First, microtubules within the arms are stable but the pigment has reversed its transport direction regardless. Second, repolarization of arm microtubules does occur even in the presence of taxol. To distinguish between these possibilities we examined the polarity of microtubules within the drug-treated severed arms to ensure that the reorientation/repolarization of microtubules is indeed delayed by taxol. We treated severed arms with $10^{-5}$ M taxol as described earlier then assayed the polarity of their microtubules 5 h later without ever inducing pigment aggregation. In the three arms examined, we found that only 12% of the proximal arm microtubules had changed their polarity (Table III). This number is similar to that seen in intact taxol-treated arms (Table II), indicating that taxol does stabilize a large majority of the arm microtubules for up to 5 h. Next, we examined the polarities of microtubules in taxol-treated arms that had been exposed to taxol for extended time periods after severing and in which pigment eventually aggregates. Four different arms were treated with $10^{-7}$ M, $10^{-6}$ M, $10^{-5}$ M, and $10^{-4}$ M taxol, respectively, for 8 h. When exposed to epinephrine, these arms aggregated pigment bidirectionally into their centers. A microtubule polarity assay revealed that 85% of their proximal microtubules had reversed polarity with their minus ends situated at the arms' centers (Table IV). Thus it appears that taxol is able to retard but not prevent the reversal of microtubule polarity within severed arms.

## Discussion

In this study we have examined, in vivo, the dependence of a reversal in pigment transport direction upon alterations in microtubule polarity. Understanding the order of these events in severed arms is important for predicting the role of microtubules in directing transport. This was accomplished by first examining if severed arms reorganize their microtubules before or after aggregating pigment to their centers. A reversal in the direction of pigment transport after the reorganization of microtubules would support the notion that microtubule polarity confers direction on transport. However, a reversal in the direction of transport before a microtubule repolarization would repudiate this concept. We have found that severed arms containing dispersed pigment will repolarize their microtubules without ever being stimulated to aggregate (Table I). Thus, the microtubule rearrangement exhibited by isolated arms occurs before, and is not depen-



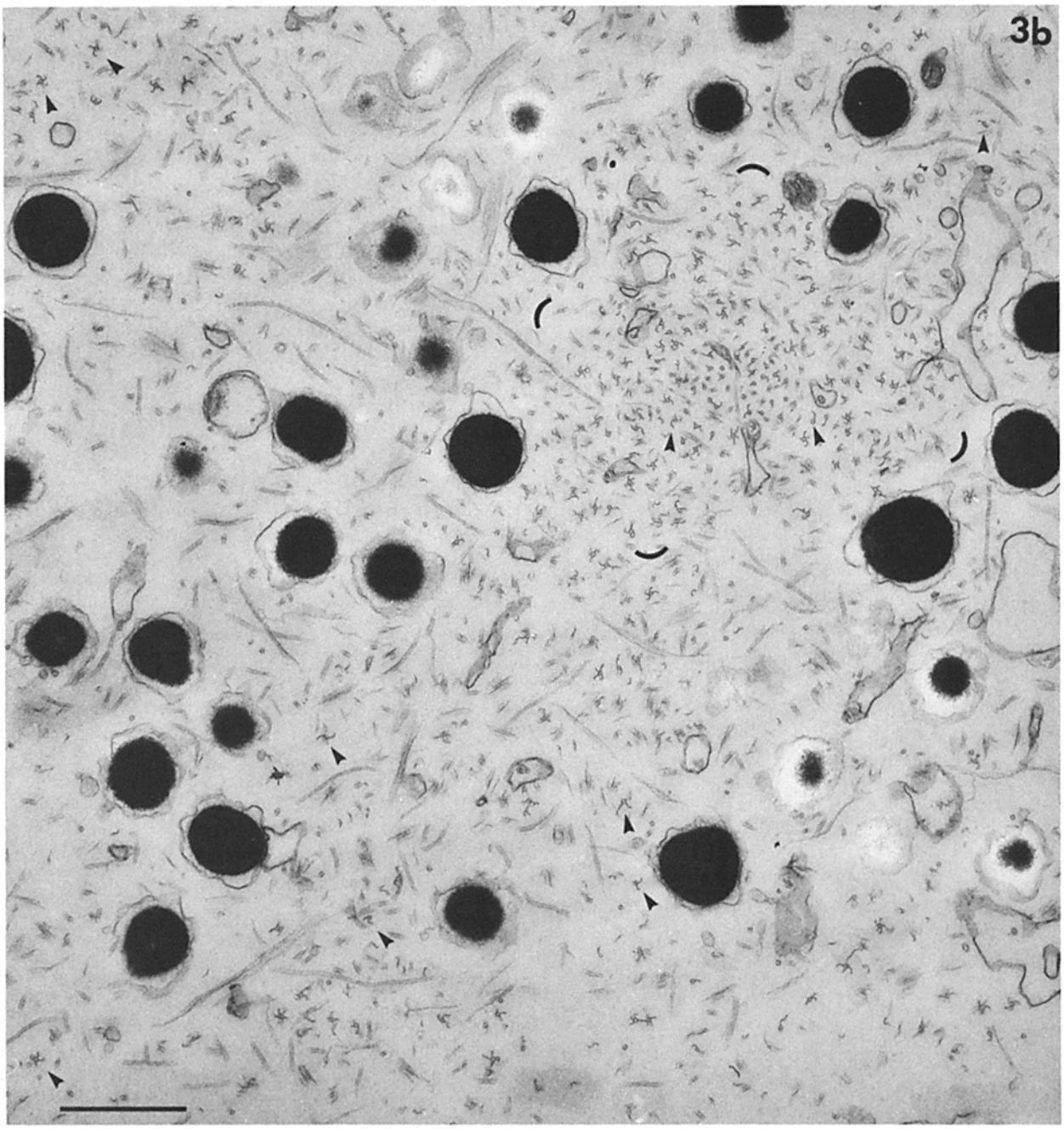




*Figure 3.* (*a*) Phase light micrograph of an intact melanophore arm which has been treated with $10^{-5}$ M taxol for 2 h. Numerous microtubule bundles can be seen as clear lines which displace the melanosomes. (*b*) The same melanophore which has been subjected to the microtubule polarity assay, fixed, embedded, and then sectioned from the distal arm tip into the cell center. The clockwise hooks on the majority of the microtubules represent the "plus ends." The misoriented microtubules with minus ends showing (counterclockwise) are marked with arrows. One of the large microtubule bundles seen in the light micrograph (*a*) is present. Bar, (*a*) 10 μm; (*b*) 0.5 μm.

*Table II. The Effects of Taxol on Microtubule Polarity in Whole Intact Melanophores*

| | Clockwise microtubules | Counterclockwise microtubules | Clockwise | Counter-clockwise |
|---|---|---|---|---|
| | | | % | % |
| *Cell A* | 107 | 7 | 93 | 7 |
| *Cell B* | 154 | 25 | 86 | 14 |

* Polarities of microtubules in unsevered melanophore arms treated with $10^{-5}$ M taxol for 5 h.

dent upon, the process of pigment aggregation. Second, we attempted to stabilize chemically the arm microtubule population with taxol to test whether the pigment aggregation into the arm center is delayed or prevented all together.

### *Effects of Taxol on Pigment Movement*

The taxol treatment of severed melanophore arms produced some unexpected results. We found that taxol does have a stabilizing effect upon the polarity of arm microtubules and prevents repolarization for the 5-h time period tested (Table III, *A–C*). Coinciding with this, there is a delay in the reversal of pigment transport direction into the arm center. The duration of the delay is dependent on the concentration of taxol used (Fig. 5) and is rapidly reversible when the drug is removed (Fig. 4). The repolarization of microtubules within the arm after rinsing is not totally surprising, since it coincides with earlier work demonstrating that taxol-stabilized spindle microtubules become labile after rinsing with drug-free media (33). Taxol, then, does not appear to be a microtubule fixative. The very rapid reorganization of microtubules after taxol removal (30 min) suggests that the severed arms have been "primed" during the drug incubation after severing. Such a priming process may represent the establishment of a new centrosome-like structure within the arm center which can be used as soon as the drug is removed. It is surprising that even saturating concentrations of taxol can not prevent the switch in microtubule polarity or the reversal in transport direction. Arms which aggregate pigment into their centers after 8 h in taxol have repolarized >80% of those microtubules (located proximally) so their minus ends are at the center and plus ends extending out (Table IV). This level of reorganization is close to that seen in non-severed taxol-treated arms from control cells (compare cells in Table II with cells in Table IV) which contain some

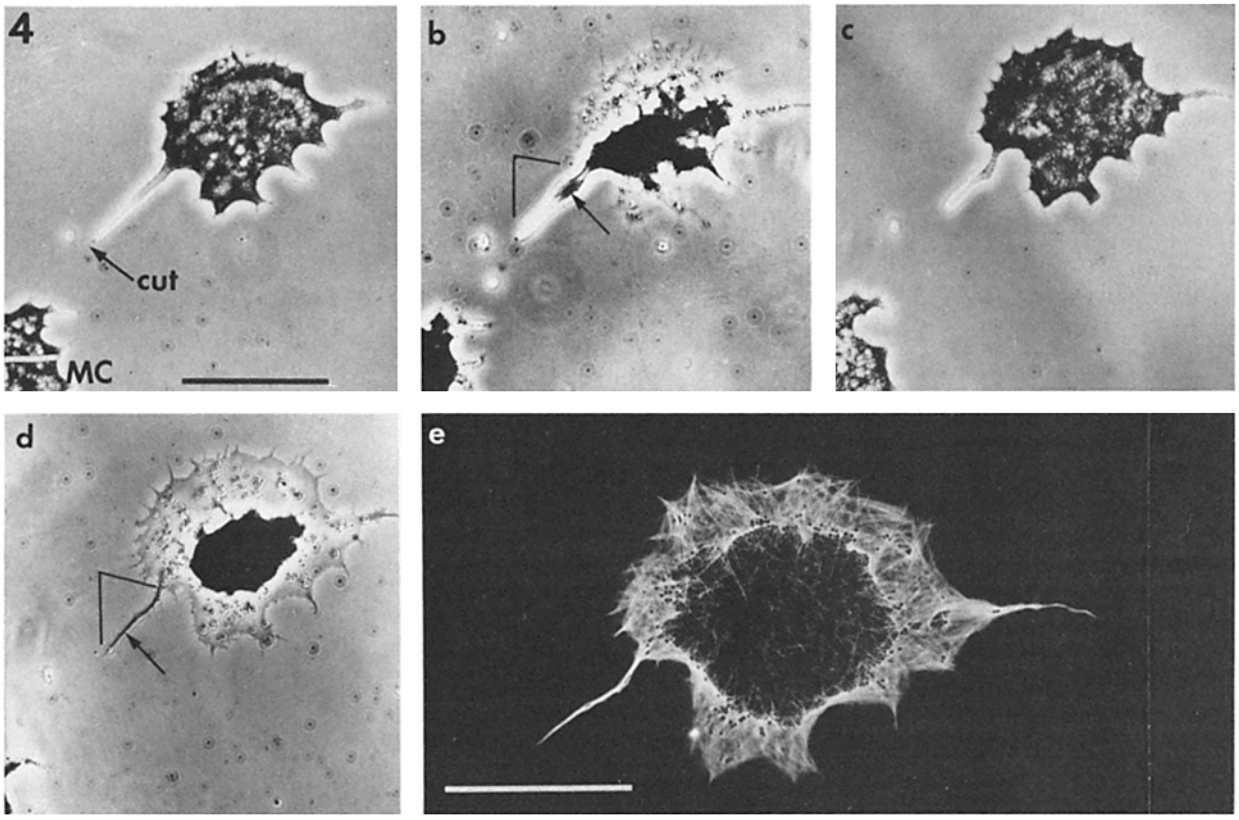



*Figure 4.* The taxol-induced delay of pigment aggregation to an arm center can be reversed with rinsing. A phase photomicrograph series of an isolated melanophore arm treated with $10^{-5}$ M taxol. (*a*) A large dispersed melanophore arm was severed from the main cell (*MC*). (*b*) 4 h later epinephrine was added to the taxol-containing medium to induce aggregation. Pigment was transported from the distal periphery and formed a linear column which moved unidirectionally toward the cut. The thin proximal arm region (bracket), which is already filled, is bulging (arrow) in an attempt to accommodate all of the pigment from the distal arm region. It is important to note that none of the pigment in the proximal arm region has moved away from the cut. (*c*) Epinephrine was removed, the arm then redispersed in caffeine and rinsed for 30 min in drug-free medium. (*d*) The arm was again stimulated to aggregate. This time pigment was transported bidirectionally, emptying the arm's thin proximal region (bracket) and forming a large mass at the arm center. (*e*) The same arm stained with fluorescent tubulin antibodies. There are large numbers of microtubules, many which appear to extend outward from the pigment mass toward the cortex. Some of the microtubules are oriented randomly. Bars, 10 μm.

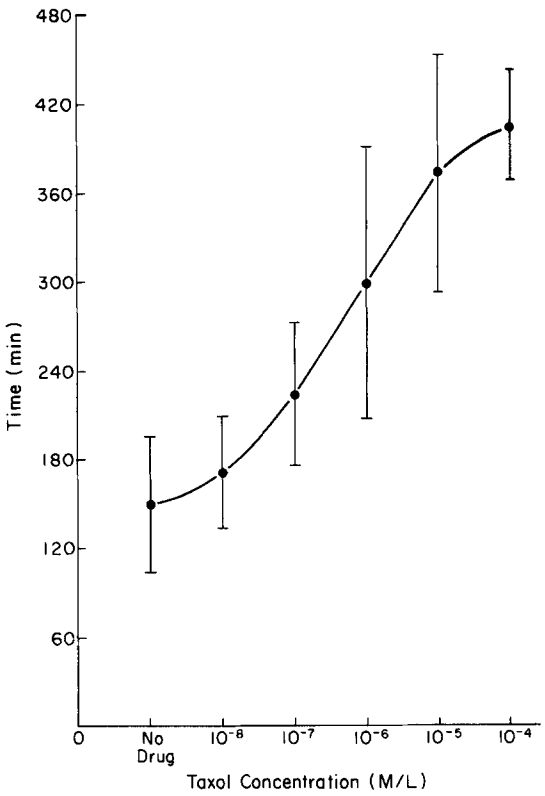



*Figure 5.* Taxol delays aggregation of pigment into the center of isolated arms. The graph qualitatively shows the effect of increasing taxol concentrations on the incubation time needed for arms to reverse their direction of pigment transport after severing. High taxol concentrations will delay the reversal for over 8 h and may prevent a full aggregation of pigment into the arm center all together. For each concentration of taxol tested, 10 severed arms were monitored and stimulated to aggregate pigment every 30 min until transport reversed direction.

randomly polarized microtubules despite the presence of a centrosome. Apparently taxol induces the formation of some microtubules with random polarities even when a centrosome is present (Table II). We find it remarkable that arm cytoplasm without an evident centrosome can repolarize its microtubules even in the presence of taxol. This is in contrast with past work which has demonstrated that purified microtubules treated with $5 \times 10^{-6}$ M taxol are insensitive to 4°C cold, 4 mM calcium, and antimitotic drugs (15, 20, 34). How, then, do severed arms counteract the stabilizing effects of taxol? Work by De Brabander et al. (8) has shown that $PtK_2$ cells incubated in $10^{-5}$ M taxol form noncentrosome-associated cytoplasmic microtubule bundles. Some drug-treated cells enter mitosis, disassemble the bundles, and form multiple asters instead of a normal spindle. Several hours later the asters disappear while the microtubule bundles seen earlier are reconstructed. These results suggest that the cellular mechanism used for assembling and disassembling microtubules is more effective than any influence we can exert exogenously. Taxol may interfere with the assembly of a highly ordered structure such as the mitotic spindle but not with less complex microtubule arrangements such as asters.

### *The Direction of Pigment Movement in Isolated Arms Is Homogeneous*

The fact that severed melanophore arms do not alter the direction of their pigment transport in the proximal zone until they have reoriented their microtubule framework suggests that these two processes are intimately linked. Our data indicates that the direction in which a melanosome is transported appears to be dependent upon the polarity of the microtubules it interacts with. Thus, all melanophore arms that transport pigment to and from the cell center of intact melanophores, the cut end of an arm, or a new arm center, abide by a common rule. Aggregation of pigment is always directed toward the minus end of the microtubule population while dispersion is always toward the population's plus end. We have not at any time observed any violation of this rule. It is worth pointing out that the direction of melanosome transport in severed arms is totally uniform during aggregation and dispersion despite the presence of a sizeable population of oppositely oriented microtubules. The number of these randomly polarized microtubules is small (<5%) in the peripheral regions of the arms, but may reach over 20% near the arms' centers. If each melanosome functions autonomously and translocates independently, guided by the polarity of a single microtubule, one would expect to see a percentage of pigment move in a direction opposite to the rest. This is not the case; never have we observed a melanosome to move in a direction contrary to the others. Pigment aggregation and dispersion are always 100% homogeneous. It appears then, that translocation of pigment is directed by the polarity of the microtubule majority and is not affected by small numbers of microtubules oriented in the opposite direction. This model makes the assumption that each pigment granule forms multiple interactions, or is tied to a

*Table III. Taxol Delays the Repolarization of Microtubules in Severed Melanophore Arms*

| | | Clockwise microtubules | Counterclockwise microtubules | CLW | CCLW |
|---|---|---|---|---|---|
| | | | | % | % |
| **Cell A* | Prox | 130 | 18 | 88 | 12 |
| | Dist | 165 | 25 | 87 | 13 |
| **Cell B* | Prox | 86 | 10 | 89 | 11 |
| | Dist | 68 | 24 | 74 | 26 |
| **Cell C* | Prox | 160 | 45 | 88 | 12 |
| | Dist | 113 | 25 | 82 | 18 |

* Polarities of microtubules from melanophore arms treated with $10^{-5}$ M taxol for 5 h after severing. Pigment never aggregated. *Prox*, Proximal to arm's center. *Dist*, Distal to arm's center.

*Table IV. Taxol Does Not Prevent Severed Melanophore Arms From Repolarizing Microtubules Over Long Time Periods*

| | | Clockwise microtubules | Counterclockwise microtubules | Clockwise | Counterclockwise |
|---|---|---|---|---|---|
| | | | | % | % |
| **Cell A* $10^{-7}$ M Taxol | Prox | 22 | 87 | 20 | 80 |
| | Dist | | | (no distal Mts counted) | |
| **Cell B* $10^{-6}$ M Taxol | Prox | 5 | 50 | 9 | 91 |
| | Dist | 74 | 24 | 75 | 25 |
| **Cell C* $10^{-5}$ M Taxol | Prox | 16 | 95 | 15 | 85 |
| | Dist | 73 | 16 | 82 | 18 |
| **Cell D* $10^{-4}$ M Taxol | Prox | 9 | 89 | 9 | 91 |
| | Dist | 53 | 7 | 88 | 12 |

* Polarities of microtubules in severed arms which aggregated pigment toward a new center, while in taxol, 8–9 h after severing. *Prox*, proximal to arm's center. *Dist*, distal to arm's center.

lattice-work, involving several microtubules (29,30). It is possible that the force generated by interactions with the larger population of properly oriented microtubules would predominate over the force provided by the smaller, randomly oriented, microtubule minority. Multiple granule–microtubule bridging structures have been visualized in chromatophores by both conventional fixation (7, 19) and quick-freezing methods (16).

### *Dual Mechanisms for Pigment Transport?*

The premise that the polarity of a microtubule population can direct transport raises a difficult question. How can a motility mechanism use one microtubule polarity to aggregate pigment and the opposite polarity to disperse pigment? Both Porter (28) and McIntosh (23) circumvented an analogous problem for mitosis by proposing a logical alternative. They suggested that during metaphase a dynamic matrix or net could elongate outward along uniformly oriented microtubules extending from the poles. At anaphase the matrix would expend stored energy and contract inward. The direction of this "elastic" contraction would be independent of microtubule polarity. Our data indicates that in melanophores, at least, orthograde and retrograde motion are both directed by microtubule polarity. This raises the possibility that different motility mechanisms transport pigment in opposite directions. This notion is supported by the fact that translocation of kinesin-coated beads along a unipolar population of microtubules is predominantly orthograde in direction. A second uncharacterized axonal component has been implicated in transporting axoplasmic particles in the retrograde direction.

There are noticeable differences in the characteristics of orthograde vs. retrograde movement in many cell types. Pigment dispersion in chromatophores is slow and saltatory whereas aggregation is rapid and takes place at a uniform velocity. Pigment aggregation, like retrograde axonal transport, is noticeably more sensitive to *N*-ethymalaimide and sodium vanadate than is orthograde dispersion. Other cell types exhibit these differences as well. During endocytosis in granulosa cells, endosomes saltate gently after internalization, then stop saltating and translocate centripetally to the cell center at a constant velocity (14). The axoplasmic transport of microinjected polystyrene beads is equal in rate and character to the transport of endogenous particles but only in the orthograde direction, suggesting that orthograde transport uses a different system which has less stringent requirements for the type of particle carried (1).

Most of the observations listed above are subtle and only suggest that cells use dual translocating mechanisms. Alternatively, such contrasts may represent a single motility motor that behaves differently when performing retrograde vs. orthograde translocation functions. We are optimistic that future biochemical dissections of temporally distinct transport events like pigment aggregation and dispersion will define the number of functional motility mechanisms and how such motors could use opposite microtubule polarities.

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