Chromatophores—Models for Studying Cytomatrix Translocations

MARK A. McNIVEN and KEITH R. PORTER
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

Teleost chromatophores have proven valuable in the study of the cytomatrix and its involvement in intracellular transport. These cells, which move their pigment rapidly, can be isolated from the scales of fish and maintained in culture for several days. Their transport activity can be followed with the light microscope and recorded by cinematography. Individual pigment granules can be seen moving radially toward the cell center in aggregation, then back toward the cortices during dispersion. These two motions are different. In aggregation, the granules move smoothly and rapidly (5–10 μm/s), reminding one of the behavior of chromosomes during anaphase. In dispersion, which takes twice as long as aggregation, the granules move haltingly, first out (orthograde) and then back (retrograde). This saltatory motion continues throughout dispersion but ceases suddenly at the onset of the next aggregation.

The direction of pigment motion is easily controlled by the addition of epinephrine to the medium to stimulate aggregation or of caffeine (a phosphodiesterase inhibitor) to stimulate dispersion. This relative ease in temporally separating two types of movement distinct in direction, rate, and character enhances the value of chromatophores for experimental studies on the mechanisms of intracellular transport.

All of the fish chromatophores we have studied are capable of rapid pigment transport and possess a similar internal organization (5, 18, 32). A highly structural centrosome complex consisting of a centriole pair and surrounding stratified layers of dense material occupies the cell center (31, 42). Thousands of microtubules nucleate from this structure and extend outward, organizing the pigment into linear columns.

There are few, if any, intermediate filaments in chromatophores (none in erythrophores) and only small amounts of filamentous actin, which is subcortical in distribution (3, 43). A smooth endoplasmic reticulum (SER)[1]-like membrane network encompasses the centrosome and extends outward along microtubule bundles to the plasma membrane (25, 38).

The cell constituents described above are clearly demonstrated by conventional thin sectioning; however, the structure of the surrounding cytoplasm is poorly defined and has been described only as wispy flocculent material. Whole-mount high-voltage electron microscope examinations of cultured chromatophores have shown this material to be a three-dimensional latticework of fine filaments or trabeculae (2–6 nm in diameter) that suspend the pigment (9, 24, 39). These filaments undergo a conformational change during pigment movement. With the pigment dispersed, the filaments are thin and delicate in appearance, as if extended. Upon aggregation, the trabeculae become shorter and beaded, as if contracted, and appear to clump the pigment together during aggregation.

These observations support the work of Green (18), who closely watched the effects of pigment transport on other cell components, such as the mitochondria, nucleus, and plasma membrane. She stated that "the granule-moving system is expressed by the spatial distribution of granules and is the function of a dynamic equilibrium between concentrative and dispersive forces" and concluded that a continuum "contracts" during aggregation and "expands" during dispersion and seems to be centered at or around the cell centrioles (18).

If the pigment indeed is suspended or encased in a latticework, one would predict that each granule occupies a fixed position within it. Using high-resolution bright-field optics and a video camera, we were able to follow and record the movements of dense, more prominent, granules within cultured erythrophores (38). Each granule aggregated from and redispersed to its original starting point with remarkable accuracy. In fact, even after several hours of repeated pulsations, the identifiable granules maintained their fixed positions. We interpreted this to mean that each granule occupies a fixed position within a unit structure, the cytoplast (38, 39). Although the cytoplast undergoes dramatic reversals in form during aggregation and dispersion, its structural integrity is maintained, inasmuch as any solation or disintegration would disrupt the positioning of pigment. We now realize that the uniform distribution of pigment in dispersion is essential for a complete change in the color of the fish and therefore for its protection against predators. It follows that to achieve this the pigment must be structurally contained and not free to diffuse randomly.

[1] *Abbreviations used in this paper:* HMW-MAP, high molecular weight microtubule-associated protein; SER, smooth endoplasmic reticulum.

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 NO. 1 PT. 2 JULY 1984 152s–158s
© The Rockefeller University Press · 0021-9525/84/07/0152s/07 $1.00

We were hopeful that close morphological studies of the cytoplast during pigment movement could lend insight into the force-generating mechanism. The relatively slow-working chemical fixation of glutaraldehyde may distort a highly dynamic structure such as the chromatophore cytoplast. It would be preferable to rapidly freeze cells and then chemically fix its structures while frozen. The whole-mount, freeze-substitution technique does this (37). Aggregating or dispersing erythrophores are plunged into liquid propane (−185°C). Over a 48-h period, the cell water is substituted with an osmium tetroxide–methoxyethanol mixture at −90°C. The mixture fixes cell components while extracting the carotenoid-containing pigment granules. The remaining structure clearly depicts the distribution of matrix during dispersion and aggregation. In dispersed cells, the cytoplast extends radially outward from the centrosphere and appears evenly distributed throughout the cell (Fig. 1 *c*). Cells quick-frozen during aggregation have an altered cytoplast. The pigment-bearing matrix becomes withdrawn from the cortex and forms a compacted ring encircling the cell center (Fig. 1 *d*). This matrix ring in completely aggregated cells is fully compacted, as if reaching its maximum state of contraction. High-magnification images of the contracting matrix reveal structural changes not seen before. As in conventionally fixed cells, the trabeculae have shortened and thickened, becoming beaded. In addition, the beaded trabeculae become grouped into thousands of small clusters, as if contracting to shared focal attachment sites (39). These clusters accumulate at the cell center to form the concentric matrix ring seen in aggregated cells. In addition to the motile lattice, which moves with the pigment, we observe a matrix that does not cluster but remains peripheral during aggregation. We believe this residual lattice is a nonmotile structural component which, in conjunction with microtubules, maintains cell shape and provides support for mitochondria and the SER during pigment movement. No doubt the pigment-moving matrix is a highly differentiated structure designed for a specific task, the rapid and uniform transport of thousands of pigment organelles. The residual matrix, however, may persist in all cells.

The composition of neither lattice is known. It is unlikely that actin is a component, because antibodies to actin stain only a fine cortical meshwork (43). In addition, actomyosin inhibitors (*N*-ethylmaleimide-modified heavy meromyosin) and drugs that induce actin disassembly (cytochalasin B, DNase I) or assembly (phalloidin) have no effect on pigment movement or the integrity of the lattice (3). If actin does participate structurally or functionally, then it does so by a mechanism without precedent.

Calcium and Energy

It appears that matrix contraction in chromatophores not only requires but is controlled by Ca^{++} (25). Erythrophores cannot aggregate their pigment when in Ca^{++}-deprived medium. When Ca^{++} is increased to 5×10^{-6} M in the presence of Ca^{++} ionophore, aggregation upon stimulation proceeds normally. These observations suggest that cytoplasmic free Ca^{++} is involved in the mediation of pigment aggregation. It is not known, however, whether Ca^{++} enters the cytoplasm from the external environment or from intracellular stores. The SER-like membrane network described above is a prime candidate for a Ca^{++} sink because it is distributed throughout the cytoplasm and therefore could induce a uniform matrix contraction.

The metabolic energy used for matrix contraction and elongation is less clearly defined. Studies in which chromatophores were exposed to dinitrophenol and other inhibitors of oxidative phosphorylation show that when cellular ATP is depleted, dispersed cells aggregate and cannot be induced to redisperse (22, 24). Depletion of cellular ATP induces mitotic chromosomes to behave in a similar fashion. Exposure of the diatom *Hantzchia* to dinitrophenol during prometaphase causes chromosomes to move to either pole before ceasing movement. Upon removal of the drug, chromosomes back away from the poles (35).

The tendency for chromosomes or pigment to aggregate at the poles when energy is depleted supports the concept that work is performed to extend and maintain extension of the matrix. Without ATP, the matrix returns to its lower energy state of contraction. Although these metabolic-inhibitor experiments appear straightforward, it is possible that the drugs produce spurious results by indirectly affecting the motility mechanisms. For example, depletion of cellular ATP would also turn off Ca^{++} pumps and cause internal Ca^{++} levels to become abnormally high, inducing a matrix contraction. Exposure of metabolic inhibitors to detergent-permeabilized melanophores produces different results. In contrast to the findings of the studies described above, dispersed melanophores without cellular ATP will not aggregate until exogenous ATP is added (12). One may conclude from this that both pigment dispersion and aggregation require energy.

Thus, although all experiments performed to date show that matrix elongation is an energy-dependent event, the metabolic events needed for contraction are unknown.

Role of Microtubules

Although chromatophores are considered a model for microtubule-based motility, it is not clear whether microtubules play a direct or a supplemental role in providing motive force. Schliwa and Euteneuer (41) and Beckerle and Porter (3) have performed tests to determine whether chromatophores treated with microtubule poisons maintain the capacity to transport pigment. They found that without microtubules the pigment loses its radial disposition and becomes randomly dispersed. When these cells are stimulated to aggregate, the pigment gathers into small clumps. Over a prolonged period, these clumps partially aggregate to the cell center but cannot be induced to redisperse. These observations indicate that microtubule depolymerization and repolymerization by themselves cannot induce or provide the motive force for pigment transport (42). More importantly, the clumping and slow centripetal movement of clumps suggest that a synergistic relationship exists between matrix and microtubules. The contractile matrix provides a motive force that somehow is organized and directed by the microtubules.

The microtubule-independent transport of pigment is so lethargic in comparison to normal movement that it is possible that microtubules do contribute to the movement structurally and mechanochemically as well. It is likely that proteins closely associated with microtubules mediate interactions between microtubules and the contractile matrix. Efforts to demonstrate the involvement of a dyneinlike molecule have proven interesting but contradictory. Sodium vanadate (an inhibitor of flagellar dynein ATPase) has been used as an

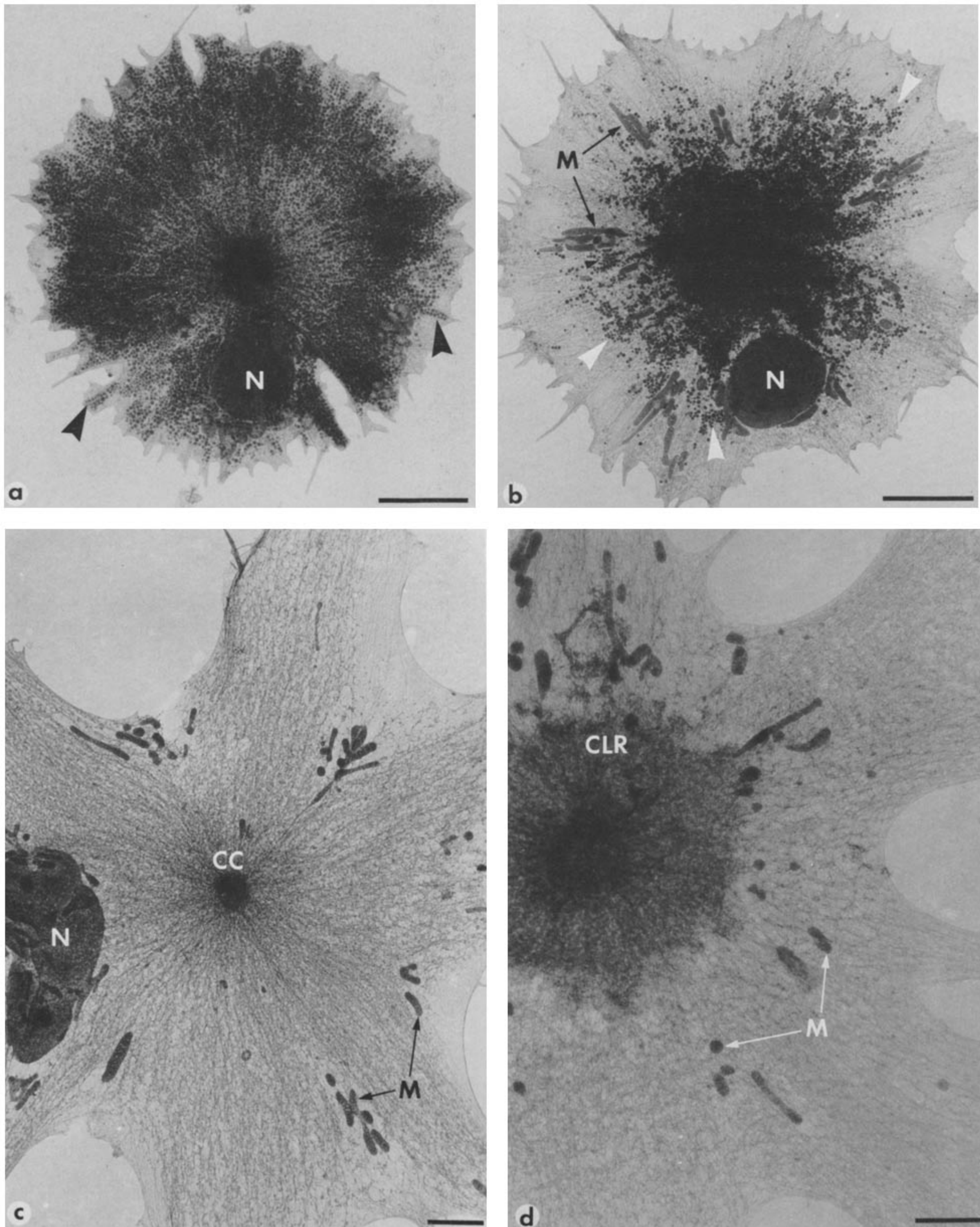

FIGURE 1 (*a* and *b*) Cultured squirrel fish erythrophores fixed with glutaraldehyde, critical-point dried, and viewed under 1 MeV. In dispersion (*a*) the pigment is organized along microtubules into long radial files (arrowheads). During aggregation (*b*) the pigment becomes less linear and clumps together as it moves inward. The mitochondria (*M*), which are also oriented along microtubules, remain on the periphery. *N*, nucleus. (*c* and *d*) Cultured erythrophores preserved by freeze-substitution. The arrangement of matrix in these cells reveals the distribution of pigment before it was extracted by the substitution solution. In the dispersed cell (*c*) the lattice extends outward uniformly from the cell center (*CC*) to the plasma membrane. During aggregation (*d*) a portion of the lattice withdraws from the periphery to form a compact ring (*CLR*) around the cell center. In fully aggregated cells, this ring becomes more compact, as if fully contracted.

in vivo probe for a dyneinlike molecule in various cell types. It has been shown to halt particle movement in cultured fibroblasts (17) and spindle elongation during mitotic anaphase (10). The injection of vanadate into cultured erythrophores inhibits saltatory motion as well as aggregation of pigment (2). Similar experiments using permeabilized chromatophore systems also have shown an inhibition of pigment movement by vanadate (13, 46). More recent work, however, has shown that injected vanadate has no effect on particle movement in fibroblasts (7) or pigment transport in melanophores (6). Although these reports do not discount earlier work, they are reminders that experiments with dynein-inhibitory drugs should be interpreted with caution.

The high molecular weight microtubule-associated proteins (HMW-MAPs) may connect matrix to microtubules. It has been shown that antibodies to HMW-MAP-1 stain the microtubule array and centrosome of cultured erythrophores (34). Stearns (45) has used fluorescent antibodies to MAP-1 and MAP-2 to monitor their distribution during pigment movement. He reports that during aggregation the MAP-2 component comigrates with the pigment, whereas MAP-1 stains a nonmotile component that remains associated with the microtubules. This differential distribution of MAPs supports the concept that there are several structurally distinct lattices that perform different functions.

Microtubules and Matrix

What structural cues within the cytoplast ensure that the matrix moves in a uniform direction during aggregation or dispersion? It is likely that microtubules and their proteins are involved because we know that pigment movement without microtubules is random in direction. We can infer that microtubules in some way provide direction to movement. Microtubules may act either as inert structures that merely columnize the matrix and its pigment as it contracts and elongates, or they may control the direction of movement more actively. It is possible that the intrinsic polarity of microtubules provides a structural template that actively directs the matrix. This polarity is defined as the difference in the rate of tubulin exchange on either end of a microtubule. The rapid exchange end is designated "plus" and the slower end "minus" (1, 4, 23, 26, 47). It has been suggested that this polarity plays a functional role in directing the motive thrust produced by sliding parallel microtubules in flagella (40) or antiparallel microtubules in the mitotic overlap (28, 29). Earlier models predicted that orthograde and retrograde movements of particles in axons and chromatophores are directed by two populations of microtubules with opposite polarities (33, 44). The advent of microtubule polarity assays eliminated this concept by demonstrating that microtubules in chromatophores (16) and axons (8, 21) possess unipolar microtubules, the minus end embedded in the centrosome and the plus end extending toward the cortex. How then could a unipolar set of microtubules ensure homogeneous movement for both aggregation and dispersion? There are two plausible explanations. The first and most obvious is that only one of the two movements utilized microtubule polarity. Earlier models proposed that during mitotic metaphase the lattice would interact with polar microtubules to "ratchet" or "crawl" outward to transport chromosomes to the metaphase plate. An elasticlike matrix contraction during anaphase would not need directing and would be indifferent to microtubule polarity (29, 36). A second alternative is that the microtubule surface lattice, in tandem with associated proteins, provides a separate directional template for each movement direction. For example, matrix contraction would read a plus to minus polarity, while elongation would read minus to plus. To test these concepts, we have developed an in vivo model system using surgically isolated cytoplasm from cultured melanophores.

Pigment Movement in Isolated Melanophore Arms

Melanophores are among the largest chromatophores seen on the fish scale. They are stellate and possess long cytoplasmic extensions or arms that may be 100 μm long. Melanosomes are transported along microtubules out into the arms during dispersion and back into the cell center during aggregation. As early as 1931, Matthews (27) surgically isolated pigment-filled arms from melanophores in *Fundulus* scales. He observed that each arm, when severed, acted autonomously and transported pigment readily. He concluded that pigment movement was not centrosome dependent. We[2] have severed arms from cultured angelfish melanophores to test whether the microtubules within the arms maintain or alter their original polarity and spatial organization. If some free-ended microtubules do disassemble and reassemble with a reverse polarity, would this affect the direction of pigment transport? Because orthograde and retrograde motion in chromatophores are temporally separate and easily controlled, it would be obvious if pigment direction were altered in any way.

We found that arms severed from a cell, then immediately stimulated to aggregate, transport pigment uniformly retrograde, forming a pigment clump at their cut ends (Fig. 2, *a* and *b*). The rate of this movement is equal to that seen in uncut arms. The number and disposition of microtubules in these arms seems unchanged (Fig. 2 *c*). Arms isolated from melanophores, then incubated for several hours, alter their pigment transport. When stimulated to aggregate, these arms move pigment bidirectionally from their distal and proximal ends and form a pigment mass at their center. The rate of this aggregation is normal, whereas redispersion proceeds at one-half the normal rate. The movement within these severed arms appears less ordered, as if the microtubules had lost their radial organization or had disassembled. Fluorescent tubulin antibodies and whole-mount high-voltage electron microscopy have shown that microtubules within these arms indeed have an altered distribution. These microtubules no longer appear to extend from the proximal to distal arms ends as before (Fig. 2 *c*) but instead seem to emanate outward from the central pigment mass (Fig. 2 *f*) as if a new minicell had formed. Some of the microtubules (~5%) are oriented randomly and may be the reason for the less organized mode of pigment movement.

We wanted to determine whether the polarity of microtubules within these centrally aggregating arms had changed. If microtubules in the proximal arm region maintained their original polarity, one could infer that contraction or elongation of the lattice is indifferent to the polarity of microtubules. If microtubule polarity did reverse, it would suggest that the

[2] McNiven, M. A., M. Wang, and K. R. Porter. Microtubule polarity and the direction of pigment transport reverse simultaneously in surgically severed melanophore arms. *Cell.* In press.

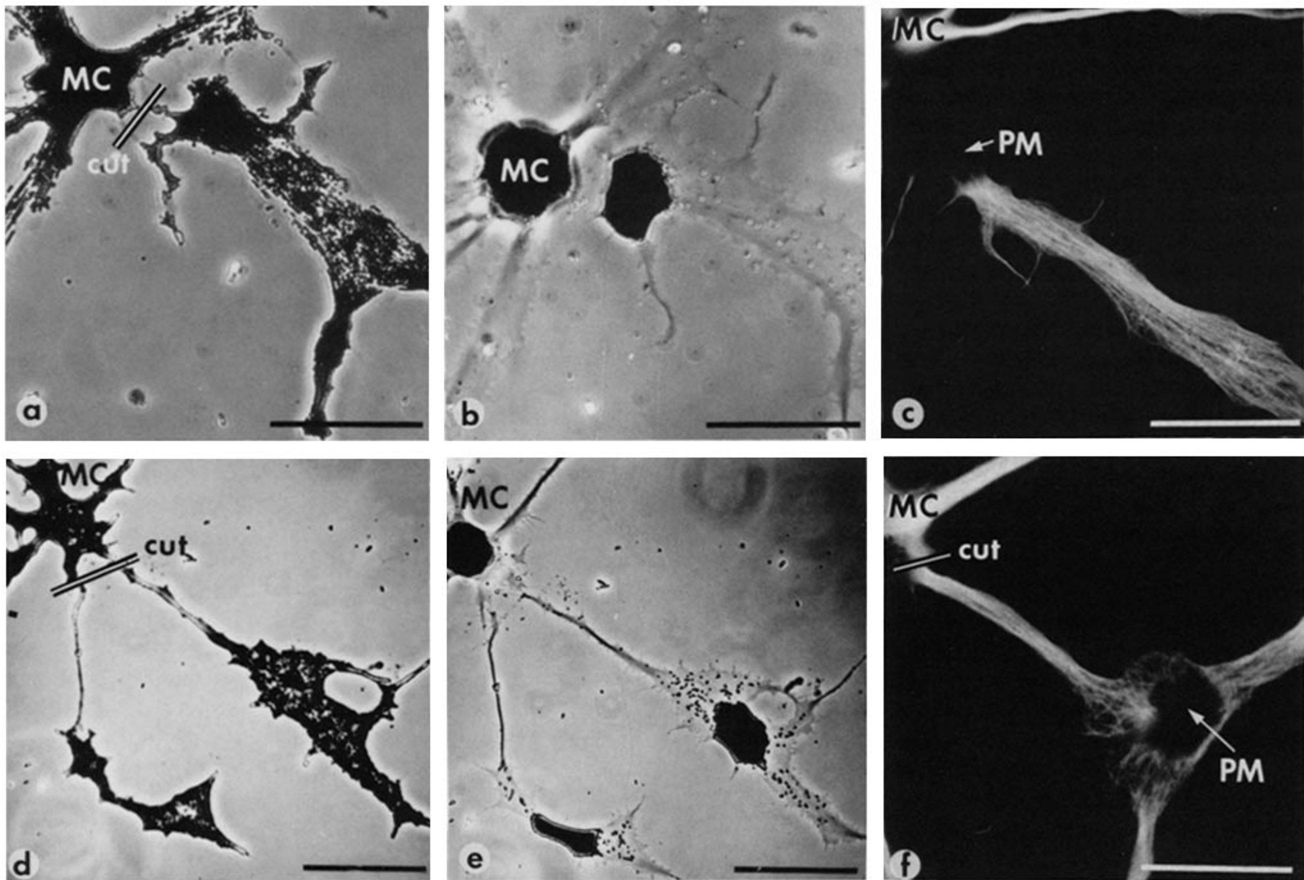

FIGURE 2 Phase-contrast series showing pigment transport within isolated melanophore arms. (*a*) An arm filled with pigment is surgically severed from the main cell (*MC*) and then immediately stimulated to aggregate. (*b*) Pigment is transported retrograde forming a clump at the cut end. (*c*) A similar arm that has aggregated pigment at the cut is then stained with fluorescent tubulin antibodies. A normal complement of microtubules extends from the proximally located pigment mass (*PM*) outward. (*d*) Two pigment-filled arms severed from a melanophore. The cell was returned to the incubator for 4 h and then stimulated to aggregate. (*e*) Pigment is transported from proximal and distal arm ends to the middle, forming a centrally located pigment mass. (*f*) A similar cut arm that has transported pigment to its center is fixed and stained with fluorescent tubulin antibody. Microtubules appear to extend in all directions outward from the pigment mass (*PM*). Bars, 10 μm. (*a–c* and *f*) × 2,000. (*d* and *e*) × 1,600.

direction of pigment lattice translocation is controlled by microtubules.

Polarity of arm microtubules was determined by a modification of the Heidemann and McIntosh (20) hook assay. The addition of exogenous brain tubulin suspended in 0.5% PIPES buffer and 2.5% DMSO induces the formation of C-shaped hooks on the walls of preexisting cytoplasmic microtubules. Depending on the polarity of the microtubule and the direction of thin sectioning, the hooks point either clockwise or counterclockwise. This technique has been used to determine the polarity of microtubules in a variety of cell types (8, 14–16, 20, 21) and is believed to be statistically accurate (30). We have found that severed arms that transport pigment unidirectionally to their cut ends possess a unipolar population of microtubules, minus ends toward the cut and plus ends extending outward (Fig. 3 *a*). These microtubules are 90–95% uniformly polar, which is slightly less than that seen in intact arms (95–99%). Arms that transport pigment bidirectionally to a new center possess two populations of microtubules with opposite polarities. Microtubules in the distal arm region maintain their original polarities, minus ends disappearing into the central pigment mass and plus ends extending out distally. Microtubules in the proximal arm region have a reversed polarity; minus ends disappear into the pigment mass and plus ends extend outward to the cut end (Fig. 3 *b*). Microtubule polarity counts of seven different arms show that 90% of the microtubules in the proximal arm end reverse polarity.

These data, in conjunction with the ultrastructural observations reported above, indicate that over time isolated melanophore arms form minicells. The cytoplast apparently reorganizes after an arm is severed so that its microtubules and pigment extend outward from its new center. This center is not a point equidistant between proximal and distal arm ends but instead is a region of greatest arm mass. For instance, the two severed arms shown in Fig. 2 *d* are wide in the distal region and narrow at the proximal. During aggregation, granules must travel over 30 μm from the proximal end to the pigment mass, whereas distal granules need travel only 10 (Fig. 2 *d*).

Transport Direction and Microtubule Polarity

The simultaneous changes in microtubule polarity and direction of pigment transport imply that microtubules confer direction to movement. With our observations, we have con-

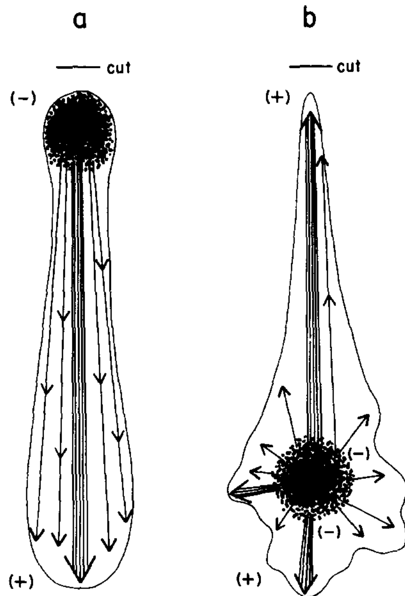

FIGURE 3 Illustration depicting involvement of microtubule polarity in directing pigment motion. In all instances that we have observed, matrix contraction is directed from the microtubules' plus ends to their minus ends, while expansion is from minus to plus. This rule is maintained in whole cells or arms that transport pigment unidirectionally to their cut ends (*a*) or bidirectionally to their centers (*b*). Arrowheads and plus signs indicate plus ends and minus signs indicate minus ends.

structed a model for matrix-microtubule interaction. In all instances (intact cells or isolated arms that transport pigment to their cut end or center), contraction of the matrix is directed from microtubules plus ends to minus ends. Matrix expansion is directed minus ends to plus ends (Fig. 3, *a* and *b*). We have not observed an exception to this rule.

There still remains the possibility that matrix contraction is independent of microtubule polarity. For example, when the matrix is severed from its centrosomal anchor, it would become free to contract isotropically to its center at the arm middle. We think this is unlikely, because the changes in polarity and transport direction are simultaneous. We intend to try to separate these two events by determining whether taxol stabilization of isolated arm microtubules delays or fully inhibits the change in transport direction. If the change is prevented until the taxol is removed, we can conclude that the direction of matrix contraction does not change until the polarity of the microtubules is reversed.

Formation of a New Center

The unexpected reorganization of microtubules from the center of cut arms indicates that cytoplasm isolated from the centrosome maintains its ability to organize microtubules. At present, we can only speculate on how and why an arm does this. Most likely there is a peripheral protein that both nucleates and bundles microtubules. This protein may be distributed throughout the cell, because a single melanophore arm severed three times will form three autonomous minicells. It is possible that the nucleating components comprising the pericentriolar satellite material extend throughout the cytoplasm, where they are usually undetected. Peripheral nucleating elements have been found in axons (11), axopodia (48), and some plants (19). We presently are looking for a nucleating material in melanophore arms. It seems unlikely that it is associated with the membrane or is freely soluble. That arms and whole cells both organize microtubules from their centers of mass suggests that the material may be associated with the dynamic matrix. Tension exerted on the matrix by interaction with membrane, or other cytoskeletal elements, could determine a central focal point, that is, the point at which tension exerted from all directions is approximately equal, such as the hub of a spoked wheel. It will be of interest to determine whether a new centrosomelike structure is formed at this point and whether its formation depends upon the pigment-bearing matrix. The reorganization of microtubules in arms emptied of pigment and associated matrix before severing would indicate that a structural nonmotile component is responsible.

We have presented both old and recent evidence for a dynamic matrix in chromatophores. This matrix requires energy for expansion, is controlled by Ca^{++}, is non–actomyosin dependent, and requires microtubules for support and direction. This pigment-moving matrix is indeed a highly differentiated organelle and it is unlikely that such a structure is unparalleled in nature. Although variations may exist, we believe that the basic unit structure is expressed in all cells and aids in such basic cellular motility functions as vesicle transport and separation of chromosomes.

The authors are pleased to acknowledge grant support from the American Cancer Society and assistance with the manuscript from Mary Ulrich and Joyce Albersheim.

REFERENCES

1. Amos, L. A. 1979. The structure of microtubules. *In* Microtubules. K. Roberts and J. S. Hyams, editors. Academic Press, New York. 2–64.
2. Beckerle, M. C., and K. R. Porter. 1982. Inhibitors of dynein activity block intracellular transport in erythrophores. *Nature (Lond.).* 295:701–703.
3. Beckerle, M. C., and K. R. Porter. 1983. Analysis of the role of microtubules and actin in erythrophore intracellular motility. *J. Cell Biol.* 96:354–362.
4. Bergen, L. G., and G. G. Borisy. 1980. Head-to-tail polymerization of microtubules in vitro. *J. Cell Biol.* 84:141–150.
5. Bikle, D., L. G. Tilney, and K. R. Porter. 1966. Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus. Protoplasma.* 61:322–345.
6. Buckley, I. 1983. Microinjected vanadate fails to inhibit hormone-induced melanosome movements in living cultured melanophores. *J. Cell Biol.* 97(2, Pt. 2):467*a*.
7. Buckley, I., and M. Stewart. 1983. Ciliary but not saltatory movements are inhibited by vanadate microinjected into living cultured cells. *Cell Motility.* 3:167.
8. Burton, P. R., and J. L. Paige. 1981. Polarity of axoplasmic microtubules in the olfactory nerve of the frog. *Proc. Natl. Acad. Sci. USA.* 78:3269–3273.
9. Byers, H. R., and K. R. Porter. 1977. Transformations in the structure of the cytoplasmic ground substance in erythrophores during pigment aggregation and dispersion. *J. Cell Biol.* 75:541–558.
10. Cande, W. Z., and S. M. Wolniak. 1978. Chromosome movement in lysed mitotic cells is inhibited by vanadate. *J. Cell Biol.* 79:573–580.
11. Chalfie, M., and J. N. Thomson. 1979. Organization of neuronal microtubules in the nematode *Caenorhabditis elegans. J. Cell Biol.* 82:278–305.
12. Clark, T. G., and J. L. Rosenbaum. 1982. ATP-dependent aggregation of pigment in detergent-permeabilized melanophores of *Fundulus heteroclitus. J. Cell Biol.* 95(2, Pt. 2):330*a* (Abstr.)
13. Clark, T. G., and J. L. Rosenbaum. 1982. Pigment particle translocation in detergent-permeabilized melanophores of *Fundulus heteroclitus. Proc. Natl. Acad. Sci. USA.* 79:4655–4659.
14. Euteneuer, U., and J. R. McIntosh. 1980. Polarity of midbody and phragmoplast microtubules. *J. Cell Biol.* 87:509–515.
15. Euteneuer, U., and J. R. McIntosh. 1981. The structural polarity of kinetochore microtubules in PtK cells. *J. Cell Biol.* 89:338–395.
16. Euteneuer, U., and J. R. McIntosh. 1981. Polarity of some motility-related microtubules. *Proc. Natl. Acad. Sci. USA.* 78:372–376.
17. Forman, D. S. 1982. Vanadate inhibits saltatory organelle movement in a permeabilized cell model. *Exp. Cell Res.* 141:139–147.
18. Green, L. 1968. Mechanism of movements of granules in melanocytes of *Fundulus heteroclitus. Proc. Natl. Acad. Sci. USA.* 59:1179–1186.
19. Gunning, B. E. S., A. R. Hardham, and J. E. Hughes. 1978. Evidence for initiation of microtubules in discrete regions of the cell cortex in *Azolla* root-tip cells. *Planta (Berl.).* 143:161–179.

20. Heidemann, S. R., and J. R. McIntosh. 1980. Visualization of the structural polarity of microtubules. *Nature (Lond.)*. 286:517–519.
21. Heidemann, S. R., J. M. Landers, and M. A. Hamborg. 1981. Polarity orientation of axonal microtubules. *J. Cell Biol.* 91:661–665.
22. Junqueira, L. C., E. Raker, and K. R. Porter. 1974. Studies on pigment migration in the melanophores of the teleost *Fundulus heteroclitus* (L). *Arch. Histol. Jpn.* 36:339–366.
23. Kirschner, M. W. 1980. Implications for the stability and polarity of actin and tubulin polymers in vivo. *J. Cell Biol.* 83:338.
24. Luby, K. J., and K. R. Porter. 1980. The control of pigment migration in isolated erythrophores of *Holocentrus ascensionis* (Osbeck). I. Energy requirements. *Cell.* 21:13–23.
25. Luby-Phelps, K. J., and K. R. Porter. 1982. The control of pigment migration in isolated erythrophores of *Holocentrus ascensionis* (Osbeck). II. The role of calcium. *Cell.* 29:441–450.
26. Margolis, R. L., and L. Wilson. 1978. Opposite end assembly and disassembly of microtubules at steady state *in vitro*. *Cell.* 13:1–8.
27. Matthews, S. A. 1931. Observations on pigment migration within the fish melanophore. *J. Exp. Zool.* 58:471–486.
28. McDonald, K. L., M. K. Edwards, and J. R. McIntosh. 1979. Cross-sectional structure of the central mitotic spindle of *Diatoma vulgare*. Evidence for specific interaction between antiparallel microtubules. *J. Cell Biol.* 83:443–461.
29. McIntosh, J.R. 1980. Microtubule polarity and interaction in mitotic spindle function. *In* International Cell Biology. H. G. Schweiger, editor. Springer-Verlag, Berlin. 359–381.
30. McIntosh, J. R., and U. Euteneuer. 1984. Tubulin hooks as probes for microtubule polarity: an analysis of the method and an evaluation of data on microtubule polarity in the mitotic spindle. *J. Cell Biol.* 98:525–533.
31. McNiven, M. A., and K. R. Porter. 1981. The microtubule organization center in erythrophores: its 3-dimensional structure and behavior during pigment motion. *J. Cell Biol.* 91(2, Pt. 2):334*a*. (Abstr.)
32. Murphy, D. G., and L. G. Tilney. 1974. The role of microtubules in the movement of pigment granules in teleost melanophores. *J. Cell Biol.* 61:757–779.
33. Ochs, R. L., and P. L. Burton. 1980. Distribution and selective extraction of filamentous components associated with axonal microtubules of crayfish nerve cord. *J. Ultrastruct. Res.* 73:169–182.
34. Ochs, R. L., and M. E. Stearns. 1981. Colloidal gold immunolabeling of whole-mount erythrophore cytoskeletons: localization of tubulin and HMW-MAPs. *Biol. Cell.* 42:19–28.
35. Pickett-Heaps, J. D., and T. P. Spurck. 1982. Studies on kinetochore function in mitosis. I. The effects of colchicine and cytochalasin on mitosis in the diatom *Hantzschia amphioxys*. *Eur. J. Cell Biol.* 28:77–82.
36. Porter, K. R. 1976. Introduction: motility in cells. *Cold Spring Harbor Conf. Cell Proliferation.* 3:1–28.
37. Porter, K. R., and K. L. Anderson. 1982. The structure of the cytoplasmic matrix preserved by freeze-dryng and freeze-substitution. *Eur. J. Cell Biol.* 29:83–96.
38. Porter, K. R., and M. A. McNiven. 1982. The cytoplast; a unit structure in chromatophores. *Cell.* 29:23–32.
39. Porter, K. R., M. Beckerle, and M. McNiven. 1983. The cytoplasmic matrix. *In* Spatial Organization of Eukaryotic Cells—A Symposium in Honor of K. R. Porter. J. R. McIntosh, editor. Alan R. Liss, Inc., New York. 259–302.
40. Sale, W. S., and P. Satir. 1977. Direction of active sliding of microtubules in *Tetrahymena* cilia. *Proc. Natl. Acad. Sci. USA.* 74:2045–2049.
41. Schliwa, M., and U. Euteneuer. 1978. A microtubule-independent component may be involved in granule transport in pigment cells. *Nature (Lond.)*. 273:556–558.
42. Schliwa, M., U. Euteneuer, W. Herzog, and K. Weber. 1979. Evidence for rapid structural and functional changes of the melanophore microtubule-organizing center upon pigment movement. *J. Cell Biol.* 83:623–632.
43. Schliwa, M., K. Weber, and K. R. Porter. 1981. Localization and organization of actin in melanophores. *J. Cell Biol.* 89:267–275.
44. Schmitt, F. O. 1968. Fibrous proteins—neuronal organelles. *Proc. Natl. Acad. Sci. USA.* 60:1092–1101.
45. Stearns, M. E. 1984. Cytomatrix in chromatophores. *J. Cell Biol.* 99(1, Pt. 2):144s–151s.
46. Stearns, M. E., and R. L. Ochs. 1982. A functional in vitro model for studies of intracellular motility in digitonin-permeabilized erythrophores. *J. Cell Biol.* 94:727–739.
47. Summers, K., and M. W. Kirschner. 1979. Characteristics of the polar assembly and disassembly of microtubules observed in vitro by darkfield light microscopy. *J. Cell Biol.* 83:205–217.
48. Tilney, L. G., and K. R. Porter. 1965. Studies on microtubules in *Heliozoa*. I. The fine structure of *Actinosphaerium nucleofilum* (Barrett), with particular reference to the axial rod structure. *Protoplasma.* 60:317–344.