Cytomatrix in Chromatophores

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Although intracellular transport is a fascinating and vital function shared by diverse cell systems, our understanding of the processes involved remains incomplete, despite the enormous amount of attention it has received over the past 40 years (46). Recent work on pigment granule transport in chromatophores (e.g., erythrophores, melanophores, and xanthophores), however, has begun to yield new information on three important aspects of the phenomenon: (*a*) the organization of the cytomatrix components involved in directed organelle transport; (*b*) the identification of proteins powering organelle transport; and (*c*) the precise determination of the physiological requirements (ions, nucleotides, pH) of transport.

The progress made in these studies is largely due to the development of methods for culturing chromatophores and is based on the striking behavior of chromatophore pigment granules. Because of the relatively flat, discoid shape of the chromatophore and because of the size, color, and rapid rate of transport of its pigment granules, pigment granule motion is easily followed in living cells. Normally, chromatophores are observed to cyclically aggregate and disperse their numerous pigment granules in a radial pattern from the cell center. These are two radically different types of motion: aggregation is rapid (20 μm/s) and uniform (not unlike chromosome transport), and dispersion is slow (5 μm/s), nonuniform, and saltatory (like cytoplasmic flow and organelle transport in nerve cells). These phenomena are temporally distinct, and chromatophores can be independently induced to aggregate (with epinephrine) or to disperse (with caffeine) their pigment, so that the factors involved in each of these processes can be separately analyzed. It is therefore possible to watch the response of pigment granules in living cells and either directly relate this behavior to changes in the physiological conditions or to fix the cells and directly relate the observations made to the organizational properties of chromatophores. In this review I will emphasize recent discoveries from studies of intracellular transport in chromatophores, especially with regard to their functional significance. A more historical perspective and detailed description of chromatophores is given in recent reviews by Porter et al. (57) and Luby-Phelps and Schliwa (37).

Cytomatrix in Chromatophores

We know with some degree of certainty that dynamic properties of the cytoplasmic matrix or cytomatrix are important for pigment motion in chromatophores. Very early studies by Marsland (39) and Marsland and Meisner (40) showed that increasing hydrostatic pressure or cold causes melanosome dispersion within melanophores of squirrelfish. The dispersion effect was counteracted with D_2O or reversed with reduced pressures and/or increased temperatures, which promoted aggregation of pigment. Marsland reasoned that cytoplasmic gels must solate at high pressure or reduced temperature to produce dispersion and gel under the opposing conditions (or in the presence of D_2O) to produce aggregation. He postulated therefore that gel-sol transitions of the cytomatrix might normally produce the aggregation-dispersion of pigment.

Marsland's early explanation for pigment motion has enjoyed continued support from the results of light, immunofluorescent, and electron microscope studies of chromatophores. Green (23), Schliwa and Euteneuer (64), Byers and Porter (10), and Obika et al. (48) have provided corroborative evidence that pigment granules are indeed suspended in a "dynamic continuum" as Marsland originally suggested. Two fundamentally important features of chromatophores were uncovered by these later investigators. Firstly, they and others (29) found that numerous microtubules (hundreds to thousands) form polarized, radial arrays that are distributed in an even fashion and that direct pigment movement from a highly structured, centrally located centrosomal complex. Secondly, it was discovered that the microtubules and pigment are suspended in a structured cytomatrix consisting of an ordered three-dimensional network of cross-linking filaments 3–9 nm in diameter that has been termed the microtrabecular lattice by Porter and colleagues (12, 35–37, 61, 64). These two basic elements constitute the principle cytoskeletal structures in chromatophores. This is clearly exhibited in the red pigment cell, the erythrophore (57).

Whole-mount high-voltage electron microscope (HVEM)[1] studies of glutaraldehyde-fixed, critical point-dried chromatophores have suggested that pigment motion is generated directly by a complex mechanism involving the cytomatrix. These studies have indicated that in melanophores (64) and

[1] *Abbreviations used in this paper:* DOC, decentralized organizing center; EHNA, erythro-9-(3-[2-hydroxynonyl])adenine; HMW-MAP, high molecular weight microtubule-associated protein; HVEM, high-voltage electron microscope; MAP, microtubule-associated protein; MTOC, microtubule-organizing center; NBD, *N*-7-nitrobenz-2-oxa-1,3-diazole; NEM-HMM, *N*-ethyl maleimide–heavy meromyosin.

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erythrophores (10, 35) the pigment granules do not travel alone on their journey toward the centrosome. Instead, the cytomatrix material was observed to coaggregate in association with the pigment. Likewise, during pigment dispersion, the cytomatrix was found to expand outward from the centrosomal complex (10, 35). These initial observations were partially modified as a result of HVEM studies of freeze-sublimated preparations. Porter et al. (57) have shown that in fact only part of the cytomatrix network comigrates with the aggregating pigment granules. They have elegantly demonstrated that a mobile "α-cytomatrix" comigrates with the pigment while an immobile "β-cytomatrix" remains stationary in association with microtubules, smooth endoplasmic reticulum, and the nucleus, presumably providing a stabilizing influence for these organelles (Fig. 1). Thus, pigment translocations seem to evolve from a continuous cyclic pattern involving the separation and reintegration of α- and β-cytomatrices.

Role of Microtubules

Microtubule organization directly effects the rate of transport, defines channels for transport, and determines the direction of pigment motion. Increased numbers of microtubules produce increased rates of transport (47) in patterns that follow and are bounded by the radial arrangement of the tubules (5, 29, 47, 62). Removal of the tubules with antimicrotubule drugs results in incoherent, nondirected pigment motion and eventually the cessation of motion (3, 61, 81). Without microtubules, pigment aggregation is no longer a resolute event in which granules move in a coordinated, linear fashion toward the cell center. Instead, an apparently random "peripheral pigment patching" occurs in response to drugs (e.g., epinephrine stimuli) that normally induce aggregation (4, 64).

Clearly, the microtubules perform a significant function in directing the polarized motion of pigment to and from the central microtubule-organizing center (MTOC). Recent work has shown further that the absolute structural polarity of microtubules reflects the polarity of pigment behavior. Euteneuer and McIntosh (18) determined the structural polarity of microtubules in angelfish melanophores by gently lysing cells in the presence of exogenous tubulin under conditions that induce the assembly of hooklike tubulin structures on existing microtubules. Because the direction of hook curvature observed in thin sections reflects the intrinsic polarity of the microtubules (43), they were able to show that chromatophores assemble their tubules with the plus end (the rapid-growing end) located distal to the MTOC. Utilizing this hook technique, McNiven et al. (44) looked to see whether changes in microtubule polarity occur when changes in the direction of pigment aggregation are physically induced in branching arms of cultured angelfish melanophores. They discovered that after the physical separation of "branching-arms" from the cell bodies there is a rapid rearrangement in both microtubule polarity and the direction of pigment migration. A new "presumptive MTOC" forms in the cut arm, and the microtubules rearrange around the new center so that their minus ends are now at the new center and their plus ends are relocated distal to the new center. With microtubule rearrangement the pigment begins to migrate in a synergistic fashion toward the newly formed center. I presume that this change in microtubule polarity and pigment migration pattern is a marker for and reflects similar changes in the inherent organization of the cytomatrix as well. Thus, it would be very interesting to know whether a reorganized cytomatrix determines the repositioning and change in polarity of the microtubules or vice versa.

Possible Organizational Features of the Cytomatrix: the Decentralized Organizing Center Theory

It seems reasonable to suppose that both the MTOC and the cytomatrix are normally involved in microtubule assembly, orientation, and pattern determination, albeit in different capacities and to different degrees. In effect, the cytomatrix can be seen as an elaborate cytoplasmic extension of a more densely ordered MTOC. Just as the MTOC works to initiate

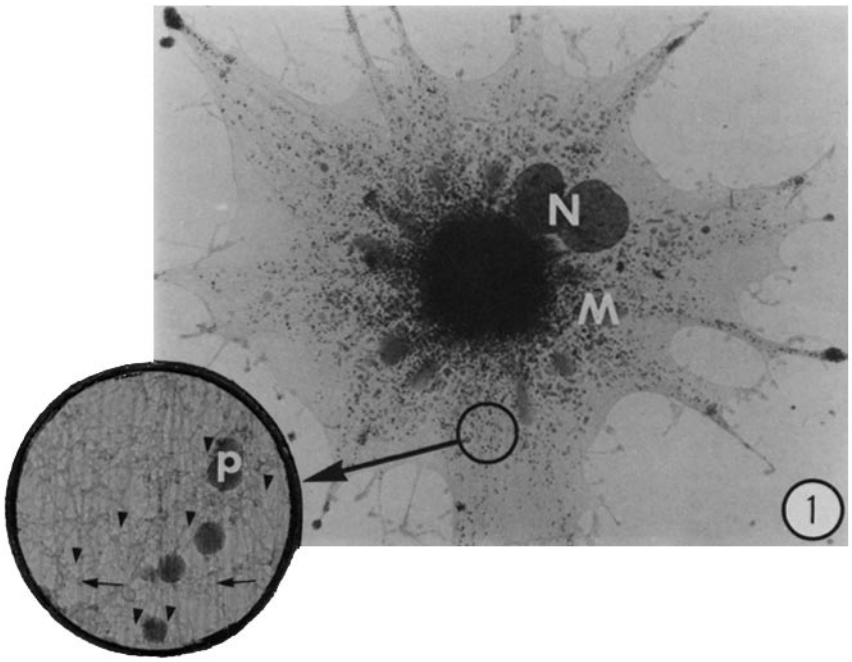

FIGURE 1 Whole-mount erythrophore glutaraldehyde fixed with the majority of its pigment aggregated at the cell center. Nucleus (*N*); mitochondria (*M*). × 2,500. (*Inset*) Parallel arrays of microtubules (arrows) and crosslinking β-cytomatrix filaments (arrowheads). The α-cytomatrix has largely migrated to the cell center and is not visible in this type of preparation. Pigment (*P*). × 22,000.

ordered microtubule arrays, the cytomatrix serves to maintain this order, even at the margins of the cell. More specifically, the cytomatrix may restrict the positioning and direction of microtubule assembly and may further influence the orientation of microtubules with respect to each other, and with respect to the other cytoplasmic organelles.

Electron microscope studies of several protozoans has strongly supported this view of the cytomatrix. In heliozoans, it has been shown (75–77) that "intermicrotubule linkers" assist in the assembly of microtubules into ordered axopodial structures during reassembly after cold or colchicine treatments. More recent studies (28) of axoneme development have demonstrated further that in two other heliozoans the microtubules are first assembled from MTOCs with a random orientation. The tubules are then linked by filaments into predetermined microtubule structures. The authors indicate that the process is complicated in that a cytomatrix network appears to draw the microtubules together so that the interlinkage of microtubules can take place. Similar cytomatrix activities might readily orchestrate the ordering of microtubules in chromatophores or the reordering of microtubules in "separated arms" of melanophores.

Does the cytomatrix have the information content for ordering the arrangement of other organelles as well? Despite the difficulty of recognizing patterns of organelle distribution, it is clear that, like cytoskeletal filaments, most organelles (e.g., pigment, ribosomes, nucleus, mitochondria) do not usually occur at random in the cytoplasm (52, 57). During aggregation and dispersion of pigment in erythrophores, Porter and McNiven (58) have observed that individual pigment granules occupy spatially defined domains. That is, granules move set distances in defined channels to and from the centrally located centrosomes. It is unlikely that this behavior is coincidental. It is more probable that the cytomatrix possesses positional information that can dictate pigment positioning. More precisely, I believe that the cytomatrix consists of a central cytomatrix-organizing center (commonly referred to as the MTOC) and numerous minutely ordered "decentralized organizing centers" (DOCs), which are distributed in predescribed patterns. The DOCs could exhibit preferred affinities for individual organelles so that in a dynamic system like the chromatophore the pigment granules move from a central docking site (the MTOC) toward the margins of the cell in an orderly pattern dictated by the DOCs. At the molecular level, individual granules could simply differ in the amounts of a particular cross-linking protein (i.e., microtubule-associated protein) attached on their surface so that they can always migrate to a particular DOC. The observed saltatory motion of granules might actually reflect a sampling process wherein granules momentarily stop while testing potential DOCs along their route. Note that this sampling process is discontinued instantaneously in response to cellular signals that trigger pigment aggregation. Here, I predict that it will be found that the affinity of granules for DOCs is uniformly minimized and that pigment binding to the contractile α-cytomatrix is maximized. Admittedly, this model of cytomatrix organization is largely based on intuition and must purposely remain sketchy until supportive evidence can be obtained.

Composition of the Cytomatrix

A better understanding of the observed morphological changes and related pigment behavior will be possible once the biochemical properties of the cytomatrix are known. Currently, our knowledge of cytomatrix composition is fragmentary or sparse, although the participation of microtubule proteins, intermediate filaments, and actin microfilaments has been partially examined. Microtubules are the struts that guide transport, but translocation of microtubules, tubulin molecules, or polymers of tubulin does not seem to contribute directly to the process of cytomatrix aggregation (contraction) and expansion. Certainly, when the microtubules are superstabilized with taxol (60), pigment transport can continue normally in erythrophores (3). It has been shown from thin section studies of melanophores of the fish *Pterophellum scalare* that the total microtubule numbers per cell can decrease by 55% during pigment aggregation (63). However, the significance of this unique example is not known, unless inadvertent changes in microtubule stability can sometimes occur as a function of changes in cytomatrix distribution.

Other possible cytoskeletal elements, such as intermediate filaments and microfilaments, do not seem to compose a major part of the cytomatrix, nor do they seem to play a pivotal role in pigment transport. Intermediate filaments are present in some chromatophores (melanophores, xanthophores) but not in others (erythrophores). In teleost melanophores, 11-nm intermediate filaments are reported to alter their distribution during pigment aggregation (30, 81), but the actual contribution of these filaments to pigment motion is not known.

Actin microfilaments, on the other hand, are present to a limited extent in all chromatophores examined. Heavy meromyosin decoration (48, 66) and actin antibody studies (66) have shown that actin is limited to surface filopodia regions. Erythrophores stained with fluorescently labeled phalloidin (NBD-phallacidin and rhodamine-phallacidin, Molecular Probes, Inc., Junction City, Oregon) show faint staining near the membrane surfaces and in filopodia (Fig. 2). As observed in melanophores, the microfilament pattern remains unchanged during all stages of pigment migration, again indi-

FIGURE 2 Rhodamine/phallacidin-labeled erythrophore. The fluorescent picture was taken with the focus at the upper cell surface and it shows that there are few or no actin microfilaments in erythrophores. × 2,620.

cating that microfilaments and changes in actin distribution are not important for pigment transport. In any case, actin is located mainly in areas where even a partial contribution to pigment motion seems improbable. Numerous drug inhibitory studies have lent support to this conclusion. For example, Beckerle and Porter (4) have microinjected DNase I and NEM-HMM and diffused in cytochalasin B to demonstrate that the erythrophores of the squirrelfish *Holocentrus ascensionus* do not require microfilaments for pigment transport.

Unfortunately, to conclude that the cytomatrix lacks intermediate filament proteins or actin filaments (or their associated proteins) is not very beneficial, nor is it justified. Because the cytomatrix is probably a complex heteropolymer consisting of a variety of contractile proteins, it remains to be shown definitively whether any of the above proteins make up part of such a cytomatrix. It cannot be ruled out, for example, that short actin microfilaments form an active component of a transport-dependent heteropolymer, especially since cytochalasin B inhibits motion in certain melanophores.

Immunofluorescent and immunoelectron microscope methods have enabled molecular dissection of microtubules and their associated structures (17, 49), and this approach should permit the positive identification of molecular structures composing α- and β-cytomatrix components. My colleagues and I have made some progress in this direction by using antibodies raised against mammalian brain microtubule-associated proteins. Our initial investigations using polyclonal microtubule-associated protein (MAP) and tubulin antibodies have revealed that microtubule proteins together form the main microtubule and MTOC cytoskeletal complex in erythrophores (49) (Fig. 3). The possible distributions and interactions of MAPs 1 and 2 in transport were subsequently analyzed using monoclonal antibodies.[2] Immunofluorescent images have revealed that tubulin and MAP-1 antibodies stain microtubule complexes (the centrosome and tubules) in a typical radial pattern that remains constant at all stages of pigment transport. In contrast, the staining pattern of MAP-2 antibodies (AP-9, AP-13) change dramatically as a result of pigment aggregation. MAP-2 antibodies diffusely label microtubule surfaces and associated cytomatrix material in dispersed cells, but label only the central centrosomal mass in cells with their pigment aggregated. These results and others (72) suggest that the cytomatrix in dispersed cells is composed of at least two compositionally and functionally separate domains, one consisting of a microtubule-associated, MAP-1-rich β-cytomatrix and the other consisting of a pigment-associated, MAP-2-rich α-cytomatrix. During pigment translocation, the cytomatrix domains appear to separate and reintegrate in harmony with pigment aggregation and dispersion. It would be worthwhile to further investigate the types and extent of MAP formations and MAP phosphoproteins (i.e., MAP-2) present in chromatophores.

Relevant Properties of High Molecular Weight Microtubule-Associated Proteins

The possible role of several high molecular weight proteins as promoters of microtubule assembly, as microtubule-associated structures, and as specialized motility proteins has been given increased attention during the past decade. High molecular weight microtubule-associated proteins (HMW-MAPs) consist of MAP-1 (300–330 kdaltons) and MAP-2 (270–300 kdaltons); both will copurify with mammalian brain tubulin in stoichiometrically constant amounts, and both can stimulate tubulin assembly as well as retard microtubule disassembly. In vitro these MAPs form filamentous projections on microtubule surfaces (16, 46) with an axial periodicity of 32 nm (2, 32). The filaments resemble projections seen in vivo by thin-section methods (69), indicating that MAPs form part of the microtubule-associated cytomatrix in cells.

The prevailing theory is that HMW-MAPs form part of a crosslinking cytomatrix between several filamentous components (microtubules, neurofilaments, microfilaments) and between these filaments (particularly microtubules) and other organelles (vesicles, granules). Immunological studies with rabbit anti-sera to MAPs (15, 49) and with monoclonal antibodies made to MAPs 1 and 2 (6, 7, 72) have clearly demonstrated that HMW-MAPs are present along microtubules in situ. Further, monoclonal antibody studies have shown that MAPs 1 and 2 indeed may crosslink the surfaces of microtubules and intermediate filaments in situ (6, 7a). Recently, Griffith and Pollard (24) and others have reported a viscometrically measurable, actin-microtubule crosslinking activity of MAPs (MAPs 2 and 1) that depends on detectable levels of MAP phosphorylation in vitro (56). They have shown that MAP-2 has 12 phosphorylation sites and that the actin crosslinking activity of MAP-2 is inversely related to the level of phosphorylation. In other words, low phosphorylated MAPs have a high actin-binding activity, while the heavily phosphorylated MAPs have a low microtubule-actin crosslinking activity. The degree of interaction of microtubules, at least with actin-bound organelles in vivo, could therefore be regulated by the level of phosphorylation of MAP-2. With respect to organelle transport, it is interesting, therefore, that Suprenant and Dentler (74) have shown that increased cAMP levels result in increased secretory granule binding to MAP-microtubules in vitro and that increased Mg-ATP levels cause granule release. Inasmuch as MAP-2 has a cAMP-dependent type-II protein kinase activity associated with it (78), it is possible that self-regulated phosphorylation states of MAP-2 directly affect its ability to bind microfilaments or vesicles in vitro.

Precisely how these findings relate to the in vivo situation and to MAP cross-linking of organelles during transport is not clear. Further work should be done to test the involvement of MAPs in the regulation of organelle-microtubule interaction. For example, in chromatophores it is not understood how the pigment-dispersing effect of increased cAMP levels can affect the motility system. It has been postulated that the phosphorylation of key motility proteins could be responsible for the "decision" to disperse. There has been one attempt to identify the substrates for putative phosphorylation events accompanying cAMP-induced dispersion of pigment (38). In this case, xanthophores from goldfish were isolated and stimulated by ACTH to disperse their pigment in the presence of ^{32}P-labeled orthophosphate. The cells were then fractionated, and labeled proteins were identified on autoradiograms of SDS polyacrylamide gels. A single 56,000-dalton band was phosphorylated in the pigment granule fraction. However, the radioactive pulse was 15 min long, so many bands were labeled in other fractions as well. Unfortunately, autoradiograms from control (aggregated) cell fractions were not shown, so it was necessary to repeat and reevaluate experi-

[2] Monoclonal antibodies raised against tubulin, MAP-1, and MAP-2 (AP 9, 13) were the generous gift of Drs. Lester Binder and Lionel Rebhun, University of Virginia, Charlottesville.

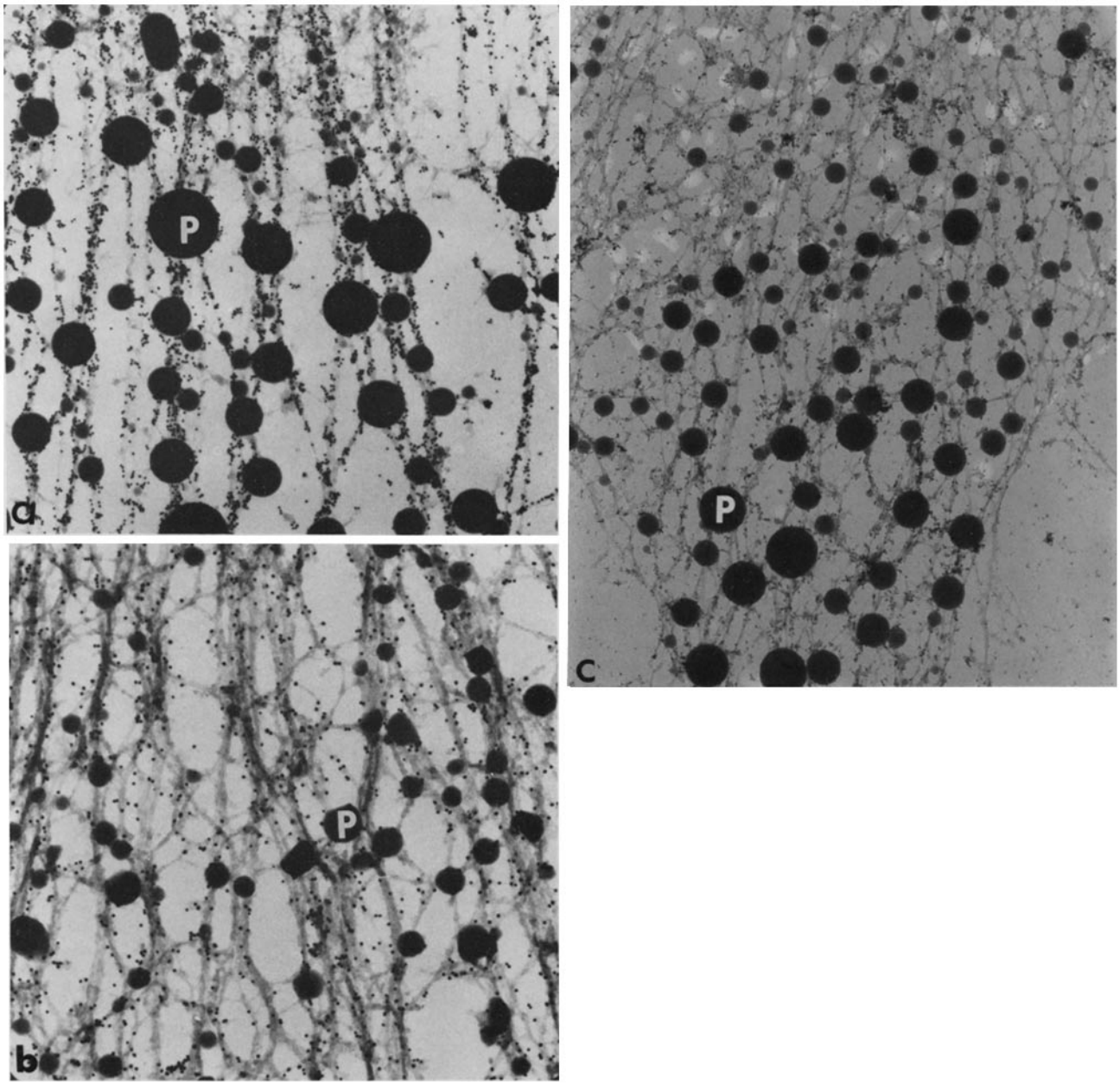

FIGURE 3 Colloidal gold/protein A–labeled erythrophore cytoskeletons prepared by glutaraldehyde fixation and critical point drying techniques. The pictures show the binding pattern of (*a*) tubulin antibody, (*b*) high molecular weight–sera antibodies, and (*c*) protein A/colloidal gold. The tubulin bodies specifically label the surfaces of microtubules, and the rabbit HMW-MAPs antibody labels the cytomatrix material coating the microtubule surface. The picture of a control preparation (*c*) shows that protein A/gold tends to clump randomly on the surfaces of pigment (*p*) cytomatrix, microtubules, and the Formvar surface but does not exhibit the regular patterns observed with tubulin and HMW-MAPs antibody. Note that with 0.1% Triton X-100 treatment of the glutaraldehyde-fixed specimens this background could be reduced by 10-fold. Fluorescent images of FITC/protein A/colloidal gold-antibody–labeled cells showed similar results. (*a*) × 30,000. (*b*) × 38,000. (*c*) × 20,000.

ments revealing a phosphorylated, granule-associated band. Palazzo et al. (51) appear to have successfully completed this reevaluation and have found results similar to those originally reported (38).

Motility in Detergent-Opened Model Systems

Detergent-permeabilized models have been used extensively in studies of microtubule-dependent motility in diverse cell systems. This basic approach has helped elucidate the mechanisms of motility in the flagellum (21), the axostyle (45), and chromosome movement (12, 13). Organelle transport in lysed chromatophores, fibroblasts, and axons also has been investigated (11, 14, 19, 20, 71). The results indicate that motility can involve dynein-ATPase and HMW-MAP microtubule interactions, actomyosin coupling, and/or Ca^{2+}-regulated proteins.

Extensive work on the mechanism of flagellar beat has established that dynein (a complex of high molecular weight proteins that possesses ATPase activity) normally interacts with the surface of an axonemal microtubule to generate motive force (21, 73). Because flagellar dynein ATPase activ-

ity is inhibited by both erythro-9-(3-[2-hydroxynonyl])adenine (EHNA) and vanadate in the +5 oxidation state (vanadate +5) (8, 33, 53), these reagents have been used to probe the involvement of dynein ATPase in intracellular motility events that are suspected to require such enzymatic activity (68). Recent studies (3, 19, 20, 71) have shown that vanadate and EHNA at micromolar levels can block saltatory motion, the chief operational process of pigment dispersion. Vanadate and EHNA have various effects on pigment aggregation; vanadate inhibits aggregation in melanophores and in erythrophores; EHNA does not inhibit aggregation in erythrophores but does inhibit it in melanophores. In nonpigmented cells, microinjected EHNA and vanadate block ciliary beating but do not block saltatory motion (9). Finally, millimolar EHNA has been found to reduce the velocity of retrograde transport but to have no effect on anterograde transport in isolated, saponin-treated, or intact lobster axons (20). These results suggest that cytoplasmic dynein-ATPases either do not exist or that a rather complex transport mechanism exists in which dynein powers motion in one direction (dispersion) and other phosphoproteins (i.e., MAP-2; 79) control other aspects of motion.

The inhibitory effects described above may result from the nonspecific inhibition of other cellular enzymes. We can rule out the inhibition of Ca-ATPase because it (either membranous or sarcoplasmic reticulum–like) would produce an increase in intracellular calcium and thereby induce pigment aggregation, and this effect is distinct from that observed for vanadate or EHNA. Likewise, inactivation of membrane pumps (e.g., Na^+/K^+ ATPase) does not appear to be the mechanism by which vanadate or EHNA affects motility. Vanadate and EHNA (unpublished results) have been found to block the saltatory motion of pigment granules in detergent-permeabilized erythrophores (71). Under these circumstances, the cell membrane is no longer intact, so it is clear that a membrane effect is not the cause of inhibition. If vanadate's effect were on the general cellular energy metabolism, the cells would be expected to aggregate as they do in response to metabolic inhibitors (35). Because they remain dispersed in the presence of vanadate and because excess Mg^{2+}/ATP does not relieve the inhibitory effect of the probe (71), it is unlikely that the primary effect is on the metabolic state. I contend, therefore, that the impact of the vanadate is at the level of the motility system, because the only detectable effects are on the capacity of the cells to translocate pigment granules.

Unfortunately, despite the fact that vanadate is clearly a dynein inhibitor, the block of granule motility by vanadate is still not absolute proof that the affected ATPase is dynein, and additional work is still required to show that it is. One difficulty with interpreting the vanadate results is that in vitro studies show that ionic strength can inversely affect vanadate's specificity of dynein (33). Thus, at the physiological ionic strengths found in vivo, vanadate may be much less specific than has been demonstrated in vitro. What about EHNA? Examination of the EHNA effect on a large number of ATPases, including Na^+/K^+ ATPase and myosin ATPase, indicates that EHNA is specific in its inhibition of dynein (53). EHNA does not block membrane ATPases nor a number of kinases (e.g., hexokinase, adenylate kinase, and phosphofructokinase), and these characteristics lend credibility to the interpretation that the EHNA effect in vivo truly represents an effect on dynein. The major liability of using EHNA as an in vivo probe appears to be its effect on cellular protein carboxylmethylase activity (55). This disadvantage can be circumvented by including competitive inhibitors of this methylation enzyme in digitonin permeabilization solutions (Fig. 4)

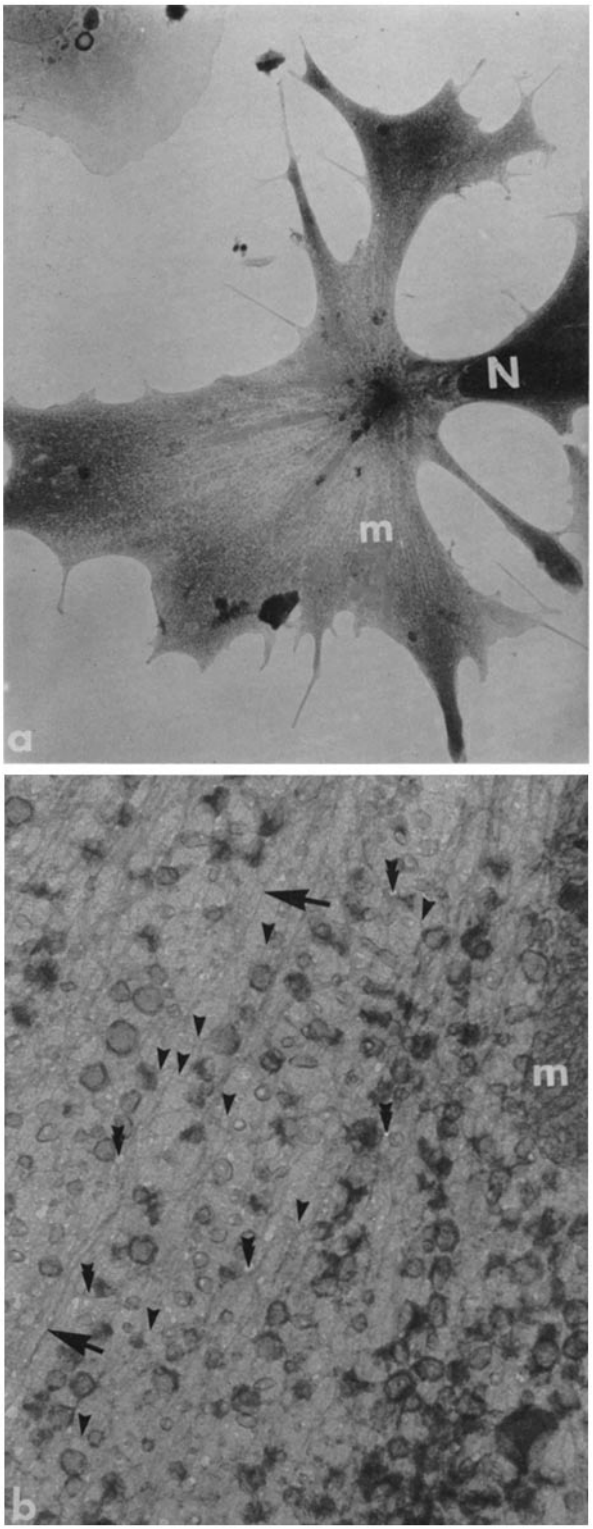

FIGURE 4 (*a*) HVEM picture of a digitonin-opened erythrophore. The cell was treated with 1 μg/ml digitonin for 2 h at 23°C, 2% gluteraldehyde fixed, and dried by the critical point method. (*b*) The cell surface was lightly coated with Pt/Pd to reveal holes (double arrowheads) in the plasma membrane. Nucleus (*N*); mitochondria (*m*); microtubules (arrows); cytomatrix filaments (arrowheads). (*a*) × 1,900. (*b*) × 20,000.

and thereby eliminating the possibility that EHNA's effect on a cell's methylation state is mistaken for an EHNA effect on dynein. Thus, in continuing work, vanadate and EHNA can be used to more rigorously examine the possibility that dynein is a component of the erythrophore intracellular motility system.

Potential Transport Regulatory Molecules

In chromatophores, it is possible that several molecular modifications (e.g., calcium binding, phosphorylation state) regulate cytomatrix separation and reintegration events. We know that increased calcium ion levels can trigger the "resolute" aggregation phase of pigment motion in erythrophores (36), and it is clear from studies of digitonin-permeabilized chromatophores that calcium acts directly on the cytomatrix to effect this change. The dispersion or reexpansion event is produced in turn with reduced Ca^{2+} levels (36) and is promoted by cAMP in an ATP-dependent manner (35, 71).

Does calmodulin regulate intracellular dynein-dependent motility in erythrophores? Calmodulin, as well as dynein, has been identified as a component of ciliary axonemes (22), and it has been suggested (22, 41) that calmodulin, via association with calcium, could serve to attenuate a dynein-dependent motility response. Numerous agents, most notably trifluoperazine, have been used to identify calmodulin in vitro and study its role in vivo. Trifluoperazine has been shown to bind calmodulin with fair specificity (67) and block its activation of phosphodiesterase (34). The drug also blocks cell spreading (31) and phagocytosis (26) and perturbs cytoarchitecture (50), effects that led these investigators to postulate a pivotal role for calmodulin in these cell functions. I wish to know whether anti-calmodulin or trifluoperazine inhibit aggregative motility in erythrophores and, thus, whether calmodulin is a regulatory element in these cells. Obviously, such experiments can be readily performed in permeabilized models, and these studies are currently in progress in my laboratory.

A Model for Pigment Motion

In summary, it is possible to envisage a cytomatrix consisting of at least two classes of proteins: a class of contractile proteins (spasminlike) that aggregate and shorten to produce pigment aggregation and class of HMW-MAPs (including the dyneinlike ATPases) that serve the crosslinking and/or the saltatory transport of pigment granules along microtubules. When an erythrophore is stimulated to aggregate, calcium influx occurs; this calcium associates with calmodulin, and the complex serves to inhibit dynein activity, thus preventing saltatory movement. MAP-2 cross-linking of microtubules is also diminished and MAP-2 interactions with pigment are maximized. The cessation of shuttling and change in MAP-2 affinities aid the progress of aggregation, which is triggered by Ca^{2+} binding to α-cytomatrix networks. During postaggregation, calcium is pumped from the cell to lower the intracellular concentration to a point at which dynein is no longer inhibited. MAP-microtubule interactions are restored and the dispersive saltatory motility resumes in conjunction with expansion of the α-cytomatrix material. During pigment dispersion HMW-MAPs (i.e., MAP-2) possibly interconnect the reexpanding cytomatrix and associated pigment granules with microtubules and the subplasmalemma. Here, a cAMP-dependent phosphorylation of dynein-ATPases and/or aggregated MAP-2 phosphoproteins molecules (79) conceivably lowers the activation energy of "dynein-ATP-driven" saltations and α-cytomatrix expansion processes to produce pigment dispersion. Some complexity possibly is introduced into the process by the existence of an additional microtubule-stabilizing MAP-1-rich β-cytomatrix component that serves to maintain nonmotile organelles (microtubules, smooth endoplasmic reticulum, mitochondria) in orderly arrays. Information from whole-mount microscopy and monoclonal antibody studies of MAP-1 and 2 distributions in my laboratory supports this model.

The model does not consider the central centrosomal structure and whether it also changes in its HMW-MAPs composition or organization as a function of pigment motion. This aspect of the problem is potentially interesting in light of thin-section observations by (65) showing that melanophore MTOCs can expand or disperse their contents during pigment aggregation.

The behavior of the cytomatrix in chromatophores may reflect a more widespread phenomenon in diverse cell types. Several notable examples are presented here. In heliozoans, and in the ciliates *Vorticella* and *Stentor*, the axopod or stalk rapidly contracts and reextends during feeding. Not unlike pigment motion, this process involves a filamentous network of proteins (e.g., spasmin in *Vorticella*) whose Ca^{2+}-triggered contractile behavior is guided largely by bundles of microtubules (1, 27, 80). Similarly, recent morphological evidence suggests that an extensive kinetochore-associated cytomatrix may produce chromosome migration by interacting with spindle microtubule structures. In recent reviews, Pickett-Heaps et al. and McIntosh (42, 54) have discussed the possible involvement of this cytomatrix network in chromosome motion in detail. Finally, in cultured neuroblastoma cells, I (70) have shown that a Ca^{2+}/Mg^{2+}-sensitive cytomatrix is involved in the saltatory transport of organelles along microtubules. In this report, I predict that an organelle-associated cytomatrix undergoes a molecular process of segregation and reintegration with a microtubule-associated cytomatrix in a manner that supports the continuation of saltatory motion. These observations are strongly supported by structural studies of fast-frozen, deep-etched, rotary replica-shadowed crayfish axons in which it has been confirmed that a crosslinking cytomatrix maintains numerous interlinkages between the microtubules and motile vesicles (25).

Note Added in Proof: Murphy and Grasser (1984, *J. Cell Sci.* 66:353–366) have recently shown that intermediate filaments (8 nm) might comprise a major component of the erythrophore and melanophore cytoskeleton that is found surrounding the pigment granules. Although intermediate filaments are known to be present in melanophores, this is the first report of intermediate filaments in erythrophores. Unfortunately, the data is unconvincing, since chromatophore preparations isolated by their procedures also contain epithelial cells and iridiphores. Obviously the contaminating cells could release 8-nm filaments that then interact with or bind to the pigment granules nonspecifically. Further work utilizing either antibodies to intermediate filament proteins and/or electron microscopy should demonstrate whether intermediate filaments are indeed present in erythrophores.

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