# Functional coordination of microtubule-based and actin-based motility in melanophores

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**The fish melanophore has been considered the exemplar of microtubule-based organelle transport. In this system, a radial array of uniformly polarized microtubules [1] provides a framework on which dynein-related and kinesin-related motors drive pigment granules toward the minus or plus ends, respectively [2–4]. Stimulation of minus-end motors accounts satisfactorily for aggregation of granules at the cell center. Rapid dispersion is clearly microtubule-dependent; however, the uniform distribution of granules throughout the cytoplasm is paradoxical because stimulation of plus-end motors is predicted to drive the granules to the cell margin. This paradox suggested that the transport system was incompletely understood. Here, we report the discovery of a microtubule-independent motility system in fish melanophores. The system is based on actin filaments and is required for achieving uniform distribution of pigment granules. When it is abrogated, granules accumulate at the cell's margin as predicted for microtubule plus-end motors acting alone. The results presented here demonstrate the functional coordination of microtubule and actin filament systems, a finding that may be of general significance for organelle motility in cytoplasm.**

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## Results and discussion

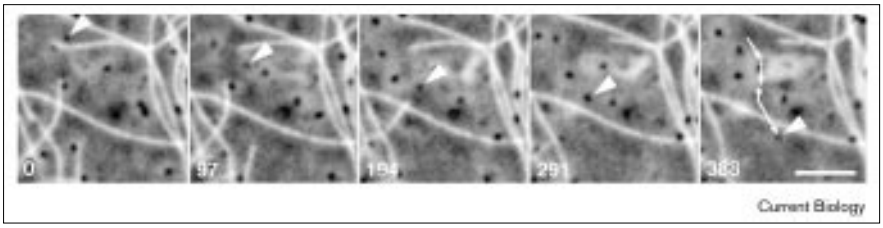
Black tetra melanophores were cultured and induced to aggregate and disperse pigment as described [5]. In the dispersed state of untreated cells, granules displayed a 'to-and-fro' movement evident upon playback of time-lapse image sequences as originally reported by Green [6]. To test whether granules in the dispersed state were transported exclusively along microtubules, the motion of pigment granules was examined in cells with microtubules labeled with fluorescently tagged tubulin [5]. However, because melanophores contain a dense array of cytoplasmic microtubules and the concentration of pigment granules is also very high, it was difficult to decide whether any individual granule was transported along microtubules alone.

To overcome these difficulties, cytoplasmic fragments of melanophores partially depleted of pigment granules were created by dissecting fragments in the course of pigment aggregation (1–2 minutes after adrenalin addition) from cells with prelabeled microtubules. Pigment granules in the fragments were subsequently redispersed (by caffeine treatment) and images of granules and microtubules were obtained in time-lapse mode. Playback of image sequences showed that some granules displayed apparent, bidirectional motion along microtubules (data not shown). Remarkably, however, granules often detached from microtubules and continued their motion along irregular tracks (Figure 1). This behavior of the granules could not be explained by motion along stable microtubules that failed to incorporate labeled tubulin, since injected cells were incubated for at least 3 hours, a time sufficient for labeling of all microtubules in black tetra melanophores [5]. These results suggested the existence of a microtubule-independent transport system in melanophores.

We thus examined the behavior of pigment granules in melanophores with microtubules completely depolymerized by a combined cold and nocodazole treatment [7]. In melanophores lacking microtubules, the shuttling motion continued and was apparently random throughout the cytoplasm. The instantaneous speed of shuttling motion in the absence of microtubules ($0.07 \pm 0.05$ μm/sec) was indistinguishable from the motion in the presence of microtubules, both at the periphery ($0.06 \pm 0.05$ μm/sec) and at the center ($0.05 \pm 0.04$ μm/sec) of control cells. Tracks of individual granules, although highly irregular (Figure 2a), frequently exhibited long processive runs. Thus, thermally driven, Brownian motion did not seem a likely explanation. To test if an active mechanism was involved, microtubule-free melanophores were depleted of ATP by combined treatment with sodium azide and deoxyglucose [8]. Motion completely stopped (data not shown), establishing that the microtubule-independent shuttling of granules was energy dependent.

The possibility that the shuttling motion was dependent on actin filaments [7] was tested by treating cells for 60 minutes with the inhibitors of actin polymerization, cytochalasin D (2 μM) [9] and latrunculin A (5 μM) [10]. Neither compound inhibited adrenalin-stimulated aggregation or caffeine-stimulated dispersion in melanophores containing microtubules, consistent with previous studies

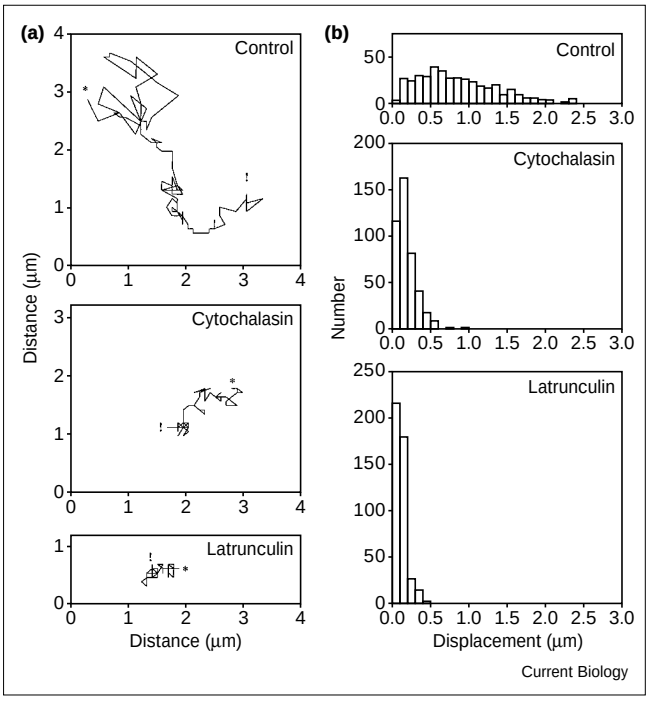
**Figure 1**




Motility of individual pigment granules in a granule-depleted melanophore fragment with fluorescently labeled microtubules (see text). A granule (arrowheads) moved along an irregular track that did not correspond to a microtubule. Numbers in the lower left corner of each frame indicate time in sec; arrows in the last frame show distance covered by the granule within each 97 sec time interval; the scale bar is 5 μm.

employing actin inhibitors [7,11]. Neither compound affected the distribution of microtubules in control cells as assayed by tubulin immunofluorescence (data not shown). However, in melanophores lacking microtubules, both compounds inhibited shuttling motion as assayed by analysis of individual granule tracks (Figure 2a) and by analysis of net displacement after a 30 second interval (Figure 2b). (Quicktime movie sequences of the shuttling motion and the effects of latrunculin are available at http://borisy.bocklabs.wisc.edu.) Cells treated with cytochalasin D showed some residual shuttling activity but shuttling motion was blocked almost completely by latrunculin. The inhibitory effects were fully reversible upon washout of the compounds. These results suggested that the shuttling motion of pigment granules was dependent upon actin filaments.

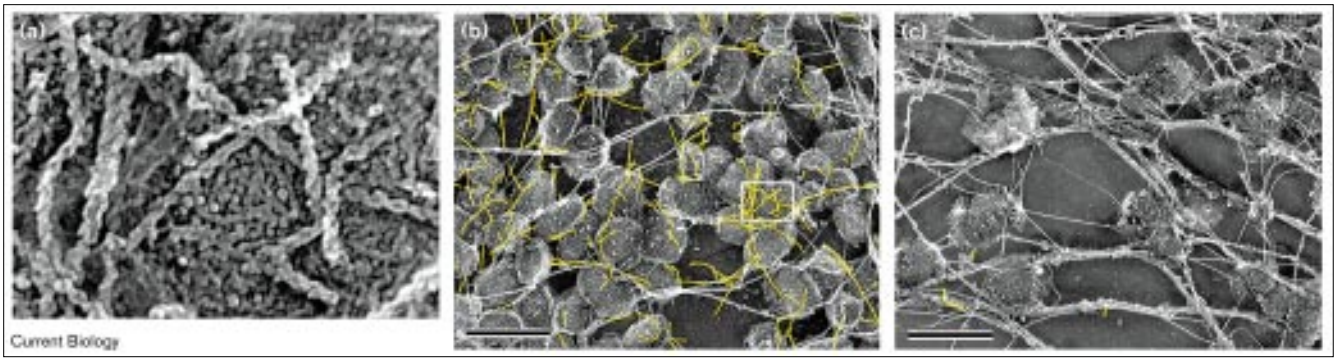
**Figure 2**




Microtubule-independent motility of pigment granules. Shuttling motion of pigment granules was quantified in cells lacking microtubules. **(a)** Tracks of individual granules. Granule positions were determined every 3 sec for 300 sec; tracks shown are for 100 steps. The asterisk indicates the beginning and the exclamation mark the end of a track. **(b)** Net displacement in 30 sec. Net displacement was determined as the straight-line distance between starting and ending coordinates for each 30 sec interval; $n$ = 500 intervals for 50 granules in each condition. Granules in control cells showed extensive motion whereas granules in cells treated with 2 μM cytochalasin D or 5 μM latrunculin showed reduced or virtually no motion, respectively.

The strong effect of the inhibitors on the shuttling motion, their rapid reversibility, and their lack of effect on the microtubule-dependent processes of aggregation and dispersion indicated specificity in their action and a presumptive actin target. To evaluate this suggestion, we concentrated on the action of latrunculin because of its completely inhibitory effect. Actin filament levels were determined by quantitative fluorescence staining with rhodamine phalloidin [12]. Phalloidin staining in latrunculin-treated cells was 20.1% of the fluorescence value in control cells ($n$ = 29; s.d. = 6.5%), consistent with substantial removal of actin polymer. Actin filaments have been shown to exist in the periphery of melanophores [13]. We visualized them by decoration of cytoskeletons with myosin subfragment S1 and subsequent preparation of platinum replicas for electron microscopy [14] (Figure 3a). Short actin filaments (0.2–3 μm long) were abundant in the cytoplasm of melanophores either containing or lacking microtubules. The density of actin filaments was greater at the cell periphery than at the center and ranged over values of 0.1–1.5 μm total length per square micrometer of cytoplasm. Many, if not most, pigment granules were in contact with at least one actin filament and the actin filaments showed no preferential orientation (Figure 3b). As assayed by replica electron microscopy, latrunculin treatment removed almost all actin filaments (Figure 3c), reducing levels to approximately 5% of control cells. Other cytoskeletal components, including microtubules and intermediate-like filaments were not affected by the latrunculin treatment. Thus, structural analysis established the presence of randomly arranged actin filaments in the cytoplasm of melanophores and that latrunculin treatment specifically removed these filaments.

**Figure 3**

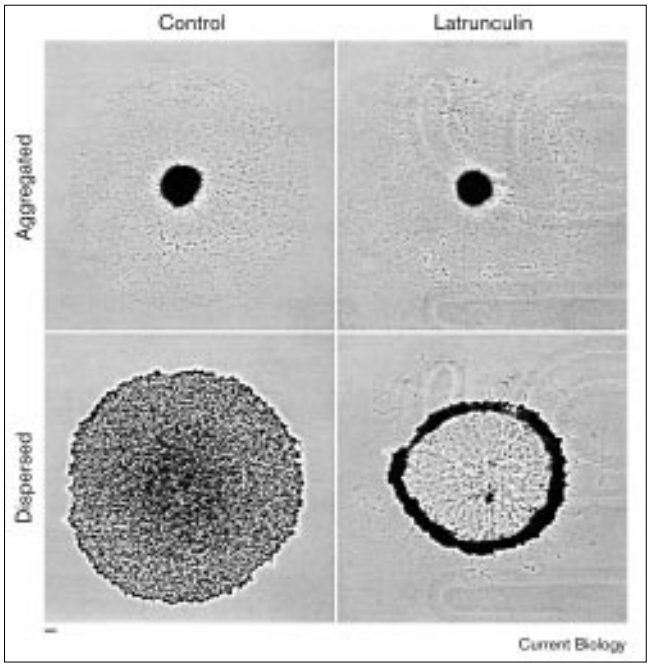



Actin filaments in melanophores. Actin filaments were identified by decoration with the S1 subfragment of myosin and platinum replica electron microscopy. (a) Control cell. High magnification view of the boxed region in (b), showing the characteristic asymmetric 'rope-like' appearance of decorated actin filaments. The thicker end of an individual rope element points toward the pointed end. (b) Low magnification view of control cell showing distribution of actin filaments (yellow). Actin filaments frequently contact pigment granules and show no preferential orientation. (c) Latrunculin-treated cell. Virtually no actin filaments (yellow) remained. The scale bars are 1 μm.

Redispersion of pigment after aggregation generally leads to uniform distribution of granules throughout the cytoplasm. If the randomly arranged actin filaments were necessary for achieving this uniform distribution, removal of actin by latrunculin would be predicted to alter the distribution of pigment. To test this possibility, melanophores containing microtubules were induced to aggregate and then challenged to redisperse their pigment either without or after latrunculin treatment. Control cells redispersed their pigment uniformly as normal (Figure 4a) but, in latrunculin-treated cells, pigment granules moved centrifugally all the way to the cell margin (Figure 4b). Tubulin immunofluorescence showed that microtubules extended all the way to the rim as in untreated cells (data not shown). Cell arborization and partial retraction were also induced by latrunculin, but the important point is that the normal uniform distribution of pigment was drastically altered — the granules moved to the rim of the cell as would be predicted by the activity of microtubule plus-end motors acting without constraint. Latrunculin exerted a similar but less dramatic effect on melanophores in the dispersed state. Although the normal distribution of pigment granules was perturbed, treated cells retained the capacity to aggregate and redisperse granules with close to normal kinetics (data not shown).

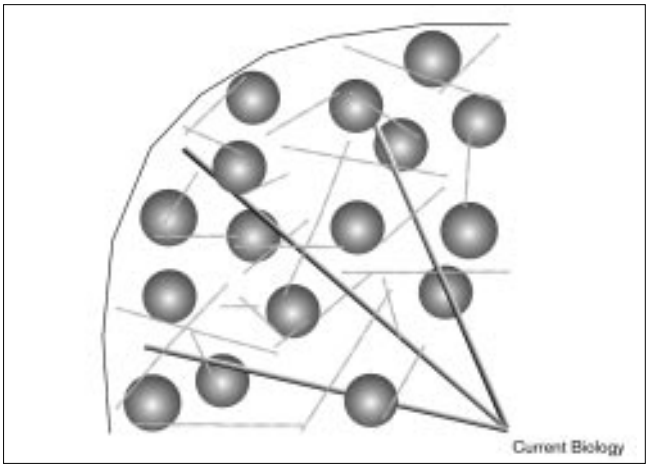
Eukaryotic cells contain two principal systems of motility: microtubule dependent and actin-filament dependent. Studies of transport in cell extracts [15,16] and *in vivo* [17,18] showed that the same membrane organelle could move along both microtubules and actin filaments. To explain the relative roles of microtubules and actin filaments as tracks for these movements, it has been suggested that microtubules serve as routes for transport over long distances, while actin filaments support movement to local sites [19,20]. The melanophore's role in causing color change may provide a dramatic illustration of this

**Figure 4**




Actin filaments are necessary for achieving uniform distribution of pigment. Aggregation of pigment granules was stimulated with 10 μM adrenalin and dispersion with 5 mM caffeine. In control cells, aggregation proceeded to a tight focus within 5 min and dispersion to a uniform distribution within 15 min. For latrunculin-treated cells, 5 μM latrunculin was added for 15 min after normal aggregation; dispersion was then stimulated by addition of caffeine. The image acquired 10 min after caffeine addition shows pigment granules accumulated at the cell margin. Some retraction of the cell margin has also occurred. The scale bar is 20 μm.

**Figure 5**




Model for dispersion of pigment granules in melanophores. During dispersion, pigment granules (balls) initially move to the periphery along radial microtubules (thick lines) but then leave microtubule tracks and distribute homogeneously in the cytoplasm by motion along randomly arranged actin filaments (thin lines).

idea. We suggest that dispersion of pigment granules is accomplished by functional coordination of the two principal motility systems. Microtubules provide tracks for initial fast motion toward the periphery, but the final uniform distribution of pigment granules is achieved by a two-dimensional 'random walk' on actin filaments (Figure 5). A problem not addressed by our observations is the anchoring of actin filaments. If they were not anchored in some way, they would be predicted to move on the granules rather than the granules moving on them.

The existence of actin-dependent transport suggests that granule motility can be driven by myosin as well as by kinesin-related and dynein-related motors. This possibility is experimentally established in the accompanying paper [21] by the demonstration of *in vitro* motility of *Xenopus* pigment granules driven by myosin-V. The specific manner in which microtubule and actin motility systems interact may depend upon the contingencies existing for different cell types. However, the results in melanophores, previously considered as a model system exclusively for microtubule-dependent transport, suggest that functional coordination of the two motility systems may be a general principle of organelle transport in cytoplasm.

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