Actin-Dependent Pigment Granule Transport in Retinal Pigment Epithelial Cells

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Introduction

Since diffusion of particles as large as vesicles or organelles is constrained by the high viscosity of cytoplasm, directed translocation of these particles from one cytoplasmic site to another must be achieved by cytoskeletal intervention. In most cases, this is achieved by attaching the particle to a molecular motor which then navigates along a microtubule or an actin filament track (Fath and Burgess, 1994). Although microtubule-dependent transport in animal cells has been more extensively studied, important roles for actin-dependent transport have also recently been recognized (Langford, 1995).

Results and Discussion

Our laboratory has been using the migration of membrane-bound melanin pigment granules in the teleost retinal pigment epithelium (RPE) as a model system for studying intracellular particle transport (Burnside et al., 1983; King-Smith et al., 1995, 1996). The RPE is a simple epithelium that lines the back of the vertebrate eye and lies between the photoreceptors and the choroidal blood supply. In the fish RPE, pigment granules undergo dramatic migrations in response to changes in light condition: they aggregate into the RPE cell body in the dark and disperse into the cell’s long apical projections in the light, migrating distances as great as 100 μm. Several properties of RPE cells make them technically advantageous for a study of particle transport. In these cells, pigment granules migrate within fixed apical projections; thus transport-related cytoskeletal dynamics can be examined in the absence of change in cell shape. Either pigment granule aggregation or dispersion can be triggered at will by incubating RPE cells with cAMP or dopamine, respectively, and pigment granule translocation is slow enough to permit experimental intervention or biochemical isolation while granules are traveling in either direction (Burnside and Basinger, 1983; Dearry and Burnside, 1988). Pure preparations of RPE sheets (containing a single cell type) can be isolated in different functional states in sufficient amounts to permit both molecular and biochemical analyses. Finally, normal aggregation and dispersion can be induced in single isolated cells in vitro, thereby permitting analysis of transport kinetics under different experimental conditions by time-lapse videomicroscopy (King-Smith et al., 1995, 1996).

Video analysis has shown that pigment granule movements in RPE cells are slower but otherwise similar to those previously reported for dermal melanophores (unpubl. obs.). During dispersion, pigment granule movement is saltatory and bidirectional, with individual pigment granules moving independently; mean centrifugal and centripetal velocities were 3.2 and 1.5 μm/min respectively. The net pigment dispersion rate in isolated cells (1.8 μm/min) is similar to that observed in vivo (2.2 μm/min). After full dispersion is achieved, bidirectional saltations of pigment granules continue within the apical projections. When aggregation is triggered by cAMP, all granules undergo a coordinated, smooth, non-saltatory centripetal movement, with a mean velocity of 3.6 μm/min, which is comparable to the mean in vivo velocity of 3.4 μm/min. In the time-lapse movies, other forms of motility are visible in the apical projections, including bidirectional translocations of mitochondria,
formation and migration of cytoplasmic bridges between projections, and modest extensions and retractions of the tips of the projections.

To identify the cytoskeletal mechanisms of RPE pigment migration, we investigated whether disruption of microtubules with nocadazole would block pigment granule movement in isolated sunfish RPE cells in vitro. Neither aggregation nor dispersion was inhibited by the complete disruption of the microtubules of the apical projections (unpubl. obs.). Microtubule disruption had no effect on the mean velocities of individual granules, or on the rate or extent of pigment granule aggregation or dispersion. Maintenance of the aggregated or dispersed states was also unaffected by microtubule disruption. These observations strongly suggest that microtubules are not required for pigment granule migration in isolated RPE cells.

As a first step toward evaluating the role of actin filaments in RPE pigment granule transport, we also investigated the effects of cytochalasin D on pigment granule migration in isolated RPE cells (unpubl. obs.). Net pigment granule aggregation and dispersion were both strongly and reversibly inhibited by cytochalasin D, the IC50 for dispersion (0.5 μM) being lower than that for aggregation (2.5 μM). Video analysis revealed that cytochalasin has a surprising effect on the movements of individual pigment granules. Although most granules stopped moving altogether within minutes of exposure to cytochalasin, several granules in each projection began to undergo very rapid (up to 40 μm/s), bidirectional excursions. When cytochalasin was applied to isolated RPE cells in which the microtubules had been previously disrupted by nocadazole, all pigment granule movement stopped, suggesting that the very rapid bidirectional excursions observed in cytochalasin-treated cells are microtubule-dependent.

These inhibitor studies suggest that actin filaments play important roles in both aggregation and dispersion, although the mechanism of actin filament participation is not clear. Since the effects of cytochalasin D on aggregation and dispersion have different IC50s, the force-producing mechanisms of the two processes may differ. The low IC50 for dispersion suggests that interfering with plus-end assembly of actin filaments is sufficient to block centrifugal transport. The higher IC50 for aggregation, on the other hand, suggests that additional effects of cytochalasin, such as disruption of actin filament organization, may be necessary to block motility in this case. The implication that both centrifugal and centripetal pigment granule movements are actin-dependent is somewhat surprising, since all known myosin motors move only toward the plus ends of actin filaments. The roles of myosin motors and actin filament dynamics in pigment granule transport are not yet clear.

In parallel with physiological analyses of RPE transport, we have also identified myosin motor proteins expressed in fish RPE cells (unpubl. obs.). Using degenerate primers based on conserved sequences in the myosin motor domain for RT-PCR, we have identified 11 myosin motor proteins that are expressed in teleost RPE. These include one apparently novel myosin selectively expressed in RPE and retina, and two selectively expressed in RPE. We are currently making isotype-specific antibodies to these motors for isolation of native proteins and subcellular localization of each myosin isotype. Ultimately we plan to analyze the roles of these myosin motors in pigment granule transport and other motile processes of RPE cells.

Acknowledgments

Supported by NIH grant R37-EY03575.

Literature Cited


