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Actin-based organelle movement

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Abstract. Evidence for actin-dependent organelle movement was first obtained from studies of cytoplasmic streaming in plants. These studies, together with cell-free organelle motility studies and biophysical analyses of muscle myosin, support a model whereby organelle-associated motor molecules utilize the energy of adenosine triphosphate binding and hydrolysis to drive movement along F-actin tracks. Recent studies indicate that this mechanism for organelle movement may be responsible for organelle and vesicle movement during secretion, endocytosis and mitochondrial inheritance in a variety of eukaryotes.

Key words. Actin; myosin; motor molecules; secretion; endocytosis; mitochondria; organelle inheritance.

Multiple cellular processes such as neuronal outgrowth, transport of materials across an epithelial layer and cell migration require establishment of cell polarity. Eukaryotic cells generate asymmetric distribution of cellular constituents by rearranging cytoskeletal elements and utilizing cytoskeletal fibers for organelle and vesicle transport. Recent studies suggest that organelle-cytoskeletal interactions and organelle movement along inherently polar cytoskeletal tracks are mediated by motor molecules. In many eukaryotic cells, microtubules and the microtubule motors kinesin and dynein support long-distance movements of vesicles and organelles [1, 2]. However, evidence has emerged for a role of the actin cytoskeleton in control of organelle position and movement. Here, we review specific examples of actin-dependent organelle movements with an emphasis on the functional significance of these motility events and on the components of the motile apparatus that makes them possible.

Cytoplasmic streaming

Actin-dependent organelle movement was first identified in plant cells. In large cells such as *Chara* or *Nitella*, which are up to 10 cm long, transport of nutrients and metabolites from one end of the cell to the other by simple diffusion could require days or weeks. Therefore, these and other plant cells utilize an active transport system to insure effective cytosplamic mixing. This system, known as cytoplasmic streaming, consists of two opposing currents of endoplasm which undergo vigorous rotational streaming around a large central vacuole at velocities of approximately 60 μm/s [3].

Several studies suggest that actin bundles are required for cytoplasmic streaming. First, actin bundles are found in the cortical regions of *Nitella*. Actin filaments within these bundles are oriented with the same polarity, and with their long axis parallel to the direction of cytoplasmic streaming [4]. Second, physical injury to the actin-rich cellular cortex of *Chara* or *Nitella* arrests cytoplasmic streaming [3]. Third, destabilization of actin structures by cytochalasin B, a fungal metabolite which blocks barbed-end polymerization and severs actin filaments [5], inhibits cytoplasmic streaming in *Nitella* and *Avena* [6].

A mechanism of force generation for cytoplasmic streaming was proposed based on studies in whole cell and cell-free systems. Kachar and Reese [7] described continuous movement of large, tubovesicular, branching structures along stationary actin bundles in live *Chara* cells at rates similar to those of cytoplasmic streaming (50–60 μm/s). These structures were identified as rough endoplasmic reticulum using a combination of video-enhanced light and electron microscopy. In addition, organelle movement was reconstituted using actin cables, organelles and other components found in dissociated *Chara* endoplasm [8]. These observations support a model whereby active movement of endoplasmic reticulum along polarized actin bundles generates the drag force necessary to propel cytoplasmic streaming.

Organelle movement in *Chara* and *Nitella* is adenosine triphosphate (ATP)-dependent and polarized towards the barbed end of actin filaments. In addition, proteins that cross-react with myosin antibodies or that have myosin-like activity have been identified in *Nitella* [9–11]. These observations indicate that the motor for cytoplasmic streaming may be a member of the myosin superfamily. Myosins, a family of at least 12 classes of

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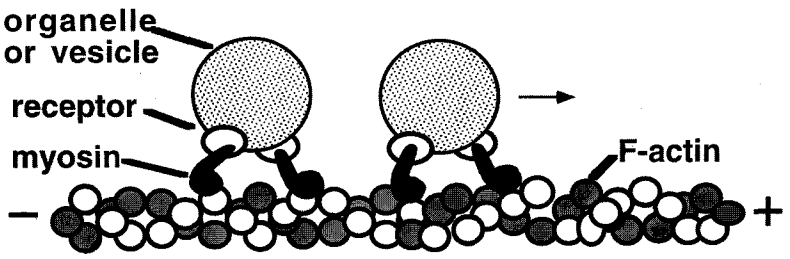

Figure 1. Model for actin-dependent organelle movement (see text for details).

homologous proteins, contain conserved amino-terminal motor domains with ATP- and actin-binding sites, and variable tail domains which are specialized for dimerization, signal transduction and/or membrane binding [12]. The neck domain connecting the motor and tail domains contains IQ motifs, sequences which bind to calmodulin and calmodulin-like light chains [13]. Myosins were implicated in organelle movement on the basis of observations that (1) muscle and non-muscle myosins display ATP-dependent, barbed end-directed motor activity [14, 15]; (2) vesicles and organelles that bind to or move along F-actin contain proteins that are immunologically cross-reactive with myosin [10, 16–18]; and (3) anti-myosin antibodies and mutations of myosin genes inhibit actin-dependent particle movement in whole cells and cell-free systems [19–21].

These observations led to a model for actomyosin-driven organelle movement (fig. 1). According to this model, myosin motors utilize tail subdomains to bind to their organelle passengers. Myosin head groups, which project from organelle surfaces, bind to the lateral surface of actin filaments and transduce the energy derived from ATP hydrolysis to drive movement along F-actin tracks. The precise mechanism of myosin-driven movement is not completely understood. Studies on muscle myosin indicate that ADP-bound myosin interacts with actin filaments with high affinity. Upon exchange of ADP for ATP, myosin is released from F-actin. Finally, upon ATP hydrolysis myosin binds to F-actin and undergoes the 'power stroke', a conformational change which results in displacement along F-actin. Repeated rounds of myosin-mediated ATP binding and hydrolysis lead to movement of myosin towards the barbed end of actin filaments [22–24].

Intracellular movement in other eukaryotes

Actin destabilization studies provide evidence that actin-dependent organelle movement is not restricted to plant cells. For example, treatment of photoreceptor cells of arthropods with cytochalasin D inhibits light-induced reorganization of mitochondria, endoplasmic reticulum and pigment granules [25]. Inhibition of organelle movement is also observed upon cAMP-induced actin reorganization in *Dictyostelium* [20] and injection of antibodies against actin-binding proteins in fibroblasts [26]. Finally, in budding yeast, mutations in the actin gene (*ACT1*) or in genes encoding the actin-binding proteins fimbrin (*SAC6*), tropomyosin (*TPM1*) or capping protein (*CAP1* and *CAP2*), which destabilize actin structures, also result in defects in secretion, endocytosis and mitochondrial motility [27–36]. Thus, although organelle transport along microtubules is well documented in various cell types [1, 2], recent findings indicate that actin-dependent organelle movement occurs in many eukaryotes. Below, we describe what is known regarding the molecular basis of these actin-dependent processes.

Secretion

Fungi bearing mutations in myosin genes show defects in secretion. The *Saccharomyces cerevisiae* gene, *MYO2*, encodes an essential, type V myosin which localizes to sites of active cell growth [21, 37]. *myo2* mutants show vesicle accumulation, defects in reorganization of the actin cytoskeleton and synthetic lethality with late-acting secretory mutants [21, 37, 38]. Simultaneous deletion of the *MYO3* and *MYO5* genes, which encode class I myosins, also results in secretory defects and abnormal actin organization [39, 40]. Myo5p is localized at sites implicated in actin nucleation and assembly [40, 41]. These observations raise the possibility that Myo2p and myosin I proteins of yeast are required for secretory vesicle transport along F-actin tracks. However, *myo2* and myosin I mutants display abnormal actin organization, a defect that is known to cause impaired secretion. Thus, secretory errors in yeast myosin mutants may be a consequence of cytoskeletal destabilization rather than direct effects on possible vesicle motors. Finally, secretion defects and inhibition of hyphal growth in germ tubes of *Aspergillus nidulans* is observed upon deletion of *myoA*, an essential class I myosin. At present, actin organization in the *myoA* mutants, and the precise role of this myosin motor in secretion, remain to be determined [42].

In neuronal cells, evidence has emerged for a direct role of the actin cytoskeleton and actin-dependent motor molecules in vesicle movement and secretion. Although

microtubules are thought to be the major tracks for movement in axons, these cytoskeletal fibers rarely penetrate the dense cortical actin meshwork that extends beneath the plasma membrane along the length of the axon, growth cone or axon terminal [43, 44]. Therefore, it is possible that microfilaments mediate cortical motility events such as delivery of secretory vesicles to the plasma membrane or synaptic vesicles to the axon terminal [45]. Consistent with this, synaptic vesicles are found to be reversibly anchored to actin filaments in the nerve terminal of bovine brain by synapsin I [46, 47]. In addition, actin filament-dependent movement of intracellular particles is observed in growth cone lamellipodia of superior cervical ganglion neurons in culture. In some cases these particles appear to fuse with the plasma membrane at the leading edge of the growth cone [48]. Finally, linear, unidirectional and ATP-dependent movement of organelles is observed in extruded squid axoplasm [16, 49]. The tracks for this movement were identified as actin filaments on the basis of rhodamine phalloidin staining, cytochalasin D sensitivity and nocodazole insensitivity. Organelles from extruded squid axoplasm move in a barbed end-directed manner [18, 50] along polarized actin arrays [51, 52]. Thus, it appears that the neuronal vesicle motor(s) has characteristics similar to those of myosins.

Although the motors for actin-dependent organelle movement in the squid axon have yet to be identified, myosin proteins are implicated in vesicle movements and secretion in other nervous systems. Mutation of the myosin V-encoding *dilute* gene in mice results in defects in melanosome movement to hair shaft keratinocytes and thus in dilution of hair color [53, 54]. In addition, p190, a class V myosin found in Purkinje cells in the cerebellum, primary hippocampal neurons and non-neuronal cells, colocalizes with wheat germ agglutinin containing vesicles at the perinuclear region and in the distal ends of cell processes [55, 56]. Thus, p190 appears to be associated with Golgi-derived vesicles where it may play a role in secretory transport.

Further support for the model for motor-driven, actin-dependent organelle movement was obtained from studies of polarized intestinal epithelial cells which are specialized for absorption and secretion at apical and basal surfaces. In these cells, microtubules are required for efficient transport of secretory vesicles to the cell apex. Microtubules are oriented with their minus ends at the cell apex and their plus ends directed basally; thus it is possible that a minus end-directed microtubule motor (e.g. dynein) may be involved in the apical translocation of secretory vesicles [57]. However, since microtubules terminate below the terminal web and do not extend into the actin-rich microvillar surface, it is unlikely that microtubules mediate vesicle movement in the brush border. In support of this, dynein and brush border (BB) myosin I are both found on the cytosolic face of Golgi-derived vesicles in enterocytes [58]. Although the majority of BB myosin I and dynein appear in a soluble pool, a small percentage of dynein and BB myosin I appears to be specifically associated with Golgi-derived vesicles. The distribution of motors on the surface of vesicles is not equal: BB myosin I is present in a large majority of Golgi-derived vesicles, whereas dynein appears to be present only in late Golgi secretory vesicles [17, 58]. Thus, secretory vesicles may switch tracks from microtubules to actin filaments as they move into the brush border.

Finally, 95F, a myosin in *Drosophila melanogaster*, has been implicated as a motor for particle movement to the plasma membrane during development. This class VI myosin is found associated with cytoplasmic particles in syncytial blastoderm *Drosophila* embryos. The 95F-containing cytoplasmic particles are distributed throughout the cytoplasm during interphase and are transported to membrane pseudocleavage furrows during metaphase. These particles display linear, ATP-dependent and cytochalasin D-sensitive movement. In addition, microinjection of polyclonal antibodies raised against 95F inhibits linear particle movement, membrane invagination and furrow formation in *Drosophila* embryos [59, 60]. At present, the composition of 95F-associated cytoplasmic particles is not well understood.

Endocytosis and osmoregulation

Uptake of extracellular constituents into cells can occur by receptor-mediated endocytosis, fluid phase endocytosis (pinocytosis) or phagocytosis. Each of these processes involves alterations in the plasma membrane ranging from invagination for vesicle production to protrusion for entrapment of extracellular liquid or particles. The vesicles and membrane-bound compartments produced during these uptake events are transported from the cell surface to the cell interior. In many cell types, actin is enriched at the cell cortex and sites of plasma membrane plasticity. Studies in cells including budding yeast, *Acanthamoeba castellani* and *Dictyostelium discoideum* implicate specific myosin motors in endocytosis. Therefore, actin and myosin may serve as force generators for plasma membrane deformation and/or vesicle movement during endocytosis. In addition, the cortical meshwork of actin filaments may act as a barrier to modulate organelle movement. Regulation of particle movement may be controlled by actin binding and/or motor proteins that affect the integrity, organization or porosity of the actin meshwork.

Cytochalasin D treatment results in inhibition of receptor-mediated and fluid phase endocytosis in the apical portion of Madin-Darby Canine Kidney (MDCK) cells [61]. In yeast, mutation of the *ACT1* gene results in osmotic sensitivity and defects in receptor-mediated endocytosis [27]. These findings suggest a role for the actin

cytoskeleton in endocytosis. In addition, mutations in the yeast genes which encode the actin-binding proteins fimbrin (*SAC6*) and myosin Is (*MYO3* and *MYO5*) also impair endocytosis [33, 40]. However, as described above, mutation of yeast myosin I genes results in loss of polarized actin organization and defects in actin-dependent processes including secretion and polarized cell growth [39, 40]. Therefore, the precise role of yeast myosin I proteins in endocytosis remains to be determined.

Acanthamoeba organelles undergo barbed end-directed, ATP- and myosin I-dependent movement along polarized actin tracks in cell-free systems. Consistent with this, subcellular fractionation, immunoelectron microscopy and binding studies using purified motors and membranes localize amoeboid myosin Is at sites of endocytosis and/or osmoregulation. *Acanthamoeba* myosin IA is associated with actin-rich phagocytic cups and small cytoplasmic vesicles. Myosin IB is found in the plasma membrane as well as vacuolar and phagocytic membranes. Finally, myosin IC is associated with the plasma membrane, vacuole membranes and the contractile vacuole [62]. The localization of myosin IA is consistent with that of a vesicle motor [19]. However, the mechanism whereby amoeboid myosin I proteins affect the dynamics of other membranes is not well understood.

Dictyostelium myosin Is are also localized at regions implicated in pinocytosis and phagocytosis [63, 64]. Single mutations in *Dictyostelium* myosin I genes *myoB* and *myoC* result in reduced levels of phagocytosis [65]. In addition, suspension cultures of $myoA^-/myoB^-$ and $myoB^-/myoC^-$ double mutants exhibit defects in fluid phase pinocytosis, defects in rearrangement of the cortical actin cytoskeleton and reduced levels of endocytic vesicles [66]. Thus, myosin I proteins in *Dictyostelium* have partially redundant function and are required for membrane-actin dynamics including phagocytosis and pinocytosis.

Mitochondrial inheritance

Mitochondria are essential organelles that can be produced only from pre-existing mitochondria. Therefore, transfer of these organelles from mother to daughter cells during cell division is essential to ensure the survival of the progeny [67]. In mitotic yeast, mitochondrial movements are linear, long distance and polarized towards the developing daughter cell. Thus, mitochondrial inheritance occurs by active transport of organelles from mother to daughter cells. Several studies indicate that mitochondrial movements may be controlled by direct interactions with the actin cytoskeleton. Light and electron microscopy studies in mitotic yeast reveal co-localization of mitochondria with actin cables, bundles of actin filaments arranged along the mother-bud axis [34, 35, 68]. In addition, mutation of the *ACT1* gene of yeast results in abnormal mitochondrial aggregation and impaired mitochondrial motility and inheritance [34–36]. Finally, mitochondrial inheritance in meiotic yeast is also dependent on a functional actin cytoskeleton. Smith et al. [69] showed that actin mutations result in mitochondrial aggregation and loss of mitochondrial motility during prophase of meiosis I. Together, these studies indicate that mitochondria are functionally associated with the actin cytoskeleton in yeast, and that one consequence of this interaction is control of mitochondrial spatial arrangement, movement and inheritance.

Cell-free studies reveal the nature of these actin-mitochondrial interactions. Isolated yeast mitochondria bind directly to actin filaments. This binding is ATP-sensitive, reversible, saturable and mediated by a protein on the mitochondrial surface [35]. In addition, an ATP-dependent, actin-based motor activity was detected on the surface of mitochondria, using a modification of a microfilament sliding assay [36, 70]. This motor is tightly associated with the mitochondrial surface and

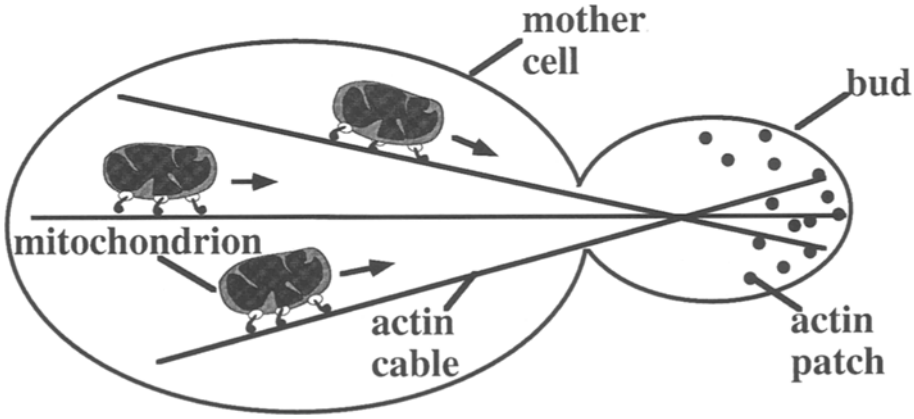

Figure 2. Model for mitochondrial inheritance in yeast (see text for details).

supports movement at a velocity similar to that of mitochondrial movement in living yeast. These studies indicate that (1) yeast mitochondria are associated with an actin-dependent motor, and (2) this motor mediates interaction of mitochondria with filamentous actin and is capable of supporting mitochondrial movement along polarized actin cables during inheritance (fig. 2). At present, the mitochondrial motor has not been identified. Phenotypic analysis of yeast myosin mutants indicates that this motor is not encoded exclusively by any of the five yeast myosin genes [36].

Conclusion and future perspectives

In cells ranging from yeast to animal cells, motor molecule-driven organelle movement along actin tracks has been recapitulated in cell-free systems. Analysis of the distribution of motile organelles and motor proteins, characterization of the dynamics of organelle movement, and analysis of the effect of actin destabilization provide evidence that this mechanism of organelle movement occurs in living cells. In addition, studies indicate that organelles and vesicles can move from microtubule tracks to microfilament tracks, and that both microtubule and actin-dependent motors are present on the surface of a single organelle population. At present, the identity of specific motors for many actin-dependent organelle movements remains ambiguous. Indeed, all of the known actin-dependent motors are believed to be plus end-directed. Future studies may reveal a different class of actin-dependent motors which are minus end-directed. In addition, very little is known regarding the mechanisms responsible for targeting motors to specific membrane populations, and for switching of organelles from microtubule to microfilament tracks. Finally, although myosin activities are known to be regulated in vitro by post-translational modifications and/or light chain interactions, regulation of motor activities in vivo is not well understood. Each of these topics is under active research and may be resolved in the near future.

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