Mini-Review

Overlapping Functions of Myosin-I Isoforms?

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Since the discovery of myosin-I (MI)1 in Acanthamoeba (32), DNA cloning and/or biochemical purification have established the presence of multiple MI isoforms in nearly every eukaryotic cell examined. Despite intensive study, the physiological role of these actin-based, molecular motors has remained a mystery, in large part, because cells express several isoforms of MI with similar biochemical properties. The main questions have been: are the cellular and molecular functions of MI? Are any isoforms essential for life? Do any isoforms perform unique functions? Recent molecular genetic experiments from the laboratories of M.A. Titus (Duke University, Durham, NC) (27) and J.A. Hammer (National Institutes of Health, Bethesda, MD) (19; this issue) provide important help in untangling the roles of four of the MI isoforms in the cellular slime mold, Dictyostelium.

MI’s are single-headed, low molecular weight members of the myosin superfamily (Fig. 1). Similar physical characteristics and conserved features of the sequences of their NH2-terminal motor domains set all of the MI’s apart from the ten other families of myosin (5, 14, 26). This NH2-terminal domain couples the hydrolysis of ATP to movement along actin filaments. Although MI’s move actin filaments more slowly than muscle myosins (26, 33), the mechanism of actin-activated ATP hydrolysis by Acanthamoeba MI is almost identical (28) to the mechanism of myosin-II (23). Therefore, the basic chemistry of the energy transducing process has been conserved since the times of the earliest eukaryotes, and unlike kinesins, low numbers of MI molecules cannot support processive movement (28).

MI tail sequences have diverged much more than the sequences of the heads, so the tails probably hold the secret to the existence of multiple MI isoforms and their specialized functions (35). Immunolocalization, cell fractionation, and in vitro motility assays (26, 33) suggest that MI participates in actin-dependent membrane-based motility, as well as more conventional contraction of actin filament networks. MI tails bind membranes (2, 8, 24) and actin filaments (22), providing the mechanical connections required for these postulated movements. Dictyostelium is a good system to examine MI function because one can inhibit MI using molecular genetics techniques and quantitate many different types of cellular and intracellular motility. The Dictyostelium genome encodes one myosin-II gene, one or two myosin-XI genes (15, 30), at least six MI isoforms (myoA through myoF), and two to five uncharacterized myosin genes. The five fully sequenced MI isoforms fall into two classes based on their divergent tail sequences (Fig. 1). The tails of myoB, myoC, and myoD are homologous to the tails of the Acanthamoeba MI’s and are referred to as long-tailed MI’s. MyoA and myoE are called short-tailed MI’s because they lack the glycine/proline rich regions and the SH3 domains found in the known Acanthamoeba isoforms (Fig. 1). The Dictyostelium MI isoforms are predominantly localized to the actin-rich cortex, including pseudopods, leading edge, and phagocytic cups (13, 18, 19, 29, 39).

Dictyostelium survives knockouts of individual MI genes, and the resultant phenotypes do not clearly identify a role for MI. Cells lacking myoC (29) and myoD (18) have only small defects (Fig. 1). Knockouts of myoA (37) or myoB (16, 39), isoforms from the two different subclasses, have remarkably similar defects (Fig. 1). It is possible that closely related isoforms in Dictyostelium can compensate for the loss of an individual MI isoform from either class.

To test for functional redundancy of MI isoforms, the Titus and Hammer laboratories used different strategies to knockout multiple MI isoforms. Impressed by the similar phenotypes of myoA− and myoB− cells, the Titus laboratory suggested that the role of a given MI isoform could not be predicted on the basis of the tail sequence. Therefore, they created and analyzed single mutants (myoA−, myoB−, and myoC−), double mutants that crossed the two MI subclasses (myoA−/myoB−), and double mutants that were within the same subclass (myoB−/myoC−). The Hammer laboratory hypothesized that if functional overlaps exist, they would most likely be between isoforms within each family. Therefore, they knocked out the long-tailed MI isoforms by creating single (myoB−, myoC−, and myoD−), double (myoB−/myoD−), and triple (myoB−/myoC−/myoD−) mutants.

Which Cellular Processes Depend on Myosin-I?

Fluid-Phase Pinocytosis. MI knockouts compromised, but did not stop fluid phase pinocytosis. Single, double, and triple knockouts progressively inhibited the rate of pinocytosis, and the losses of activity in the multiple knockouts were greater than the sum of the individual losses (Fig. 1). The lower growth rates, impaired development, altered vesicle numbers and sizes, and the lower rates of the inter-
Figure 1. Summary of Dictyostelium phenotypes resulting from MI knockouts performed by the (left) Titus and (right) Hammer laboratories. (center) Schematic diagram comparing the sequence domains in the Dictyostelium myosin-Is: (motor) catalytic motor domain; (+ +) positively charged membrane-binding region; (GPA/GPQ/PA) proline-rich, actin-binding region; (SH3) Src homology-3 structural domain. The arrows point to successive MI knockouts. Multiple arrows indicate multiple knockout mutations. The size of the horizontal bars represents the relative change in a measured cellular event.

Phagocytosis. The rate of phagocytosis is reduced in single mutants of myoB and myoC but is normal for myoA- and myoD- cells (Fig. 1). In contrast to pinocytosis, the loss of a second or third MI isoform did not further compromise phagocytosis (Fig. 1). Therefore, myoB and myoC play significant and probably distinct roles in phagocytosis.

Cell Locomotion and Streaming Behavior. MyoA- and myoB- cells each have significantly reduced rates of whole cell motility, while myoC- and myoD- cells are normal (Fig. 1). Double mutants (myoA-/B-, myoB-/C-, myoB-/D-) and triple mutants (myoB-/C-/D-) were only slightly slower than either myoA- or myoB- cells. Therefore, myoA and myoB, MI's from two different classes, play major roles in whole cell motility. The Hammer laboratory found that the protein level of myoB rises dramatically during early development, and that the myoB concentration is much higher than myoC and myoD at the same developmental stage. If each isoform contributes to the rate in proportion to its cellular concentration, this would explain why the loss of myoB is more severe than the absence of myoC or myoD (19).

The organization of large groups of cells into streams during development is delayed in myoA- (29) and myoB- (16) cells as expected from their defect in motility. MyoC- and myoD-cells stream normally. Loss of myoD from myoB- cells creates no additional defect in streaming, but the loss of myoC from these myoB-/myoD-cells further compromised streaming. Therefore, myoC appears to have a function in streaming that is not apparent in assays of whole cell motility.

What Are the Molecular Functions of Myosin-I?

We can base hypotheses about the roles of myosin-I in the formation and remodeling of membrane projections through the actin-rich cell cortex upon our knowledge of the organization of the cell cortex and the interaction of MI with actin and membranes in vitro (Fig. 2; 19, 27). We assume that (A) the actin filaments in pseudopods are long, straight, and arranged into space-filling orthogonal networks, and the barbed ends are predominantly pointed toward the plasma membrane (7, 21, 31), (B) the MI tails bind membranes (2, 24), and (C) MI is a barbed-end directed motor.

Plasma Membrane Dynamics. MI anchored to the plasma
membrane might apply tension to actin filaments that are bound to an opposing membrane in a ruffle or pseudopod. Depending upon which membrane is anchored and assuming the actin filaments are oriented in the same direction, the force will displace the membrane associated with either the MI or the actin, resulting in bending and/or retraction of a membrane projection (Fig. 2 A, left). MI may function as a regulator of the elastic modulus of the actin gel, thus influencing the extent to which the gel can change shape (18). MI-induced contraction of actin filament networks (12) within a ruffle or pseudopod might result in the retraction of the structure (Fig. 2 B, right).

Three-dimensional analyses of myoA− and myoB− cells suggest that these isoforms are not necessary for the generation of protrusive forces (37, 39). Similar analyses of the cells with multiple MI knockouts will probably confirm that myoA-E do not contribute directly to these forces (6).

**Vesicle Transport.** MI might move vesicles through the actin-rich cell cortex (10). MI directly bound to vesicles might transport vesicles through the cortex toward the plasma membrane (Fig. 2 B, left). Alternatively, MI anchored in the cortex might interact with actin filaments bound to a vesicle, facilitating the movement of the vesicle away from the plasma membrane (Fig. 2 B, right). By acting as a dynamic actin cross-linker and applying tension to actin filaments, MI might also influence the ability of a vesicle to pass through the actin-rich cortex.

**Protein Localization and Transport.** MI may link integral membrane proteins to the actin cytoskeleton, allowing for their transport and localization (Fig. 2 C, left). Cytochalasin inhibits the long translational movements of some transmembrane proteins (36), but this motility has not yet been shown to depend on myosin. Furthermore, it is not clear if the orientation and organization of the actin filaments in the cortex are appropriate to support this type of motility. Membrane-bound MI may also contribute to the continuous movement of actin filaments away from the plasma membrane as observed in fibroblasts (38) and growth cones (11; Fig. 2 C, right).

Possible functions of MI in the cytoplasm are less clear. The endosomes present in the MI mutants are smaller than in the wild-type cells (19). So it is possible that MI participates in vesicle processing and/or membrane traffic along endocytic pathways. MI is capable of supporting the transport of membranes along actin tracks in vitro (1) and therefore may transport endosomes or other organelles.

**Is Myosin-I Essential?**

All of the Dictyostelium MI knockouts are relatively healthy when grown in the laboratory. However, in the wild, cells encounter conditions that are usually not reproduced in the laboratory. MI isoforms may provide a selective advantage to Dictyostelium as the cell encounters simple changes in their normal environment. An excellent example is the endocytic defect that is seen only when the cells are grown in suspension. A second example is the myosin-IC knockout in Acanthamoeba (9). This knockout has a cell lysis phenotype only when the cell is exposed to the laboratory equivalent of a rain storm. Therefore under real life conditions MI is clearly essential.

**Does Each Myosin-I Isoform Perform a Unique Function?**

Mutagenesis of Dictyostelium MI genes makes it clear that the four MI isoforms tested do not have exactly the same physiological functions. However, despite the differences in the sequences of the tail domains, the different isoforms are involved in many of the same cellular processes (Fig. 1). The interrelationship among the different MI isoforms in these processes is one of the most puzzling aspects of this work. Do some of these isoforms perform parallel, overlapping functions (e.g., maintaining membrane structure and tension), or does each isoform contribute sequentially to a specific step in a complex process (e.g., the vesicle processing pathway in endocytosis)? The new insights about processes dependent upon MI will allow more decisive tests of the mechanisms and the relationships of the MI isoforms among themselves and with the other cellular components (including other members of the myosin superfamily) that participate in these processes.

New strategies are required to provide a more detailed picture of the cellular and molecular functions of MI. For example, the documentation of temporal changes in the localization of each isoform in live cells (25) should help to identify MI-dependent steps. Investigation of the mechanisms of cellular localization and biochemical regulation of the different isoforms (4, 20) will also continue to provide insight into the function of MI in complex processes.

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