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Avian Synaptopodin 2 (Fesselin) Stabilizes Myosin Filaments and Actomyosin in the Presence of ATP

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Abstract

Smooth muscle cells maintain filaments of actin and myosin in the presence of ATP although dephosphorylated myosin filaments and actin-myosin interactions are unstable under those conditions in vitro. Several proteins have been identified that stabilize myosin filaments and that stabilize actin-myosin interactions. Fesselin or synaptopodin 2 appears to be another such protein. Rapid kinetic measurements and electron microscopy demonstrated that fesselin, isolated from turkey gizzard muscle, reduced the rate of dissociation of myosin filaments. Addition of fesselin increased both the length and thickness of myosin filaments. The rate of detachment of myosin, but not HMM, from actin was also greatly reduced by fesselin. Data from this study suggest that fesselin stabilizes myosin filaments and tethers myosin to actin. These results support the view that one role of fesselin is to organize contractile units of myosin and actin.

Fesselin is an actin binding protein that was first isolated from avian smooth muscle (1). Fesselin was later shown to be the avian form of synaptopodin 2 (2) a member of the synaptopodin family of proteins (3), (4). The work described here uses avian muscle as its source so we will use the term fesselin to describe our studies. However “fesselin” from mammalian sources will be called synaptopodin 2.

Fesselin/synaptopodin 2 is an actin binding protein that is found in smooth (1), cardiac and skeletal muscle (5;6). Fesselin is localized primarily in the dense bodies of smooth muscle (7) and Z-lines of striated muscle (5). Synaptopodin 2 has been identified in several cell lines where it appears to shuttle between the nucleus and cytoplasm (5). Fesselin/synaptopodin 2 is primarily known as an actin binding protein (1), (5). Fesselin binds to 4 actin protomers of an actin filament with an affinity of 0.5 μ M but with small positive cooperativity ($\omega = 1.7$) giving an overall affinity to a singly contiguous site of 0.39 μ M (1). Binding to F-actin results in the formation of actin aggregates (1) or bundles (8). These bundles are ordered with a uniform polarity (9). Fesselin also binds to G-actin and stimulates actin polymerization (10). Ca^{2+} -calmodulin inhibits the ability of fesselin to stimulate G-actin polymerization (11). Ca^{2+} -calmodulin does not affect binding of fesselin to F-actin (11), (12). Therefore, Ca^{2+} -calmodulin regulates fesselin mediated actin polymerization but not the subsequent bundling of actin filaments. These functions are consistent with its cellular co-localization with actin (13).

Fesselin is intrinsically disordered (14) and, like many such proteins, has multiple binding partners including α -actinin (15), calmodulin (11), (16), zyxin (17), and myosin (18). The

interaction with myosin is interesting because of the potential for fesselin to polymerize actin, and bundle it into filaments and then hold those filaments in close proximity with myosin.

Previous research showed that fesselin binds to smooth muscle myosin with an affinity of $0.5 \mu\text{M}$ with an apparent stoichiometry of 1 fesselin per myosin head (12). Fesselin inhibits activation of myosin S1 ATPase activity by actin in a concentration dependent manner (12). That inhibition appears to result from competition with S1-ATP for binding to actin.

Because fesselin polymerizes and organizes actin we determined if fesselin has similar functions for myosin. We show evidence from pre-steady state kinetics and electron microscopy that fesselin decreases the rate of disassembly of myosin filaments in the presence of high levels of ATP. Several other proteins have been identified with this activity including kinase-related protein (19) and caldesmon (20). Those proteins stabilize dephosphorylated myosin filaments under cellular conditions.

Our studies also suggest that fesselin decreases the rate of dissociation of actin-myosin complexes by ATP. We propose that fesselin tethers smooth muscle myosin to actin. Such tethering has been observed with other proteins including caldesmon (21) (22) and C-protein (23;24). Stabilization of myosin and actin-myosin may be important for organizing contractile units in smooth muscle cells.

EXPERIMENTAL PROCEDURES

Proteins

Myosin and heavy meromyosin (HMM) were prepared from turkey gizzards (25). Myosin was stored in 0.5M NaCl 10mM MOPS 2mM MgCl_2 0.1mM dithiothreitol. Actin was prepared from rabbit erector spinae muscle (26) and stored in 4 mM MOPS, 2 mM MgCl_2 . Tropomyosin was prepared from turkey gizzards (27) and labeled with acrylodan (28). Fesselin was prepared from frozen turkey gizzards (1) and stored in 94 mM NaCl, 10 mM MOPS, 2 mM MgCl_2 , 0.1 mM dithiothreitol, pH 7. Protein concentrations were determined by absorbance measurements using the following extinction coefficients: ($\epsilon^{0.1\%}$) for 280 nm: actin, 1.15; myosin, 0.5, tropomyosin, 0.22 and fesselin, 1.0. The molecular weights of the proteins were assumed to be: actin, 42000; myosin, 480,000; HMM, 350,000, tropomyosin, 68,000 and fesselin 102,000.

Transient Kinetics

The rate of dissociation of myosin from actin following rapid mixing with ATP was measured by light scattering on a SF20 sequential mixing stopped flow spectrometer (Applied Photophysics Ltd, Leatherhead, Surrey, United Kingdom). The temperature was controlled with a circulating water bath. The excitation wavelength was set with a monochromator using slit widths of either 0.5 or 1 mm. Emission wavelengths were selected with high pass filters. Acrylodan fluorescence was measured with excitation at 391 nm with emission regulated by a Schott GG 455 (Duryea, PA) high pass filter with a 455 nm midpoint. MANT fluorescence changes were monitored with excitation at 366 and emission measured with an Oriel #51270 high pass filter. Light scattering measurements were made by increasing the emission wavelength above the working range of the excitation filter (600 nm for the 455 nm high pass filter).

Single turnover studies were done similarly to earlier studies (29), (30) but with a sequential mixing stopped flow using MANT-ATP as a fluorescent indicator of ATP and ADP dissociation. MANT-ATP was synthesized as described by Hiratsuka (31). Myosin was rapidly mixed with MANT-ATP using the sequential mixing stopped flow. After 0.1 sec the

myosin-MANT-ATP complex was rapidly mixed with a 100-fold excess of ATP to compete with the MANT-ATP and MANT-ADP that dissociated from myosin.

Electron Microscopy

Protein filaments were visualized using negative staining methodology. Carbon coated formvar nickel grids (200 mesh) were negatively charged using an EMS 100 Glow Discharge unit immediately prior to applying protein solutions. Solutions of myosin, actin and fesselin were prepared by adding concentrated stocks to 94 mM NaCl, 10 mM MOPS, 2 mM MgCl_2 , 0.1 mM dithiothreitol, pH 7. After 30 seconds of incubation on the grid, the solutions were removed by touching the edge of the grid to Whatman #1 filter paper. Rinsing was accomplished by positioning the grid against a piece of filter paper and applying 50 μl of 0.1 M ammonium acetate in 1 mM sodium phosphate buffer (pH 7) to the grid. In this manner the rinse solution flowed across the grid into the filter paper. Subsequently 10 μl of 1% aqueous uranyl acetate was applied in the same manner before an additional 10 μl of uranyl acetate was applied and allowed to incubate on the grid for 45 seconds. Excess uranyl acetate was removed with filter paper and the grid allowed to air dry. Grids were examined in a JEOL 1200EX transmission electron microscope at 80 kV accelerating voltage. Images were recorded using a SIS ImageView III CCD camera (Olympus, Tokyo, Japan).

RESULTS

The ability of myosin to bind to different effectors could be a function of the physical state of myosin. Smooth muscle myosin monomers exist as a mixture of a compactly folded and inactive (10S) and extended and active (6S) forms (32), (33). We determined the state of the unphosphorylated smooth muscle myosin at the conditions used in this study by examining the rate of product release. Myosin was mixed with the fluorescent ATP derivative (MANT-ATP) and allowed to react long enough (100 ms) to bind and to reach equilibrium between the MANT-ATP and MANT-ADP-Pi states. This complex was then rapidly mixed with an excess of unlabeled ATP to displace the fluorescent nucleotides. The decay was monoexponential with a rate constant of 0.66/s in a buffer containing 500 mM KCl (not shown). This is consistent with stabilization of the active, 6S state of dephosphorylated myosin at 0.6 M KCl (29), (34). At the conditions of our studies (94 mM KCl) we observed a biexponential release of the products with rate constants of 0.6/s and 0.036/s consistent with equilibrium between the 6S and 10S, inactive, states of myosin (Figure 1 inset). This is typical of smooth muscle myosin with the 10S state increasingly favored as the salt concentration is lowered.

We examined the possibility that fesselin binding to myosin affects the stability of myosin filaments. The time course of disassembly of filamentous dephosphorylated smooth muscle myosin by ATP was measured. ATP binding is expected to change myosin filaments to inactive folded myosin monomers. Figure 1 shows that the transition was biexponential indicating the presence of at least one intermediate in the disassembly process. Myosin alone exhibited two apparent rate constants of 1.1/s and 0.1/s. Fesselin decreased both rate constants by similar fractions. At 0.2 μM fesselin, the rate constants were reduced to 0.35/s and 0.03/s. This suggests that fesselin stabilized the myosin filaments preventing dissociation upon the addition of ATP.

A decrease in light scattering indicates a reduction in filament size and it is known from earlier work that this is due to the disassembly of myosin filaments. We examined the effect of fesselin on the structure of myosin filaments to confirm the stabilizing effect of fesselin and to determine the specific structural changes caused by fesselin. Figure 2A shows electron micrographs of dephosphorylated myosin filaments in the absence of fesselin.

Filaments were short and consistent with the bipolar structure typical of smooth muscle myosin under these conditions. Addition of fesselin increased the size of the myosin filaments (Figure 2B) although the gross appearance of the filaments appeared the same. Furthermore, myosin filaments tended to cluster together in the presence of fesselin.

Figures 2C and 2D show the length and width distributions of myosin filaments (grey lines) and myosin filaments with fesselin (black lines). Fesselin increased the average myosin filament length by 2.2-fold and the average filament width by 2.8-fold. The distribution curves for myosin plus fesselin were skewed to the right indicating the presence of very long and very thick filaments. The increase in both length and width suggests that lateral and tandem associations among myosin filament units are equally favored by fesselin. This indicates that in the rapid kinetic measurements of figure 1 the initial states were different for curves 1 and 2 with larger filaments existing when fesselin was present (curve 2).

Figure 2 showed the state of myosin filaments prior to the addition of ATP. Figure 3 shows the state of myosin filaments 30s after addition of ATP (ATP was added 30s after loading myosin onto the grid). No filaments were observed in the absence of fesselin (Figure 3A). Filaments were seen in samples containing fesselin (Figure 3B). Those filaments were similar in size to those observed with myosin alone in the absence of ATP. These images along with those in Figure 2 suggests that disassembly of the large filaments formed in the presence of fesselin occurs in steps with very large filaments disassembling to form normal myosin filaments.

We next addressed the possibility that fesselin can crosslink myosin to actin. Initial studies examined changes in light scattering after mixing of actin-myosin with ATP. ATP binding to myosin decreases its affinity for actin by several orders of magnitude and results in detachment of myosin from actin. Figure 4A shows that fesselin had a large inhibitory effect on the dissociation of actin from myosin. The dissociation curve in the absence of fesselin (curve 1) was biphasic with $k_1 = 4.2/s$ and $k_2 = 0.24/s$. Addition of $0.05 \mu M$ fesselin (curve 4) reduced the observed rate constants to $1.6/s$ and $0.16/s$, respectively or about 40% of their original values. Fesselin also reduced the amplitude indicating the possibility of multiple populations of actin-fesselin-myosin: one that dissociated at a rate 40% of the actin-myosin rate and another population that did not disassemble on the experimental time scale. Figure 4B compares the effects of $2 \mu M$ fesselin or caldesmon on the rate of dissociation at long time scales. The semi-logarithmic plot shows that fesselin is much more potent than caldesmon in reducing the dissociation of actin-myosin. The time for reduction of the signal to 50% was increased by $10\times$ by caldesmon and by several hundred times in the presence of fesselin.

Fesselin had a minimal effect on dissociation of HMM from actin (Figure 4C). A single exponential process with a rate constant of $320/s$ was observed without fesselin. Addition of $0.05 \mu M$ fesselin reduced the rate constant to $230/s$ while $0.2 \mu M$ fesselin reduced the rate constant to $280/s$. Those observations suggest that fesselin does not alter the rate constants of ATP binding to myosin or the subsequent breaking of bonds between myosin and actin. Rather, it suggests that fesselin tethers actin to the light meromyosin portion of myosin.

Conformation of this tethering came from the minimal effect that fesselin had on a probe that is sensitive to the specific interaction of actin with myosin. Acrylodan labeled tropomyosin undergoes a decrease in fluorescence as myosin rapidly detaches from actin following an ATP chase (35). Figure 5A shows the biphasic decreases in acrylodan tropomyosin fluorescence when ATP was rapidly mixed with myosin-actin in the presence and absence of fesselin. The apparent rate constants were $1.6/s$ and 0.17 without fesselin and $1.4/s$ and $0.15/s$ with $0.25 \mu M$ fesselin. The lack of change argues against an effect of

fesselin on the specific ATP dependent interaction between myosin and actin. Figure 5B shows the dependencies of light scattering and acrylodan tropomyosin fluorescence on the fesselin concentration. Fesselin decreased the rapid phase of light scattering to 20% of its initial value. However, the rapid rate constant for acrylodan fluorescence decreased only to 80% of its initial value. That is, although fesselin had a large effect on the breakdown of the actin-myosin protein complex it had little effect on the ATP specific interaction between actin and myosin.

Actin-myosin complex formation and stabilization in the presence or absence of fesselin were evaluated by negative staining of filaments and examination in the electron microscope in order to confirm stopped-flow observations. Figure 6 shows that myosin applied to grids containing actin filaments was removed by washing the grids with a solution of ATP. Inclusion of fesselin with myosin resulted in retention of myosin filaments that were located in close proximity to actin filaments.

DISCUSSION

The structure of smooth muscle myosin is regulated by binding to ATP, by phosphorylation of the regulatory light chain and by binding to other ligand proteins. Dephosphorylated myosin filaments *in vitro* normally dissociate in the presence of ATP to form folded monomers (36), (37), (33). That folded state or 10S state is inactive with Mg-ADP-Pi trapped at the active sites (29). The reduced ability to bind actin and hydrolyze ATP is the result of a specific type of interaction between the two myosin heads (38), (39), (40), (41). The inability to polymerize may be due to the folding of the myosin tail region, again in a specific manner (25).

In relaxed smooth muscle, myosin filaments continue to persist (42) in spite of the presence of mM concentrations of ATP. The stability of these filaments is thought to be due to other ligand proteins that associate with myosin such as smooth muscle kinase-related protein (43) and caldesmon (20). The kinase related protein accomplishes this by promoting the unfolded state of myosin (44). Fesselin/synaptopodin 2 may also function to stabilize myosin filaments in the relaxed state.

When smooth muscle myosin was mixed rapidly with ATP there was a biexponential time dependent decrease in light scattering (Figure 1). Fesselin reduced both apparent rate constants. The transition from myosin filaments to 10S myosin monomers is thought to occur by the scheme shown below:

Binding of ATP to 6S myosin filaments (M_F) leads to formation of a collision complex (TM_F^*) that isomerizes prior to dissociation to form ATP bound 6S monomers (TM_{6S}) and finally 10S monomers (TM_{10S}) (45).

Binding of ATP to myosin was much faster than the succeeding transitions, under our conditions, and did not affect the kinetics. In the absence of fesselin, the curve was well described by $k_2 + k_{-2} = 1.2/s$, $k_3 + k_{-3} = 58/s$, $k_4 + k_{-4} = 0.09/s$ although other combinations of rate constants were possible. That is, our results are consistent with the Rosenfeld et al. model (45).

Myosin filaments formed in the presence of fesselin tended to be longer and thicker than normal filaments. Fesselin shifted the peaks of the size distribution curves to >2-fold higher

values and skewed the distribution to allow the formation of very large filaments. Filaments formed in the presence of fesselin had an average length of 0.72 μm with lengths ranging from about 0.34 μm to 1.7 μm . Filaments formed in the absence of fesselin had a tighter distribution with an average length of 0.32 μm . Native myosin filaments in smooth muscle homogenates had an average length of 1.8 μm with a range of 0.8 to $>3\mu\text{m}$ (46). Fesselin may contribute to both the stability of myosin filaments and to the larger size.

The increased resistance to ATP was likely the result of crosslinks between individual myosin molecules and between groups of myosin filaments as shown in Figure 7. Crosslinks between filaments must occur both side by side and end to end. Packing is assumed to occur so as to maximize the number of surfaces from adjoining filaments that are in contact with each other. Figure 7 also shows the proposed crosslinking of myosin to actin.

Evidence for crosslinking myosin to actin came from the decreased kinetics of dissociation of actin-myosin upon mixing with ATP. The effects of fesselin on actin-myosin crosslinking were large; a fraction of the actin-fesselin-myosin did not dissociate on the experimental time scale. The observed effects of fesselin were much greater than those of caldesmon, a known tethering protein (47). Fesselin did not greatly reduce the rate constant for breaking the association between actin and myosin. That is, fesselin had little effect on the rate of dissociation of actin from HMM. Also, fesselin had little effect on acrylodan tropomyosin fluorescence that changes in response to changes in myosin attachment to actin. Rather, fesselin appeared to tether myosin and actin together after the normal bond between myosin and actin was broken. Similar observations have been made for caldesmon (48), (49), calponin (50) and C protein (24). Electron micrographs showed a tendency of myosin filaments to be localized along actin filaments in the presence of fesselin and ATP (Figure 6). Fesselin has micro molar affinity to both myosin and actin. That affinity is on the order of 100 fold greater than actin-myosin affinity during ATP hydrolysis.

The present work adds to the list of effects of fesselin/synaptopodin 2 on actin structure. Fesselin binding to G-actin is followed by rapid polymerization that is probably the result of stabilization of actin nuclei (10). That nucleation is inhibited by Ca^{2+} -calmodulin (11). The resulting F-actin forms parallel bundles (1), (8) in the absence of myosin. The bundling is unaffected by Ca^{2+} -calmodulin. These effects are consistent with the localization of fesselin in dense bodies and Z-lines.

We show here that fesselin may have other functions related to stabilization of myosin filaments. The large myosin filaments formed appeared to be crosslinked with actin based on kinetic studies. Electron micrographs show a close association of myosin with actin filaments although crosslinks were not directly seen. When added to a mixture of both myosin and actin, fesselin appears to preferentially bundle myosin and tether myosin to actin filaments. Fesselin inhibits binding of the S1 portion of myosin to actin with the resulting loss of ATPase activity (18). However binding of fesselin to a fraction of the actin sites may be sufficient to crosslink actin to myosin. The remaining actin sites are free to interact normally with myosin and stimulate ATP hydrolysis. Low concentrations of caldesmon enhance movement of myosin past actin in the *in vitro* motility assay (51) indicating that tethering need not impede motility. It is possible that fesselin behaves in the same way.

While it is clear that fesselin/synaptopodin 2 has a role in organizing actin we must consider the possibility that fesselin also organizes myosin filaments. There is no available evidence of fesselin binding to cellular myosin. However, staining of gizzard muscle with anti-fesselin IgG showed diffuse background staining in addition to intense staining of dense bodies (7). That diffuse staining may have been due to the association of fesselin with smooth muscle myosin.

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Abbreviations

MOPS	3-(N-Morpholino)-propanesulfonic acid
MANT-ATP	2'(3')-O-(N-methylanthraniloyl)-ATP

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Figure 1.

Fesselin reduces the rate of ATP-induced, smooth muscle myosin disassociation. Biexponential fits to the light scattering transients are superimposed on the data. Absence of fesselin (curve 1) $k_1 = 1.1/s$, $k_2 = 0.10/s$. Presence: of $0.05 \mu\text{M}$ fesselin (curve 2) $k_1=0.51/s$, $k_2=0.05/s$; $0.1 \mu\text{M}$ fesselin (curve 3) $k_1=0.42/s$, $k_2=0.04/s$; $0.2 \mu\text{M}$ fesselin (curve 4) $k_1=0.35/s$, $k_2=0.03/s$. Conditions: $0.05 \mu\text{M}$ smooth muscle myosin, 94 mM NaCl, 10 mM MOPS, 2 mM MgCl_2 , 1 mM dithiothreitol pH 7.0, 10°C . The inset shows MANT fluorescence as a function of time (s) as the products (MANT-ATP and MANT-ADP) detach from smooth muscle myosin under the conditions described above $k_1=0.6/s$ and $k_2=0.036/s$.

Figure 2.

Electron microscopic analysis shows fesselin increases the length and width of smooth muscle myosin filaments. A and B are representative electron micrographs of 0.05 μM smooth muscle myosin in the absence (A) and presence (B) of 0.1 μM fesselin. Distributions of filament lengths (C) and widths (D) were determined by measuring the filaments with NIH Image J. These measurements were ordered by size and plotted as size against the fraction of filaments at that size. 133 filaments were measured in the absence of fesselin and 126 filaments were measured in the presence of fesselin. Grey shaded regions are in the absence of fesselin, average filament length of 0.32 μm and width of 0.017 μm . Black lines are in the presence of fesselin, average filament length of 0.72 μm and width of 0.048 μm .

Figure 3. The ATP resistance of fesselin-myosin filaments shown using electron microscopy. A) Smooth muscle myosin + ATP. B) Smooth muscle myosin-fesselin + ATP. Conditions: 0.05 μ M smooth muscle myosin, 0.1 μ M fesselin, 2 mM ATP, 94 mM NaCl, 10 mM MOPS, 2 mM MgCl_2 , .1 mM dithiothreitol, pH 7.0, 10°C. ATP was added to myosin immediately before adding to the grid.

Figure 4.

Fesselin increased the resistance of actin-myosin toward dissociation by 2mM ATP. A) Light scattering transients of 0.2 μ M phalloidin-actin and 0.05 μ M smooth muscle myosin. Curve 1: absence of fesselin, $k_1 = 4.2/s$, $k_2 = 0.24/s$. Curves 2-4 with fesselin: 0.0125 μ M fesselin (curve 2) $k_1 = 2.3/s$, $k_2 = 0.23/s$; 0.025 μ M fesselin (curve 3) $k_1 = 1.7/s$, $k_2 = 0.13/s$; 0.05 μ M fesselin (curve 4) $k_1 = 1.6/s$, $k_2 = 0.16/s$. The reduced amplitude of curve 4 suggests that most actin-fesselin-myosin complexes were stable on this time scale. Conditions: 94 mM NaCl, 10 mM MOPS, 2 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.0, 10°C. B) Fesselin was more effective than caldesmon in reducing the rate of dissociation. Note the logarithmic time axis. Curve 1: 0.05 μ M myosin, 0.2 μ M phalloidin-actin with no addition.

Curve 2: with 1 μM caldesmon. Curve 3: with 1 μM fesselin. Conditions: same as A except 0.2 mM ATP. C) Light scattering transients of actin and smooth muscle HMM. Curve 1: no fesselin, $k_1=320/\text{s}$. Curve 2: presence of 0.05 μM fesselin, $k_1 = 230/\text{s}$. Curve 3: with 0.2 μM fesselin, $k_1=280/\text{s}$. Conditions: same as A.

Figure 5.

Fesselin had little effect on acrylodan tropomyosin fluorescence following the rapid addition of ATP to actin-myosin. A) Acrylodan fluorescence transients in the absence of fesselin (curve 1; $k_1 = 1.6/\text{s}$, $k_2 = 0.17/\text{s}$) and with $0.25 \mu\text{M}$ fesselin (curve 2; $k_1 = 1.4/\text{s}$, $k_2 = 0.15/\text{s}$). The fitted curves are shown as white lines on the data traces. B) Plots of the apparent rate constant for the rapid phase, k_1 , against the fesselin concentration for light scattering (open circles) and acrylodan fluorescence (open squares). Hyperbolic fits are shown for both processes. The best straight line fitted to acrylodan fluorescence is also shown as a dashed line. Conditions: 94 mM NaCl, 10 mM MOPS, 2 mM MgCl_2 , 0.1 mM dithiothreitol pH 7.0, 10°C with $0.05 \mu\text{M}$ muscle smooth myosin.

Figure 6. Electron microscopy showing myosin filaments binding along actin filaments in the presence of ATP only when fesselin is present. Smooth muscle myosin and actin +/- fesselin were added to the grid and ATP was added directly. A) actin-myosin + ATP. B) actin-myosin-fesselin + ATP. 0.2 mM actin was used with 0.05 μ M myosin and 0.1 μ M fesselin. Conditions: 94 mM NaCl, 10 mM MOPS, 2 mM $MgCl_2$, 0.1 mM dithiothreitol pH 7.0, 10 $^{\circ}C$.

Figure 7. Fesselin organizes myosin and actin-myosin filaments. A) Schematic showing possible crosslinks (black bars) between short myosin filaments (rectangles) and between myosin and actin (circles). An example of an individual myosin filament is shown in the upper left rectangle. Crosslinks within myosin filaments may also occur. B) Electron microscopy image of 0.05 μM myosin, 0.05 μM fesselin showing the build-up of a large myosin filament. Conditions: same as Figure 2.