The Alteration of Myosin Isoform Compartmentation in Specific Mutants of Caenorhabditis elegans

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Abstract. Myosin isoforms A and B are located at the surface of the central and polar regions, respectively, of thick filaments in body muscle cells of Caenorhabditis elegans, whereas paramyosin and a distinct core structure comprise the backbones of these filaments. Thick filaments and related structures were isolated from nematode mutants that have altered thick filament protein compositions. These mutant filaments and their complexes with specific antibodies were studied by electron microscopy to determine the distribution of the two myosins. The compartmentation of the two myosin isoforms in body wall muscle thick filaments depends not only upon the intrinsic properties of the myosins but their interactions with other components such as paramyosin and their relative quantities determined by synthesis.

The compartmentation of different protein isoforms in distinct structures within the same cell is a common biological phenomenon. Proteins exhibiting this property include cytoskeletal constituents such as actin, myosin, and microtubule-associated proteins (2, 19), and enzymes such as creatine kinase (18). The mechanisms regulating such compartmentation have not been determined. The location of myosin A and B within different zones of thick filaments in the body wall muscle cells of Caenorhabditis elegans (12) and the availability of mutants that specifically affect thick filament assembly in this nematode (1, 3, 23) provide a potential model for the study of protein isoform compartmentation.

The nematode body wall myosins are composed of chemically and immunologically distinct heavy chains that are the products of different genes, A:myo-3 V and B:unc-54 I (12). The backbone of these filamentous proteins contain paramyosin and a separate polar core structure in addition to the myosins (4). The different locations of the myosin isoforms within the same thick filaments have suggested that the two classes perform different roles in the structure and assembly of the filaments (Fig. 1). Myosin A alone would pack in an antiparallel fashion to form the bipolar central bare zone that may contain the site for initiation of assembly, but would also pack in parallel in the remainder of the central 1.7-1.8-μm-long region. Myosin B would pack only in a parallel manner to form the polar flanking zones during subsequent elongation and termination reactions in the assembly of the thick filaments.

A long-standing question concerning the assembly of thick filaments is whether the final structure is determined by the intrinsic properties of the myosin molecules or whether other specific components and processes within the muscle cells are necessary. This problem is particularly cogent in the case of thick filaments from nematode body wall that are assembled from paramyosin and polar core structures as well as the two myosins (Fig. 2) (4). In this paper, this issue is clarified by the localization of myosin isoforms within thick filaments and related structures isolated from appropriate mutants of C. elegans. The location of each myosin isoform can be altered characteristically within specific mutants that affect the presence and amounts of other thick filament components. Under specific genetic conditions in vivo, both myosin isoforms can assemble in antiparallel within the bipolar bare zone and in parallel within the long polar regions. The presence of core structure in the genetically induced absence of either myosin B or paramyosin confirms its distinct molecular nature within the polar regions of the thick filaments.

Materials and Methods

Nematode Growth and Strains

Strains of C. elegans, variety Bristol, used were N2 (1), CB190 (e190, unc-54 I) (1), CBI24 (e224, unc-35 I) (21), CB669 (e669, unc-52 II) (9), CBI407 (e407, sup-3 V) (15), and HE200 (su200, unc-52 II) (9). Gram quantities of animals were grown on eightfold peptone-enriched nematode growth medium plates (17).

Thick Filament Isolation

Several modifications of previous procedures were used (4, 7, 12). 1 g of packed worms mixed with 2.0 ml of OCT Tissue Tek II (Miles Scientific, Naperville, IL) was poured into 3-ml plastic molds and frozen in liquid N2. Cryostat sections were dissolved in 100 ml of 6.7 mM potassium phosphate (pH 6.36), 100 mM KC1, 10 mM MgCl2, 1 mM EDTA, 5 mM ATP, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 1 μg/ml soybean trypsin inhibitor, 1 μg/ml chymotrypsin, 1 μg/ml pepstatin, and 1 μg/ml leupeptin. After the sections were washed, Triton X-100 was added to 0.9% in above buffer, and the mixture incubated at 0°C for 30 min. The sections were spun 4 min at 200 g, resuspended, and incubated again. Sucrose was
added to 0.2 M. Triton X-100 was added to 0.5% in original buffer, and 50 ml of the mixture was incubated for 10 min at 0°C. The mixture was homogenized in a 70-ml Dounce glass homogenizer by five strokes, repeated twice. The homogenate was spun for 4 min at 200 g. The supernatant containing thick and thin filaments, nuclei, ribosomes, and membrane vesicles was used for all studies in this paper. The KCl dissociations in this paper were as in Epstein et al. (4), except that the protease inhibitors were added as above.

**Electron Microscopy**

The procedure of Epstein et al. (4) was followed.

**Antibodies**

The monoclonal antibodies, 5-6 (antimyosin A) and 28.2 (antimyosin B), were used as described previously (12).

**Results**

**Long, Bipolar Thick Filaments Containing Myosin A Only**

CB190 is the canonical mutant strain that marked the *unc-54* I locus (1). These mutant nematodes produce no myosin heavy chain B or the resulting myosin isoenzyme (10, 16) due to an internal deletion within the *unc-54* structural gene (11). The mutants are severely paralyzed and have markedly disrupted myofibrillar organization; however, thick filament-like structures are observed in sectioned body walls, albeit at reduced levels from the wild-type (3, 10).

Thick filaments with lengths >10 μm have been isolated from wild-type N2 and mutant CB190 nematodes by recent improvements in procedure (Fig. 3). In our earlier studies, significant shearing was generated during isolation so that shorter fragments were isolated from both strains (7, 12). Although some caution is necessary in describing what the precise lengths are in situ, the lengths of ten longest isolated N2 and CB190 thick filaments from single grids are very similar, with means and standard errors of 10.6 ± 0.2 μm and 10.9 ± 0.4 μm, respectively. It is possible that the true lengths are even greater since the tapered ends may be relatively fragile. These values are ~10% higher than 9.7 μm, the estimate based upon polarized light microscopy of the muscle A bands in situ (7). As seen in these representative areas, CB190 has fewer thick filaments than N2. In both strains, shorter structures that are likely sheared filaments may be seen. No structures resembling partially assembled filaments with paramyosin surfaces or exposed core structures are observed in the native CB190 preparations.

CB190 thick filaments appear to be bipolar with structural bare zones devoid of myosin cross-bridges near their centers (Fig. 4). These observations are apparent in negatively stained filaments without antibody decoration (Fig. 4, A and C) or labeled with specific antimyosin A antibody that reacts with a determinant in the myosin subfragment-2 region (Fig. 4, B and D). This antibody labels the rest of the entire length of the CB190 thick filaments. In wild-type, the zone of labeling is 1.74 ± 0.8 μm (Table I), similar to our previous reports (4, 12). Myosin A, therefore, is present in both the central and polar regions of these mutant filaments, in contrast to its more restricted location in N2.

**Core Structures Do Not Contain Myosin B or Paramyosin**

Myosin B and paramyosin are dissociated processively from the polar regions toward the central zones of N2 thick filaments by increasing KCl concentrations from 0.1 to 0.45 M (4). Core structures with diameters of ~15 nm are observed under these conditions. These structures are smoother in appearance than the myosin surfaces of native thick filaments and do not exhibit the periodicities typical of paramyosin assemblies. These structures did not react with monoclonal antibodies to either myosin isoform or to paramyosin. Core structures of dissociated filaments exhibit lengths up to 4 μm from the central myosin A stubs. Negative stain penetrates the interior of the cores, indicating that they are at least partially hollow. The polar ends of thick filaments in cross sec-

**Table I. Myosin A Titration**

| Strain | Myosin heavy chain A | Mechanism | Myosin A zone length* |
|--------|----------------------|-----------|------------------------|
| N2     | 3.5-4.0:1 (14, 24)#   | Normal    | 1.74 ± 0.08 (n = 31)   |
| CB669  | 3.0-3.4:1 (25)       | Decreased myosin heavy chain B | 2.05 ± 0.06 (n = 33)   |
| HE200  | 2.6-2.9:1 (25)       | Decreased myosin heavy chain B | 2.18 ± 0.01 (n = 39)   |
| CB1407 | 1.8-2.0:1 (14, 24)   | Increased myosin heavy chain A | 3.26 ± 0.18 (n = 30)   |
| CB190  | 0.1 (16)             | No myosin heavy chain B | 10.9 ± 0.41 (n = 10)   |

* Mean ± SEM
# Ratios were calculated from data in noted references.
† Myosin A is distributed along full length of CB190 filaments.
Figure 3. Wild-type and mutant filament isolations. A and B show equivalent areas (about one-quarter of 400-mesh grid square) of N2 and CB190 preparations, respectively. Large arrows, examples of longest filaments. Small arrows, shorter fragments of filaments. Bar, 5 μm.

Sections of N2 body wall muscles appear electron-lucent and have diameters ~15 nm, suggesting a close relationship to the isolated core structures.

CB190 thick filaments can also be dissociated by increasing KCl concentration. Core structures similar to those in N2 are revealed at both poles of the dissociated filaments (Fig. 5, A and B). At higher magnification, Fig. 5, C and D shows unlabeled and antmyosin A-labeled myosin regions, respectively, at the left and the core structures at the right. These latter structures are smooth, ~15 nm in diameter, and show penetration of stain. These observations support our previous conclusion that myosin B is not contained within the core structure (4). Myosin B does not appear necessary for the assembly of the core structures in these mutants. Further, myosin A is not located by antibody reaction on the core structures (Fig. 5 D).

Several aspects of these results suggest significant differences between the behavior of the CB190 and N2 thick fila-
CB190 thick filaments. A and C were negatively stained. B and D were reacted with specific antimyosin A, monoclonal antibody 5-6, goat anti-mouse IgG antibody, and then negatively stained. Arrows, bare zones near filament centers. Bars, 0.5 μm.

Figure 4. CB190 thick filaments. A and C were negatively stained. B and D were reacted with specific antimyosin A, monoclonal antibody 5-6, goat anti-mouse IgG antibody, and then negatively stained. Arrows, bare zones near filament centers. Bars, 0.5 μm.

The pattern of dissociation of myosin A and paramyosin from CB190 thick filaments is not always symmetrical or homogeneous. Some of the mutant filaments may have unequal dissociation from both poles (Fig. 5, A and B). In Fig. 5 B, the structural bare zone appears ~1 μm from the right end and 2 μm from the left end of antimyosin A-staining region. Other filaments show dissociated regions alternating with myosin-covered areas. However, bipolar structures with

Figure 5. Dissociated CB190 thick filaments. The KCl concentration was 0.45 M. A, B, and D were reacted with antimyosin A as in Fig. 4. C was negatively stained. Arrows, junctions between myosin-containing regions and exposed cores. Bars, 0.5 μm.
central myosin A stubs and polar core structures are obtained also (Fig. 5, A and B). Reduced dissociation of myosin A occurs at 0.45 M KCl in CB190 filaments, compared with our previous results with N2 (4). The partially dissociated structures of Fig. 5 exhibit central myosin A regions, 3-4 μm long, in contrast to the 0.9-μm lengths seen in N2. Myosin B is not detectable in 0.45 M KCl-treated N2 filaments (4). Myosin A, therefore, appears to be binding more tightly to the polar substructures under conditions in which myosin B would dissociate from native filaments. This observation could reflect some abnormal interaction between the myosin A molecules and sites that ordinarily interact with myosin B or the possibility that myosin A has an intrinsically higher affinity than myosin B for these sites. In the latter case, some mechanism other than the self-assembly of the two myosins would be needed to explain their location in wild-type thick filaments. Although the affinity of myosin A could be greater than that of myosin B for the underlying polar substructure, myosin A molecules in central zones bind still more tightly than those in the more polar regions. This observation suggests differences in the interactions of identical myosin molecules in distinct regions of the thick filaments.

Structures resembling wild-type cores in diameter, flexibility, and relation to central myosin-containing stubs can be isolated from the paramyosin-deficient mutant, CB1214 (Fig. 6, A and B). In this case, the absence of paramyosin and the markedly reduced polar binding of myosin B (see below) permit observation of the cores without KCl dissociation. These observations support the previous conclusion that paramyosin is not a component of the core structure (4).

Long, Bipolar Myosin-containing Structures in the Absence of Paramyosin

CB1214 is an apparent null mutant for paramyosin in C. elegans (23). The responsible allele, e1214 unc-15 I, is suppressible by amber suppressor mutants (20, 21), indicating that the absence of paramyosin is due to premature polypeptide chain termination during synthesis. Paramyosin is a major component of nematode muscle (6, 22). The unc-15 mutants are severely paralyzed and show markedly disrupted myofibrillar organization. Earlier work using less gentle procedures demonstrated short, bipolar structures of mean length 1.5 μm and diameter 32 nm that were similar morphologically to vertebrate thick filaments (7). The presence of 15-nm diameter electron-lucent filaments in cross sections of CB1214 body wall muscle had been noted (7, 23), but comparable structures could not be identified in the earlier isolated preparations (7).
Using our present techniques, we can obtain long bipolar structures from CB1214 that contain distinct regions similar to the 32-nm-thick stubs and the 15-nm lucent filaments. Fig. 6, A and B show such negatively stained CB1214 structures. The main structure of Fig. 6 A, although not symmetrical, shows a 1.0-μm-long thicker stub region with one 15-nm structure extending 2.5 μm to the right and another 15-nm structure extending at least 1.0 μm to the left. A similar structure, although antibody-labeled, is in Fig. 6 F. Fig. 6, B-E all have only one thinner structure protruding with lengths from 2.0-3.5 μm. Most of the structures isolated from this mutant are of the shorter, thicker type (see Figs. 6 and 7), as reported previously (7). We believe that CB1214 structures in situ are likely to be symmetrical versions of the long bipolar structures seen here. These structures would be highly fragile due to breaking in their long 15-nm-wide regions. All of the CB1214 structures we observe here would have been sheared at least once for the longest and at least twice for the rest during homogenization or handling. The alternative possibility that CB1214 makes a variety of thick filament–related structures cannot be eliminated by our present observations.

As stated above, the 15-nm structures of CB1214 are very similar in appearance to the core structures of KCl-dissociated N2 and CB190 (Fig. 5) filaments. The observation indicates that the core structures or entities very similar to them do not contain paramyosin or require paramyosin for their assembly. The gross similarity of the CB1214 structures shown here to the KCl dissociated filaments described previously (4) suggests that the absence of paramyosin may have led to blocking of proper thick filament assembly. The isolated CB1214 structures may be intermediate structures or related side products of thick filament assembly.

The CB1214 structures contain both myosins A and B as determined by labeling with specific monoclonal antibodies (Fig. 6, C-F). Myosin B is localized in both the 15-nm polar structures and the bipolar thicker regions. The reaction is much stronger in the latter; the amount of myosin B binding to the 15-nm structures is probably very slight since their appearance in the absence of antibody is not typical of myosin surfaces. Myosin A is localized clearly only in the thicker stubs. The reaction of either antibody demarcated a bare zone near the center of the thick regions; at this level, no distinction between the locations of myosins A and B in these regions could be made.

**Both Myosins A and B Can Pack Antiparallel in Bare Zones**

The observation that antimyosin A and antimyosin B labeled the thick bipolar regions of CB1214 structures above suggested that in these mutant filaments the two myosins might both contribute to antiparallel interactions of the structural bare zone. In N2, only myosin A is present in the analogous region (see Fig. 1). CB1214 filaments were reacted with either antimyosin monoclonal antibody to more closely evaluate the distribution of the two myosins. Both antimyosins used in these experiments react with the subfragment-2 regions of their respective isoforms (12). This property implies that the antibodies should not react with the light meromyosin portions of the myosins that pack antiparallel in the bare zones.

Fig. 7 contains higher magnifications of CB1214 structures as a further verification of the contribution of myosins A and B to the central bare zones of the filaments in this mutant. Fig. 7 A is of an unlabeled, negatively stained bipolar CB1214.
structure. Fig. 7 B is of a CB1214 bipolar structure reacted with antiamyosin A and secondary antibody. Fig. 7 C shows the reactions of antiamyosin B and secondary antibody with a mutant filament. Bare zones and myosin-rich thick regions are clear in all structures. The bare zone and flanking regions are more sharply demarcated in the presence of antibody due to the increased mass. Note in Fig. 7 C that the polar 15-nm structure reacts with antiamyosin B. These results indicate that in CB1214, both myosins A and B contribute to the antiparallel packing of the bare zone as well as to the parallel interactions of the flanking regions.

The locations of myosins A and B in bare zone-containing regions indicate that both isoforms are structurally comparable with antiparallel and parallel packing. The absence of an additional thick filament component, paramyosin, and the reduced binding of myosin B in the polar regions of CB1214 filaments constitute the conditions in situ under which myosin B interacts at least qualitatively similarly to myosin A in the bare zone regions. However, these experiments do not indicate whether myosin B alone would form stable bipolar structures in vivo. In contrast, under wild-type conditions, that is, in the presence of paramyosin, myosin B is restricted to parallel packing in the more polar regions of the thick filaments. A possible mechanism to explain the behavior of myosin B in CB1214 is that more free myosin B is available due to decreased binding to polar structures and that these myosin B molecules can now compete with myosin A for bonding within the bare zone regions. Although myosin A may have higher affinity for such interactions than myosin B, the latter is in about fourfold excess. As suggested by the properties of myosin A in CB190, the behavior of myosin B in CB1214 supports the hypothesis that the intrinsic properties of the individual myosins are not sufficient to explain their restricted location in N2 wild-type thick filaments.

The Locations of Myosins A and B Vary with Their Relative Amounts

The findings that myosins A and B could change their locations and presumably their behavior during assembly due to the absence of other thick filament components, myosin B and paramyosin, respectively, suggested that mechanisms other than the intrinsic bonding properties of the two myosin isoforms may be necessary to explain their highly restricted locations in wild-type body wall muscle. One such mechanism would be the regulation of the synthesis of the myosin isoforms. Several mutant strains produce both myosins A and B, but at ratios distinct from the wild-type. The unc-52 II mutants, CB669 and HE200, exhibit decreased levels of myosin heavy chain B in both accumulation and synthesis experiments (25). The sup-3 V mutant, CB1407, shows increased accumulation of myosin heavy chain A (14, 21).

Filaments were isolated from these mutant strains and from N2 and CB190 as normal and null myosin B endpoints, respectively. Long bipolar filaments are obtained from each of these strains. Antiamyosin A monoclonal antibody was reacted with each preparation to test if the variation of myosin heavy chain A and B ratios produced a change in the localization of myosin A. Table I shows the results of this myosin A titration. Myosin A zone length as determined by antiamyosin A reaction increases proportionately as the ratio of myosin heavy chain B to A decreases. Thus, the relative levels of the two myosins have a direct effect upon the location and behavior during assembly of myosin A. The regulation of different relative concentrations of the two myosins and their distinct apparent affinities for filament substructures are necessary for determining their location.

It should be noted that in the CB190, CB669, and HE200 strains used in this experiment, as well as in the CB1214 strain, the body wall muscles have varying degrees of structural disorganization due to the specific mutations. It could be argued that the alterations in behavior of myosin A or myosin B observed above were due to some secondary consequence of the disorganization. However, CB1407 is mutant in that it suppresses other body wall mutations, most especially mutation of unc-15 I (the paramyosin structural gene) and of unc-54 I (the myosin heavy chain B coding gene) (15). CB1407 moves normally and exhibits wild-type structural organization in the body wall muscle cells. The alterations in myosin heavy chain A content and myosin A location of CB1407 could not be due, therefore, to secondary consequences of myofibrillar disruption. Interestingly, the exact wild-type distribution of myosins does not appear to be required for apparently normal muscle structure and function in CB1407.

Discussion

The thick filaments of body wall muscle cells in wild-type nematodes contain two myosin heavy chain isoforms, A and B (8, 12), that are encoded by myo-3 V and unc-54 I (13). These heavy chain isoforms associate into homodimeric myosin molecules designated myosins A and B (16, 17). By electron microscopy of complexes of isolated thick filaments and isoform-specific antibodies, the locations of myosins A and B are found to be restricted to the central and flanking polar regions, respectively, of the thick filaments. The compartmentation of the two myosins implies differences in their interactions during filament assembly. Myosin A is uniquely involved in the antiparallel packing within the central bare zone, whereas myosin B would interact only in a parallel manner in the flanking polar regions of the thick filament.

A complex substructure appears to underlie the differentiated myosin surface of the thick filament in C. elegans body wall muscle (4). In this model, the myosin B domain would be arranged about a paramyosin-containing domain that in turn would be coaxial with a distinct polar core structure (Fig. 2). Myosin B and paramyosin are selectively dissociated by addition of KCl, leaving the remaining substructures. The core appears by electron microscopy to be a 15-nm-diameter tubular structure with an internal density that decreases from the center to the poles. The central myosin A domain appears by electron microscopy to have a uniformly dense internal structure but its composition has not yet been biochemically resolved. The existence of at least three coaxial structures within these thick filaments indicates that some definite sequence in the formation of core structure, paramyosin, and myosin domains must occur during assembly.

In the present work, structures isolated from specific mutants of C. elegans are studied to establish what conditions are necessary for proper compartmentation of myosins A and B during normal thick filament assembly. CB190 is an unc-54 mutant that produces no myosin heavy chain B but normal
amounts of myosin heavy chain A (5, 16). Fewer thick filaments are found in the body walls of CB190 than in wild-type, in rough proportion to the myosin A present relative to the total amount of myosins A and B of wild-type (10). CB1214 is an unc-15 mutant that produces no paramyosin (23). No thick filaments are observed in its body wall muscles, but 32-nm-wide myosin-containing stubs and 15-nm-wide "lucent" tubes have been identified (7). CB669 and HE200 are unc-52 mutants that exhibit decreased levels of myosin heavy chain B synthesis and accumulation concomitant with retarded myofibrillar synthesis in larval and early adult maturation (9, 25). CB1407 partially suppresses specific unc-15 and unc-54 myofibrillar-defective phenotypes (15) and shows a twofold increase in myosin heavy chain A (14, 24). Thick filaments or related structures isolated from these mutants and the complexes of monoclonal antibodies specific to myosin A or B with these filaments were studied by electron microscopy. The compartmentation of myosins A and B found in wild-type nematode thick filaments is characteristically altered in each of these mutants. The different patterns of antibody labeling in specific mutant thick filaments are consistent with our previous conclusions (4, 12) that the true locations of the myosins are identified rather than the consequences of position upon the reactivity of the myosins with the different antibodies. A model in which the epitopes of either myosin A or myosin B are accessible in certain zones and not accessible in other zones to the specific antibodies is less likely. Such a model would require burying of myosin A heads and hinge regions by myosin B or burying of myosin B heads and hinge regions by myosin A that would vary between wild-type and specific mutants and would not be affected by KCl dissociation in wild-type because the wild-type labeling patterns are obtained with isofrom-specific antibodies to both head and hinge regions (12). No model of myosin packing has been published in which myosin heads are buried. Instead, only regions of myosin rods are believed to contribute to the buried regions of the thick filament backbones.

Myosin A forms the entire myosin domain in long, bipolar thick filaments isolated from the myosin B-deficient CB190 mutant. When myosin B to myosin A ratios fall beneath the 4:1 of wild-type, as in the CB669, HE200, and CB1407 strains, myosin A spreads from the centers of the thick filaments into normally myosin B regions. Myosin B becomes coextensive with myosin A in the central zone equivalents of the thick filament-related structures of the paramyosin-deficient mutant, CB1214. Both myosins A and B can participate therefore in either the antiparallel interactions of the central bare zone or the parallel interactions of the long, tapering polar regions of thick filaments. The primary effects of the analyzed mutants are not upon the structure of either myosin isoform itself but upon the presence or levels of the other myosin isoform or paramyosin.

It is possible that the mutant alterations could secondarily produce functionally significant modifications of the structures of the individual myosins, but no evidence presently exists for such interactions in the nematode. Such protein modifications would themselves be the result of the action of additional components, myosin-specific enzymes, and these activities would then be sensitive either to the levels of thick filament proteins or to the state of their assembly. Initiation of thick filament assembly does appear to be linked specifically to myosin concentration. CB190 with only myosin A and therefore, lower total myosin, produces fewer filaments than wild-type, but they appear to be completed structures. CB1214, with both myosins present normally, but lacking paramyosin, exhibits many incomplete or altered filament-like structures.

The findings of polar core structures in KCl-dissociated CB190 thick filaments and of very similar 15-nm lucent tubes in isolated CB1214 structures provide genetic evidence that neither myosin B or paramyosin are core components. These observations are consistent with previous studies indicating that paramyosin is quantitatively solubilized under the dissociation conditions in which cores are revealed. Further, monoclonal antibodies specific to myosin A, myosin B, and paramyosin do not react with the isolated cores (4).

Higher KCl concentrations are required to liberate myosin A from the polar regions of CB190 thick filaments than myosin B from homologous N2 regions, suggesting that myosin A may bind more tightly than myosin B to all regions of the filament substructure. Within the CB190 filaments as in N2, myosin A remains more tightly bound to the central zones than to the polar regions. The central substructure of the thick filament appears to have a higher affinity for myosin A than the polar substructures. In the absence of paramyosin, myosin B but not myosin A can bind at low levels to the core-like structures of CB1214. In wild-type filaments, this weak interaction of myosin B is not observed and paramyosin appears intermediate between myosin B and the core structures throughout most of the polar regions (4). The mechanism for determining the proper sequence of myosin B, paramyosin, and core structure interactions is not known.

From these observations, we conclude that myosins A and B are likely to have intrinsic differences in their intermolecular interactions. Other thick filament components, including paramyosin and polar core structure, have differential interactions with the two myosins as well. The available levels of each myosin isoform determine which interactions are favored in assembly. The regulation of synthesis of these isoforms, their interaction with nonmyosin components, and their intrinsic properties appear necessary for their proper compartmentation into different zones of the thick filament. The results of the work presented here as well as the earlier dissociation experiments (4) suggest that a sequence of molecular interactions and formation of substructures occurs during the assembly of thick filaments in C. elegans. The exact sequence remains to be determined. Resolution of the molecular composition and internal organization of the polar core structures is necessary for further analysis of this sequence.

The proper compartmentation of myosins A and B during assembly appears coupled to their differential synthesis and to their interactions with other thick filament components. These conditions of thick filament assembly in the nematode may be related to more general mechanisms of sorting specific isoforms of other proteins into particular compartments and structures within cells.

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