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The pathway of myofibrillogenesis determines the interrelationship between myosin and paramyosin synthesis in *Caenorhabditis elegans*

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Summary

Examination of null mutants in myosin B and paramyosin yields insights into the complex mechanisms that regulate expression of the three major components of *Caenorhabditis elegans* body-wall muscle thick filaments myosin A, myosin B and paramyosin. In the absence of myosin B, paramyosin accumulation is reduced, although neither its synthesis nor that of myosin A is affected. This implies that the interaction of myosin B with paramyosin inhibits paramyosin degradation. By contrast, the absence of paramyosin results in reduced synthesis and accumulation of myosin B but has no effect on myosin A synthesis. The non-reciprocal effects of the null mutants on turnover and synthesis are best understood as an epigenetic phenomenon that reflects the pathway of thick filament assembly. The synthesis of myosin A and paramyosin, which are involved in the initial steps of thick filament formation, is independent of myosin B; however, a properly assembled paramyosin-containing thick filament core is essential for efficient synthesis of myosin B.

Key words: myosin B, myosin A, paramyosin, *Caenorhabditis elegans*, mutant, thick filament, myofibrillogenesis.

Introduction

Myofibril assembly is a tightly regulated integrative process. It requires the coordinated synthesis of contractile proteins, activity of chaperonins that mediate protein assembly and expression of scaffolding proteins that determine the spatial organization and periodicity of the myofilament lattice. The rigid stoichiometric and structural constraints involved in generating such a complex quaternary structure have led many to study myofibrillogenesis in model organisms, such as *Caenorhabditis elegans* ([Maupas 1889] Dougherty 1955), that can be manipulated genetically and biochemically.

Brenner's initial description of *C. elegans* as a genetic system (Brenner, 1974) revealed more than 25 genetic complementation groups that affected *C. elegans* movement. These included the genes for the two major proteins of body-wall muscle thick filaments, paramyosin (*unc-15*) and the myosin B heavy chain (*unc-54*) (Epstein et al., 1974; Waterston et al., 1977). Since then, screens for motility defects, suppressor mutations and embryonic lethals have increased the number of genes implicated in muscle assembly, organization and function several fold (Francis and Waterston, 1991; Waterston, 1989a; Williams and Waterston, 1994; Zengel and Epstein, 1980a). Mutants in genes encoding major and minor structural components of the myofilament lattice, as well as proteins that direct the organization and assembly of *C. elegans* muscle, have been characterized and mapped (Barral and Epstein, 1999; Benian et al., 1989, 1999; Moerman and Fire, 1997; Ono and Benian, 1998). The existence of a diverse collection of motility-defective mutants, coupled with the sequencing of the *C. elegans* genome (Hodgkin et al., 1995) and extensive ultrastructural analysis of its striated muscle, led us to initiate investigations on the interrelationship between myosin and paramyosin synthesis and the underlying structural integrity of *C. elegans* thick myofilaments.

C. elegans body-wall muscle thick filaments form bipolar structures consisting of a core of paramyosin and filagenins (Epstein et al., 1985; Liu et al., 1998; Miller et al., 1986; Muller et al., 2001) that serves as a scaffold for the binding of two myosins: A and B. These myosins are homodimeric with respect to their heavy chains (Schachat et al., 1977, 1978) and are differentially distributed on thick filaments. Myosin A represents approximately 20% of the body-wall muscle myosin and is restricted to the central bipolar H-zone of thick filaments, while myosin B composes approximately 80% of the body-wall muscle myosin and is localized to the polar arms (Miller et al., 1983). Mutants in the paramyosin and myosin B heavy chain genes impair movement and exhibit aberrant thick filaments and A-band organization, although electron microscopic observations show that the thin filament lattice is well-defined and appears to be essentially intact (Bejsovec and Anderson, 1988; Epstein et al., 1974; Mackenzie et al., 1978;

MacLeod et al., 1977b; Waterston et al., 1977). Mutations in the myosin A gene (*myo-3*) result in embryonic lethality, probably because myosin A is required for early events in thick filament initiation and muscle cell elongation (Waterston, 1989b). In addition, recent studies have provided insight into the interactions between paramyosin and myosin that may direct assembly of the central and distal regions of thick filaments. In particular, Muller et al. (2001) demonstrated that there are significant segmental differences in both protein composition and structure within the paramyosin-containing thick filament core, and Hoppe and Waterston (2000) characterized a 322-amino-acid region within the rod portion of myosin A that is required for its interaction with paramyosin.

Because molecular interactions among the components of the thick filament are likely to dictate important steps in the assembly pathway, we investigated the effects of eliminating the interactions between myosin B and paramyosin. Previously, few studies of *C. elegans* have addressed the question of whether mutations in one thick filament protein directly affect the expression of others. Garcea et al. (1978) found that a small C-terminal deletion in myosin B does not affect the synthesis of myosin A, even though it results in improperly assembled thick filaments and partial paralysis; and paramyosin mutants did not appear to affect myosin accumulation (Waterston et al., 1977). But whether other mutations, particularly those that eliminate the expression of myosin B or paramyosin, have similarly benign effects on the synthesis of the contractile proteins they interact with remains an open question.

Here, we report that a null mutation in myosin B has no discernible effect on paramyosin synthesis, while a null mutation in paramyosin specifically decreases myosin B synthesis. The non-reciprocal nature of these effects is consistent with an ordered pathway of thick filament assembly in which myosin B synthesis and incorporation into nascent thick filaments is dependent on the presence of a properly assembled, paramyosin-containing thick filament core.

Materials and methods

Nematode strains

The following strains were obtained from the *Caenorhabditis* Genetics Center at the University of Missouri: N2 Bristol (the wild-type strain), CB190 [*unc-54* (myosin B) null], CB1214 [*unc-15* (paramyosin) null] and CB1215, CB73 and HE2000 (all *unc-15* missense mutants). Nematodes were grown using standard culture methods, as described previously (Garcea et al., 1978; Schachat et al., 1978b).

Radiolabeling and preparation of myofilament proteins

C. elegans were grown at 25°C on 8P NGM (nematode growth media) plates (Schachat et al., 1978) with a bacterial lawn of the *E. coli* strain OP50. For accumulation studies, animals were harvested and transferred to NP NGM plates and fed low specific activity (2.3×10^9 Bq mmol^{-1} l^{-1}) ^{35}S-NA22-labeled *Escherichia coli* for 72 h, as described previously (Schachat et al., 1978). Homogenates were prepared using a French Press Cell at 54 500 kPa. Myofibrils were prepared by low salt washes (50 mmol l^{-1} NaCl, 10 mmol l^{-1} Tris, pH 7.4, 15 mmol l^{-1} mercaptoethanol, 2 mmol l^{-1} EGTA, 2 mmol l^{-1} $MgCl_2$, 0.1 mmol l^{-1} diisopropyl fluorophosphate and 10 μg ml^{-1} each of the peptide protease inhibitors leupeptin, antipain and chymostatin). Aliquots were saved from each step of the myofibrillar preparation procedure. Previous studies had shown that virtually all the soluble proteins are in the supernatant (G. White, unpublished observations). The final pellet from a myofibrillar preparation was diluted into sample buffer for electrophoresis. Serial dilutions of samples were run on 8.0% sodium dodecyl sulfate–polyacrylamide (SDS–PAGE) gels using the Laemmli buffer system (Laemmli, 1970) to be certain that exposures were in a linear range, and the resulting gels were dried and exposed to Kodak X-ray film for 6–48 h. Digital images were captured using an Adobe Photoshop-driven Howtek Scanmaster 3, and quantitative densitometry was performed as described previously (Thys et al., 1998). Total actin, which results primarily from unaffected tissue, was used as an internal standard for normalization to obtain relative rates of accumulation and synthesis. To estimate the fraction of actin that arises from non-muscle and body-wall muscle, the actin-to-myosin protein ratio in squid transverse arm muscle (1.8±0.1; N=3; Kier and Schachat, 1992), which exhibits thick filament dimensions and sarcomere organization comparable to *C. elegans* (van Leeuwen and Kier, 1977; Mackenzie and Epstein, 1981), was compared with the ratio in *C. elegans* wild-type 3rd instar larvae (6.3±0.6; N=3). Calculation indicates that no more than 30% of the total actin arises from *C. elegans* muscle. Subtracting the contribution of pharyngeal muscle (20% of the total muscle), we estimate that actin from sources other than body-wall muscle comprises at least 75% of the total actin in wild-type *C. elegans*. Coupled with the electron microscopic observations that a periodic and well-packed thin filament lattice is present in CB1214 and *unc-54* mutants (Bejsovec and Anderson, 1988; Epstein et al., 1974; Waterston et al., 1977, 1980), we infer that total actin does not vary significantly in the mutants studied and is a valid standard for normalization.

Pulse labeling of nematodes

A mixed population of animals was harvested and treated with 1.25% NaOCl, 0.5 mol l^{-1} NaOH for 10 min to produce eggs. Eggs were then washed, plated and grown at 25°C. When animals entered larval stage 3, as judged by examination of the developing gonad with Nomarski optics (Sulston and Horvitz, 1977), they were transferred to plates with high specific activity (2.3×10^{10} Bq mmol^{-1} l^{-1}) ^{35}S-labeled bacteria for 2 h (Schachat et al., 1977; Garcea et al., 1978). Animals were harvested, disrupted in the French Press Cell and analyzed by SDS–PAGE as previously described. For quantification, serial dilutions of a minimum of three independent labeling experiments of each strain of *C. elegans* were analyzed. Protein translation rates were normalized to account for the different

numbers of methionines and cysteines found in each of the myofilament proteins analyzed.

Neville gel electrophoresis

To separate the two myosin heavy chain proteins A and B present in *C. elegans* body-wall muscle, SDS–PAGE gels were run as described by Neville (1971). Myofilament protein preparations, silver staining or autoradiography, and quantification were performed as described previously (Thys et al., 1998).

RNA preparation and slot blot analysis

Total RNA was prepared from asynchronous populations of each strain according to Austin and Kimble (1989). RNA was loaded onto nitrocellulose filters with duplicate loadings of 4 μg and 2 μg. ^{32}P-labeled riboprobes were prepared from myosin B and myosin A genomic fragments (Miller et al., 1986) and from an actin (*act-3*) probe (Krause et al., 1989) that hybridizes with all *C. elegans* actin genes, which we subcloned into pGEM3Z. All genomic fragments were kindly provided by Dr David Miller. Hybridization was performed overnight at 55°C. Blots were washed to very high stringency (Honda and Epstein, 1990) and were exposed with intensifying screens at –80°C for 3–18 h. Quantitative densitometry was performed as described above.

Results

Paramyosin accumulation is decreased in the absence of myosin B, and myosin accumulation is reduced in the absence of paramyosin

To investigate the interrelationship between thick filament protein expression and the integrity of the thick filament, we determined if paramyosin and myosin levels are affected by null mutations in myosin B and paramyosin, respectively. Protein accumulation was assessed in asynchronous populations of *C. elegans* wild-type and mutant strains grown for 72 h on low specific activity ^{35}S-labeled *E. coli*. These labeling conditions spanned a substantial fraction of the life cycle and resulted in uniform, steady-state labeling of the myofibrillar proteins (see Materials and methods; Schachat et al., 1977). Homogenates from these populations were subjected to SDS–PAGE, and myofibrillar protein accumulation was analyzed by autoradiography and quantitative densitometry. As shown in Fig. 1A (lanes a and b), the myosin B null CB190 exhibits an approximately 50% reduction in myosin accumulation (Fig. 1B), which is consistent with the absence of myosin B (MacLeod et al., 1977a). Densitometry also revealed that the accumulation of paramyosin is reduced by 33% in CB190 compared with the N2 wild type.

To determine whether a reciprocal reduction in myosin expression is observed in the absence of paramyosin, the paramyosin null mutant CB1214 was subjected to long-term pulse labeling as described above. Densitometric analysis showed that myosin accumulation was reduced by 28% compared with the wild type (Fig. 1A, lane c; Fig. 1B). These observations indicate that normal accumulation of myosin requires expression of wild-type paramyosin and the generation of properly assembled nascent thick filament cores.

Decreases in the rate of myosin synthesis account for the reduced accumulation of myosin in the paramyosin and myosin B null mutants

To determine whether the reduced accumulation of myosin and paramyosin in the myosin B and paramyosin null mutants results primarily from reduced synthesis or from post-translational events such as increased proteolysis, their rates of synthesis were compared to those of the wild type by pulse labeling at high specific activity for 2 h. To ensure comparability of short-term labeling in strains with different growth rates and to maximize the incorporation of radiolabel, synchronous populations of eggs were generated over a 3 h window and permitted to develop to larval stage 3, as assessed by vulval development. After labeling, homogenates were prepared and analyzed by SDS–PAGE (Fig. 2A), and the relative synthetic rates (normalized to actin) during the time interval were measured by autoradiography and quantitative

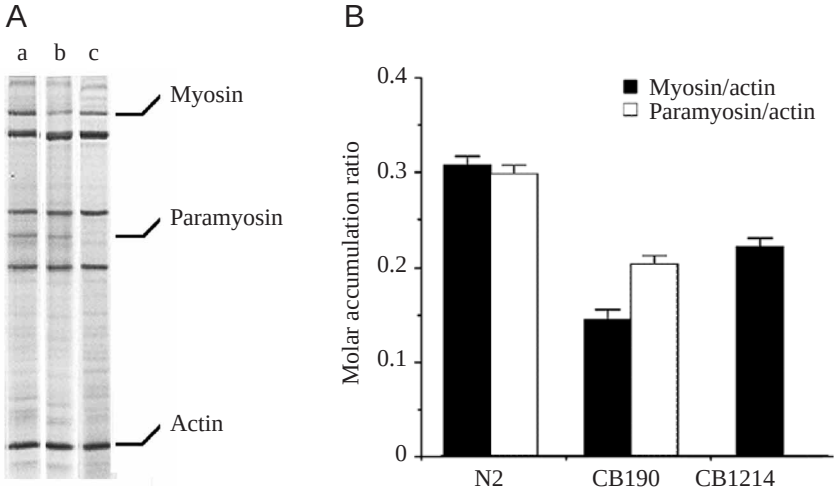

Fig. 1. Accumulation of myofibrillar proteins in the wild-type and thick filament null mutants. The accumulation of contractile proteins in myofibrillar preparations from animals continuously labeled for 3 days with low specific activity ^{35}S-labeled bacteria was quantified following autoradiography of 8% SDS–PAGE gels. (A) the autoradiogram of the wild-type N2 (lane a), the myosin B null CB190 (lane b) and the paramyosin null CB1214 (lane c). (B) Graph of the relative rates of accumulation of myosin and paramyosin normalized to actin reveals that paramyosin accumulation is reduced by 33% in the myosin null, and myosin accumulation is reduced by 28% in the paramyosin null. Quantification is based on four independent determinations on each strain, and values are expressed as means ± S.D.

densitometry (Fig. 2B). Comparison with the wild type showed a decrease in the rate of myosin synthesis in the myosin B null mutant CB190 (Fig. 2A, lanes a and b), consistent with its inability to generate myosin B. By contrast, the rate of paramyosin synthesis in CB190 was the same as in the wild type, implying that the reduction in paramyosin accumulation in CB190 (Fig. 1) is a consequence of increased degradation rather than impaired synthesis.

In the paramyosin null strain CB1214 (Fig. 2A, lane c), myosin synthesis was reduced by 33% (Fig. 2B). These data demonstrate that the 28% reduction in myosin accumulation measured in the paramyosin null mutant results from decreased myosin synthesis. Thus, the presence of wild-type paramyosin is essential for normal myosin synthesis.

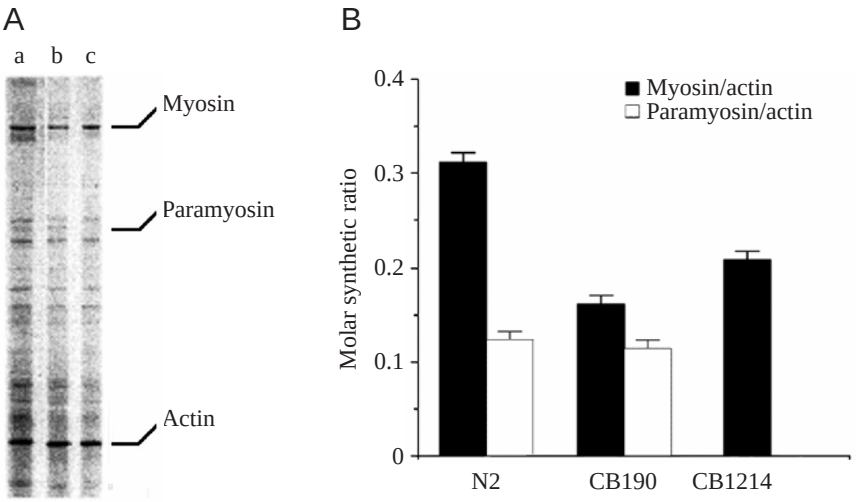

Fig. 2. Synthesis of myofibrillar proteins in the wild-type (N2) and thick filament null mutants. Synchronized animals at larval stage 3 were pulse-labeled for 2 h with high specific activity ^{35}S-labeled bacteria and the relative synthetic rates of myosin and paramyosin determined by normalization to actin. As is evident in the autoradiogram (A), compared with N2 (lane a), there is a marked reduction in the rates of synthesis of myosin in both the myosin B null CB190 (lane b) and the paramyosin null CB1214 (lane c). The results of the densitometric analysis based on four independent determinations on each strain are presented in (B). Values are means ± S.E.M.

Myosin B accumulation is differentially affected in the paramyosin null mutant

The myosin present in *C. elegans* homogenates comes from several sources. The body-wall muscle is the primary contributor, making up approximately 75% of the total myosin, while the remainder arises primarily from pharyngeal muscle. Because paramyosin mutants do not affect pharyngeal muscle structure or function (Waterston et al., 1977), we focused our attention on the two sarcomeric myosins, A and B, that are expressed in *C. elegans* body-wall muscle. These two myosins have non-overlapping functions, localize to different regions of the thick filament and are involved in different stages of thick filament assembly, and we sought to determine whether they were differentially affected by the paramyosin mutations.

Using the Neville (1971) gel electrophoresis system described by Karn et al. (1985) to resolve the body-wall muscle myosin A and B heavy chains, we found that the paramyosin null specifically interferes with accumulation of myosin B. Fig. 3 demonstrates clearly that accumulation of myosin B is reduced by almost 60%, while myosin A accumulation is not affected. Because myosin B composes approximately 50% of the total myosin, the 60% reduction accounts fully for the observed reductions in total myosin accumulation (28±4%) and synthesis (33±3%) in CB1214 (Figs 1, 2).

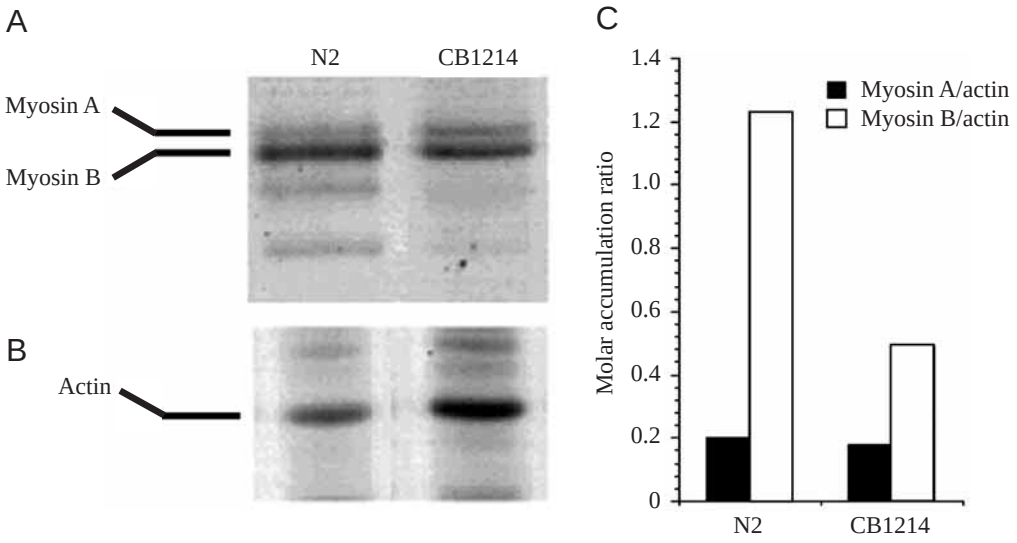

Fig. 3. Myosin B accumulation is differentially affected in the paramyosin null mutant. The Neville gel system (A) was used to quantify the relative abundance of myosin A and B, the two body-wall muscle myosins, in wild-type and paramyosin null CB1214 homogenates, and actin levels were determined by electrophoresis of identical loads on the Laemmli gel system (B). Gels were silver stained, and actin from the Laemmli gel was used as the internal standard for determining the relative accumulation of myosins A and B (C).

Myosin B mRNA levels are reduced in the paramyosin null mutant

To confirm the implications of the paramyosin null pulse-labeling studies and to determine whether the reduction in myosin B synthesis is a consequence of a reduced rate of translation or a reduction of mRNA levels, the levels of myosin A, myosin B and actin mRNAs were compared by quantitative slot blots. Myosin B mRNA was reduced by 52% in CB1214 compared with N2 controls, while myosin A mRNA levels were affected only slightly, if at all (Fig. 4). Thus, the steady-state mRNA levels indicate that the reduced rate of myosin B synthesis in paramyosin mutants is a direct consequence of lower steady-state levels of myosin B mRNA.

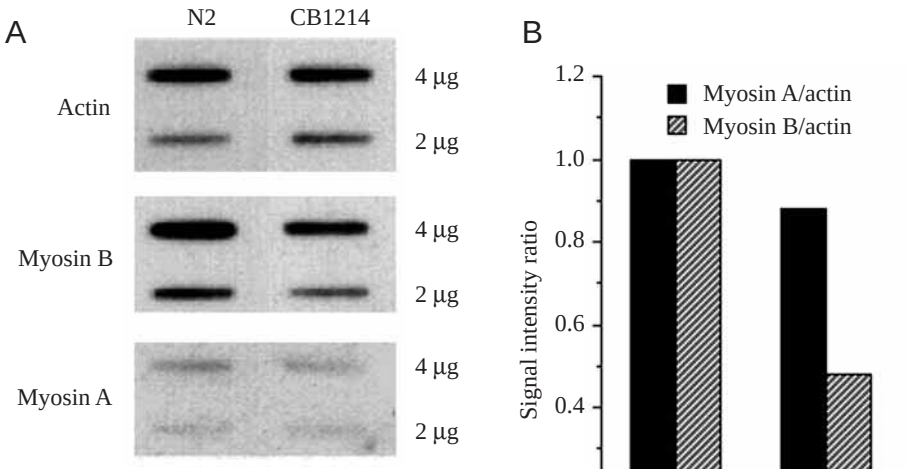

Fig. 4. Steady-state myosin A, myosin B and actin mRNA levels in N2 and CB1214. Total RNA was prepared and hybridized with gene-specific riboprobes (A), and steady-state mRNA levels were determined by normalization to total actin mRNA (B). The results demonstrate that in CB1214, the steady-state level of myosin B mRNA is approximately 60% that in the wild-type N2. The blot for the myosin A probe has a significantly longer exposure time due to the lower amount of myosin A in the muscle (Honda and Epstein, 1990). Mean values are based on four independent determinations.

Myosin B accumulation is also differentially affected by paramyosin missense mutations

To determine whether mutations that produce defective paramyosins also alter myosin B accumulation, three paramyosin missense mutants, CB73, HE2000 and CB1215, were analyzed. These mutants, selected on the basis of motility defects, all generate paramyosins with a single amino acid substitution that results in aberrant thick filament structure (Brenner, 1974; Mackenzie and Epstein, 1980; Waterston et al., 1977; Zengel and Epstein, 1980a). Neville gels (Fig. 5) show that they all exhibit reduced ratios of myosin B to total body-wall muscle myosin. So, defects in paramyosin that affect the integrity of thick filaments and motility, as well as the total absence of paramyosin, affect the expression of myosin B. These observations demonstrate that proper accumulation of myosin B depends on its interaction with paramyosin and/or the integrity of the paramyosin core of nascent thick filaments.

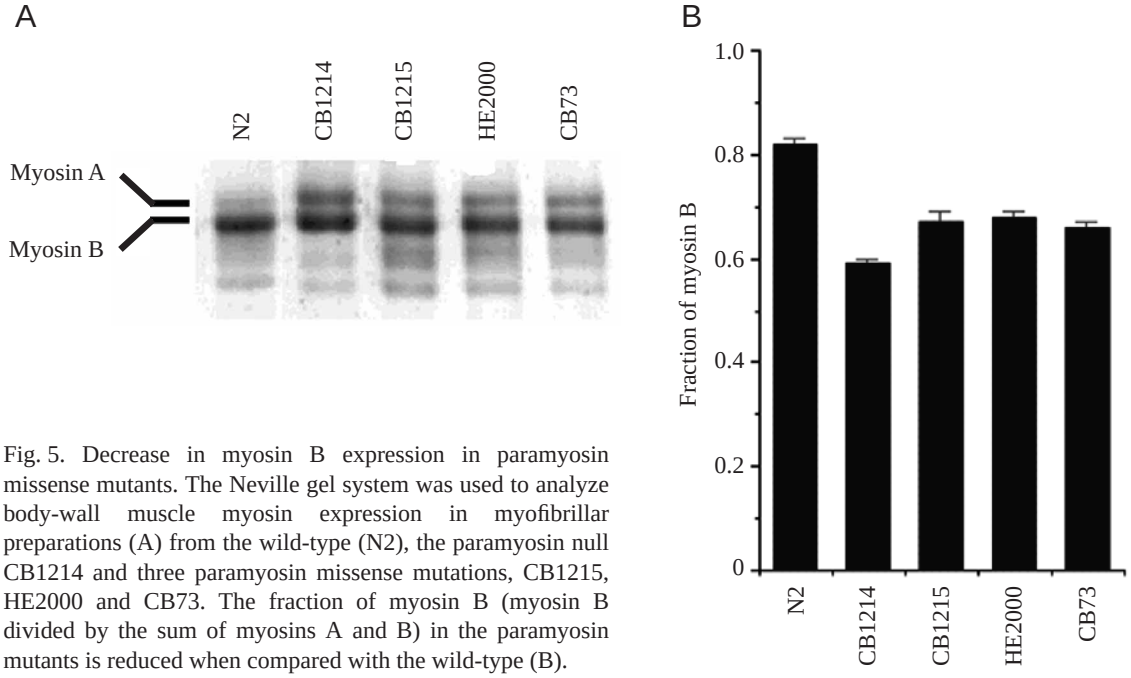

Fig. 5. Decrease in myosin B expression in paramyosin missense mutants. The Neville gel system was used to analyze body-wall muscle myosin expression in myofibrillar preparations (A) from the wild-type (N2), the paramyosin null CB1214 and three paramyosin missense mutations, CB1215, HE2000 and CB73. The fraction of myosin B (myosin B divided by the sum of myosins A and B) in the paramyosin mutants is reduced when compared with the wild-type (B).

Discussion

More than 100 genes are required for myofilament and sarcomere assembly in *Caenorhabditis elegans* body-wall muscle. The availability of mutants in these genes, as well as information on the pathway of thick filament assembly, enabled us to investigate the interdependence of myosin and paramyosin synthesis. In these studies, we find a complex relationship between the expression of the major components of thick filaments – myosin A (*myo-3*), myosin B (*unc-54*) and paramyosin (*unc-15*) – that is best explained in terms of the pathway of thick filament assembly proposed by Epstein et al. (1985, 1986).

Studies on CB675, a C-terminal deletion mutant of myosin B, demonstrated that a defect in myosin B can severely affect mobility and thick filament structure, but it does not necessarily affect either the synthesis or accumulation of myosin A (Garcea et al., 1978). Synthetic and accumulation studies on the myosin B null mutant CB190 presented here extend those observations, showing that the synthesis of myosin A as well as paramyosin is unaffected by the absence of myosin B. However, the absence of myosin B affects the accumulation of myosin A and paramyosin differently. Myosin A accumulation, like its synthesis, is independent of myosin B, but the absence of myosin B results in increased paramyosin degradation. Thus, the synthesis of myosin A and paramyosin is not dependent on interactions with myosin B; but myosin B increases the stability of paramyosin. The increased stability of paramyosin in the presence of myosin B is probably due to the physical interaction between myosin B and the paramyosin core, which stabilizes the core and/or reduces its exposure to proteases.

To determine whether the synthesis of myosin was similarly independent of its native interactions with paramyosin, myosin expression was analyzed in the paramyosin null mutant CB1214. The original studies of CB1214 demonstrated that paramyosin is essential for proper thick filament assembly and A-band formation (Epstein et al., 1974); without paramyosin, thick filaments are significantly shorter and thicker than those of the wild type (Mackenzie and Epstein, 1980, 1981). Here, we show that the absence of paramyosin and/or the consequent disruption of thick filament structure reduces the accumulation of body-wall muscle myosin by approximately 30% – a decrease that is substantial even when compared with the 50% reduction in myosin accumulation when myosin B is completely absent in CB190.

The reduced accumulation of myosin B in the paramyosin null does not result from either a generalized defect in body-wall muscle development, as occurs in myosin A or perlecan (Waterston, 1989b; Zengel and Epstein, 1980b), or an increase in the degradation of improperly assembled myosin, as occurs in dominant-negative myosin B mutations [*unc-54*(d) mutations] or in mutations of the myosin chaperonin (Barral and Epstein, 1999; Barral et al., 2002). Rather, the parallel decreases in myosin B accumulation, total myosin synthesis and steady-state myosin mRNA levels reported here demonstrate that the reduced myosin accumulation in the absence of paramyosin is due to a decrease in myosin B synthesis. Given the specific nature of the interaction between myosin B and paramyosin, the most likely explanation for the decrease in myosin B synthesis is that the efficient translation of myosin B is dependent on a nascent thick filament structure with which it or its mRNA–ribonuclear protein complex can interact.

The stability of wild-type myosin B to proteolysis in the CB1214 background is consistent with the studies of Bejsovec and Anderson (1988), who reported that wild-type myosin B was expressed at levels consistent with its gene dosage in *unc-54*(d)/+ heterozygotes. The increased proteolytic susceptibility of the dominant missense mutants of myosin B when compared with wild-type myosin B and recessive mutants, such as CB675 (Garcea et al., 1978), probably reflects differences in their ability to interact productively with the *unc-45* myosin chaperonin (Barral and Epstein, 1999; Barral et al., 2002).

It is difficult to reconcile the observations on myosin and paramyosin null mutations with a single mechanism for the regulation of contractile protein synthesis. The finding that myosin A and paramyosin synthesis are independent of the presence of myosin B is consistent with models in which the synthesis of each of the thick filament proteins is entirely independent (Saad et al., 1986). However, the specific decrease in myosin B synthesis in the paramyosin null mutant is inconsistent with such a mechanism. In the paramyosin null, myosin B synthesis is clearly dependent on the presence of paramyosin. This observation is more readily interpreted in terms of a modified ‘cotranslational assembly’ model (Isaacs and Fulton, 1987), in which synthesis of each thick filament protein is limited by its incorporation into pre-existing thick filament assemblies.

The non-reciprocal effects of myosin B and paramyosin null mutations on thick filament protein synthesis suggest that, if there is a single class of explanation, it requires consideration of other aspects of the assembly process. Our observations point directly to the pathway that Epstein, Waterston and colleagues have presented for *C. elegans* thick filament assembly. It holds that the central bipolar region containing myosin A forms first, nucleating the polar elongation of the paramyosin core, which is then followed by the addition of myosin B to the polar arms (Epstein et al., 1985, 1986; Waterston, 1989b). This pathway, combined with the structural and expression defects of mutations, is illustrated in Fig. 6. Our studies, in conjunction with those of Garcea et al. (1978) and the observation that mutations in myosin A result in an embryonic lethal phenotype (Waterston, 1989b), suggest that the effects of myosin B and paramyosin null mutations on thick filament protein synthesis are a consequence of this ordered assembly pathway. Myosin A defects that prevent the generation of a bipolar central region of the filament preclude all downstream steps in contractile protein synthesis and myofilament assembly; the paramyosin null mutant impacts only the later synthesis of myosin B, which interacts with a stable paramyosin core during thick filament elongation; and the myosin B null mutant does not affect the synthesis of either

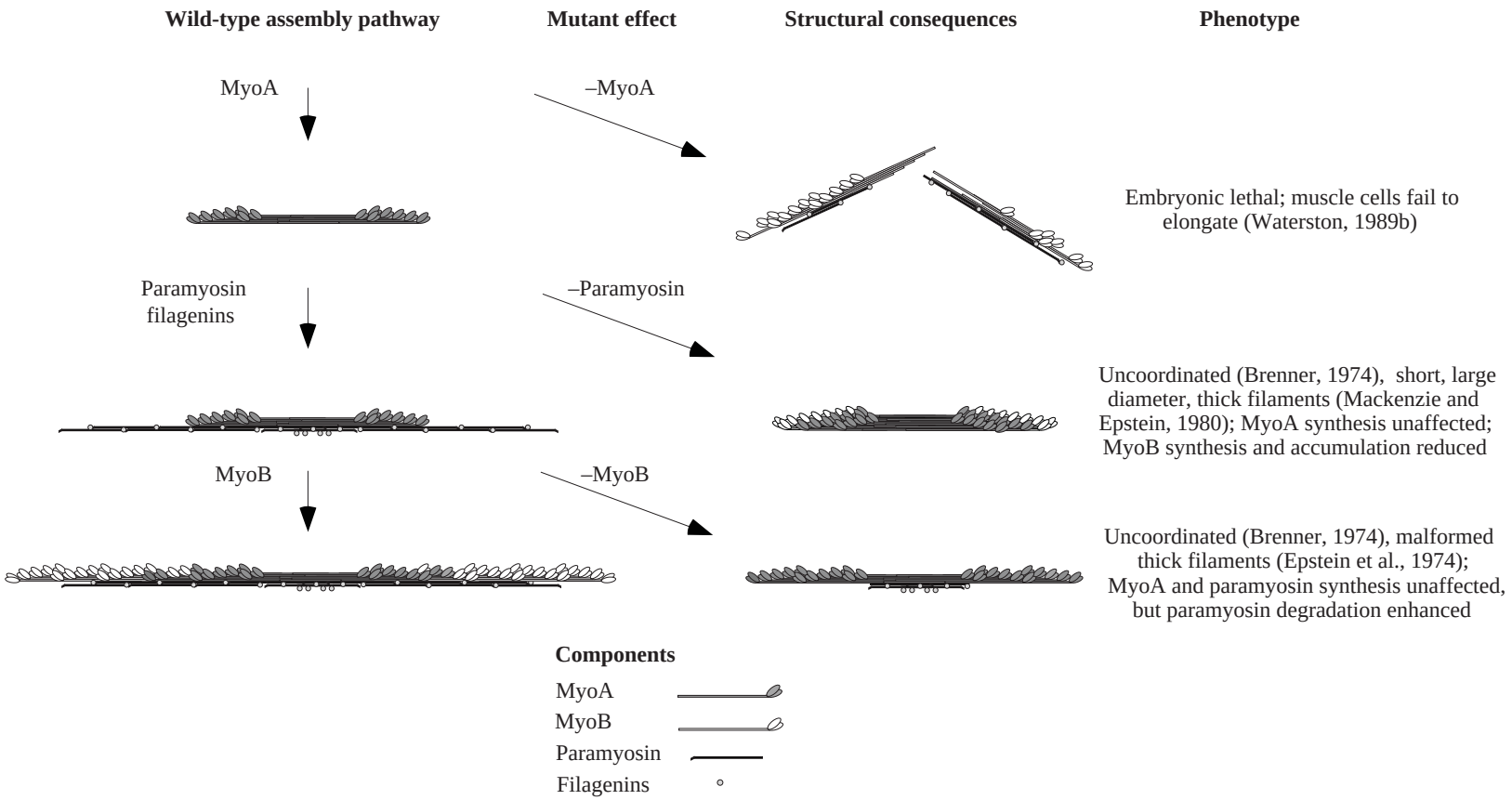

Fig. 6. The relationship between the ordered pathway of thick filament assembly, structure and expression. The ordered assembly pathway for *C. elegans* thick filaments presented by Epstein et al. (1985, 1986) and the structural and synthetic effects of mutants is presented diagrammatically. The relative positions of myosin A and B are indicated on the upper surface, and the position of paramyosin and the filagenins are indicated by exposing the core in the lower half of the filament schematic. The diagram is a modification based on Epstein et al. (1985) and Hoppe and Waterston (2000) that includes the observations on expression presented in the present study.

myosin A or paramyosin, because formation of the nascent thick filament bipolar core precedes and is independent of interactions with myosin B.

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