Myosin heavy-chain mutations that disrupt *Caenorhabditis elegans* thick filament assembly

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We have investigated *Caenorhabditis elegans* mutants in which altered *unc-54* myosin heavy-chain protein interferes with assembly of thick myofilaments. These mutants have a dominant, muscle-defective phenotype, because altered myosin heavy-chain B (MHC B), the product of the *unc-54* gene, disrupts assembly of wild-type MHC B. The mutant MHC B also interferes with assembly of wild-type myosin heavy-chain A (MHC A), the product of another MHC gene expressed in body-wall muscle cells. Because of disrupted MHC A assembly, dominant *unc-54* mutants also exhibit a recessive-lethal phenotype. Dominant *unc-54* mutations are missense alleles, and the defects in thick filament assembly result from mutant protein that is of normal molecular weight. Accumulation of mutant MHC B in amounts as little as 2% of wild-type levels is sufficient to disrupt assembly of both wild-type MHC A and MHC B. Dominant *unc-54* mutations occur at remarkably high frequency following ethylmethane sulfonate (EMS) mutagenesis; their frequency is approximately equal to that of recessive, loss-of-function mutations. This unusually high gain-of-function frequency implies that many different amino acid substitutions in the myosin heavy-chain B protein can disrupt thick filament assembly.

[Key Words: Myosin; *Caenorhabditis elegans*; thick filament assembly; dominant mutations; *unc-54*]

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Assembly of the myofibrillar lattice requires that structural components of muscle interact in a precise fashion. Biochemical studies have defined these components (for review, see Squire 1981) and immunomicroscopy has assigned their locations within the sarcomere (for example, see Sanger et al. 1984; Francis and Waterston 1985; Maruyama et al. 1985; Bennett et al. 1986). The mechanisms by which the myofibrillar lattice is assembled, however, are largely unknown. Genetic techniques have been instrumental to our understanding of other problems of macromolecular assembly, such as bacteriophage morphogenesis (Jarvik and Botstein 1973; Wood and King 1979) and assembly of flagella in *Chlamydomonas* (Luck et al. 1982). Similar approaches should be valuable for the study of muscle assembly and function. Genetic analyses of muscle development have been undertaken primarily in *Caenorhabditis elegans* (Waterston et al. 1980; Zengel and Epstein 1980) and *Drosophila melanogaster* (Mogami and Hotta 1981; Deak et al. 1982; Karlik et al. 1984; Mogami et al. 1986). Both organisms have muscle tissues that can be mutated without affecting the viability of the animal.

Genetic analysis of muscle presents a special problem, because many muscle proteins are encoded by multigene families (for example, see Fyrberg et al. 1980; Bautch and

Storti 1983; Minty et al. 1983; Romans and Firtel 1985). A mutation that reduces or eliminates gene function may have no phenotypic effect because other genes in the family supply a redundant function. In this case, dominant or incompletely dominant mutations are especially valuable (Suzuki 1970; Greenwald and Horvitz 1980). Dominant mutations may result in a visible phenotype if they create a novel function, inappropriately express the wild-type protein, or produce a disruptive product that interferes with the wild-type function (neomorphic, hypermorphic, and antimorphic mutations, respectively, in the nomenclature of Muller 1932). Dominant mutations have aided in characterizing multigene families of filamentous proteins in both *C. elegans* (Waterston et al. 1984) and *Drosophila* (Kemphues et al. 1979; Karlik et al. 1984; Hiromi and Hotta 1985).

The nematode *C. elegans* has four different muscle myosin heavy-chain (MHC) genes, and these encode four different MHC proteins (Epstein et al. 1974; Waterston et al. 1982; Miller et al. 1986). Heavy chains C and D are specific to the pharynx, while heavy chains A and B are expressed primarily in body-wall muscle cells (Mackenzie et al. 1978; Epstein et al. 1982). Heavy chains A and B assemble as homodimers (Schachat et al. 1978). Figure 1 summarizes the location of MHCs A and B within body-wall thick filaments. MHC A is restricted to the central region of thick filaments, while MHC B

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Figure 1. Schematic representation of *C. elegans* body-wall thick filaments. Zones A and B refer to regions of individual thick filaments that contain either MHC A, MHC B, or both (Miller et al. 1983). The thickness of the filament and the size of the bare zone are exaggerated in proportion to the length of the filament, which is about 10 μ . Each globular head contains one molecule of regulatory and alkali myosin light chain proteins.

overlaps partially with MHC A and extends to the filament termini (Miller et al. 1983). The MHC A-containing region includes the 'bare zone,' a region where myosin molecules interact in antiparallel fashion. Myosin interactions in this region are important for nucleating thick filament assembly (Niederman and Peters 1982).

unc-54 is the structural gene for MHC B, and several different classes of *unc-54* mutations have been previously described. Mutants of the first class, *unc-54* null alleles, are very frequent following ethylmethane sulfonate (EMS) mutagenesis. They accumulate no detectable MHC B and exhibit a muscle-defective, paralyzed (*uncoordinated*) phenotype (Epstein et al. 1974). Such mutations are recessive; loss-of-function heterozygotes are behaviorally wild type, although subtle ultrastructural irregularities can be distinguished in muscle cells. Mutations of a second class are also recessive, but occur at much lower frequency. They accumulate nearly normal amounts of a full-length MHC B and assemble a well-ordered myofilament lattice. The animals are slow, however, because of abnormal myosin function (Moerman et al. 1982; Dibb et al. 1985). Mutations of a third class contain small, weakly dominant deletions. These mutants accumulate nearly normal amounts of a MHC B that is shortened due to internal deletion (MacLeod et al. 1977; Dibb et al. 1985). Mutations of a fourth class are strongly dominant (MacLeod et al. 1977; Waterston et al. 1984; Dibb et al. 1985). Although certain atypical alleles of this class accumulate normal amounts of full-length MHC B (MacLeod et al. 1977; Dibb et al. 1985), the most frequent type of dominant mutations accumulate very little MHC B (see below).

We report here further properties of *unc-54*-dominant mutations. The mutant MHC B of these alleles fails to assemble into thick filaments, and its presence in

muscle cells inhibits the assembly of wild-type MHC B. The genetic properties of these mutations and the ultrastructure of their affected muscle demonstrate that the mutant MHC B interferes not only with the assembly of wild-type MHC B but also with that of wild-type MHC A. As a result, these *unc-54* mutations exhibit a recessive-lethal phenotype. Two features of these *unc-54* mutations are particularly surprising: (i) accumulation of mutant MHC B in amounts as little as 2% of wild-type levels is sufficient to disrupt thick filament assembly; and (ii) following EMS mutagenesis, such dominant mutations arise at a frequency approximately equal to that of recessive, loss-of-function *unc-54* mutations.

Results

Dominant *unc-54* mutations

We have isolated over 40 *unc-54*-dominant mutations. We designate these alleles *unc-54(d)* in order to distinguish them from recessive, loss-of-function mutations, designated *unc-54(0)*. The mutations were assigned to *unc-54* on the basis of two criteria: (1) they map to the *unc-54* region of linkage group I, and (2) in *cis-trans* tests they fail to complement *unc-54(0)* mutations. Since *unc-54(d)* mutations are incompletely dominant (see below), we are able to perform complementation tests with *unc-54* recessive alleles. *unc-54(d)/unc-54(0)* trans-heterozygotes have a muscle defective phenotype much more severe than *unc-54(d)/+*. *unc-54(d0)* double mutants are fully recessive (see below).

Table 1 summarizes the behavioral phenotypes of *unc-54(d)* mutants. Whereas *unc-54* null alleles are fully recessive (Table 1, lines 2 and 3), *unc-54(d)/+* heterozygotes are muscle defective and paralyzed (Table 1, line 4). The degree of paralysis varies among the different alleles, but generally *unc-54(d)/+* heterozygotes are less severely paralyzed than *unc-54(0)* homozygotes. *unc-54(d)* mutations are properly described as incompletely dominant or semi-dominant, although we will refer to them simply as dominant.

Most *unc-54(d)* mutations are lethal when homozygous (Table 1, line 5; Dibb et al. 1985), dying as late embryos or very young larvae. Lethality appears to result from an absolute lack of body-wall muscle function. Mutant embryos generally arrest at the three-fold stage, emerge from the egg, but fail to unfold from their embryonic position. Pharyngeal pumping is normal in arrested embryos.

The lethality of many *unc-54(d)* homozygotes is suppressed by *sup-3(e1407)* (Table 1, line 7). *sup-3* mutations are weak suppressors of all *unc-54(0)* alleles (Riddle and Brenner 1978). Suppression occurs because MHC A levels are increased approximately twofold in *sup-3* mutants (Waterston et al. 1982). This overexpression of MHC A is due to rearrangements affecting the MHC A structural gene (Miller and Maruyama 1986). Of 24 *unc-54(d)* alleles tested, 15 are viable and paralyzed as *unc-54(d);sup-3(e1407)* double mutants.

The lethality caused by some *unc-54(d)* mutations is

Table 1. *unc-54(d)* mutants are muscle defective when heterozygous and lethal when homozygous

Genotype	Motility phenotype
+/+	wild type
<i>unc-54(0)</i>	wild type
+	
<i>unc-54(0)</i>	paralyzed
<i>unc-54(0)</i>	
<i>unc-54(d)</i>	paralyzed; severity varies, depending on allele
+	
<i>unc-54(d)</i>	late embryonic or early larval lethal
<i>unc-54(d)</i>	
<i>unc-54(d)</i>	either severely paralyzed or lethal, depending on allele
<i>eDf10</i>	
<i>unc-54(d);sup-3</i>	either severely paralyzed or lethal, depending on allele
<i>unc-54(d);sup-3</i>	

unc-54(0) and *unc-54(d)* represent recessive and dominant alleles of *unc-54*, respectively. The phenotypes of *unc-54(0)* mutants are typical for alleles that express no MHC B. The phenotypes of *unc-54(d)* mutants depend on the allele used, as indicated in the table.

eDf10 is a deletion that eliminates the entire *unc-54* gene (Anderson and Brenner 1984); it is recessive to wild type.

sup-3 mutations overexpress MHC A approximately twofold (Waterston et al. 1982).

dependent on the number of mutant gene copies (Table 1, line 6). Certain mutations, for example *unc-54(r371)*, are lethal when homozygous but viable when heterozygous to an *unc-54* deficiency. Such animals, although viable, are severely paralyzed and have very low brood sizes. Other dominant mutations, for example *unc-54(r342)*, are inviable when hemizygous. Of 12 mutations tested, 8 are viable when hemizygous. Generally, the less strongly dominant alleles are those that are viable as hemizygotes.

Dominant *unc-54* mutations are remarkably frequent. Following EMS mutagenesis, they occur at a frequency approximately equal to that of loss-of-function *unc-54* mutations. In an experiment that was designed to recover both dominant and recessive *unc-54* mutations, we isolated approximately equal numbers of each type (see Table 2). We estimate the frequency of both dominant and recessive *unc-54* mutations to be approximately 1.0×10^{-3} per mutagenized gamete, a frequency slightly higher than that reported previously (Waterston et al. 1984). EMS-induced recessive mutations are very frequent, and they are distributed throughout the gene (Dibb et al. 1985). EMS-induced dominant mutations occur at an equally high frequency, indicating that their mutational target is also very large. These results indicate that many different nucleotide substitutions lead to an *unc-54*-dominant phenotype.

unc-54(d) alleles are missense mutations

Four lines of evidence, described below, indicate that *unc-54(d)* mutations result from amino acid substitutions in MHC B: (1) most *unc-54(d)* mutants express full-length MHC B, although at levels much less than in wild type; (2) *unc-54(d)unc-54(0)* double mutants are no longer dominant and no longer express MHC B; (3) *unc-54(d)* mutations exhibit no abnormalities on genomic Southern blots; and (4) typical *unc-54(d)* mutations accumulate near-normal amounts of full-length *unc-54* mRNA.

Figures 2 and 3 show the myosin heavy-chain proteins accumulated by representative *unc-54(d)* alleles. The amount of MHC B accumulated by different alleles is variable, ranging from below the limits of detection to ~30%. The amount of MHC B accumulated by any single allele is reproducible and characteristic for that allele. For example, in Figure 2, lanes 3, 5, and 7 show *unc-54(d)* alleles that accumulate approximately 2, 5, and 20% of the wild-type level of MHC B, respectively. The low level of mutant MHC B in *r342* (lane 3) is rarely visible when total worm homogenate is electrophoresed. However, levels of 2% can be detected when the myosin heavy chains are immunoprecipitated with polyclonal anti-actomyosin antibodies (Fig. 3). It is important to note that MHC B levels can be quantitated only for those mutants that are viable as *unc-54(d);sup-3* homozygotes. The mutant MHC B protein cannot be separated from wild-type protein for those alleles that must be maintained as heterozygotes. *unc-54(d)/+* heterozygotes accumulate an amount of MHC B that is approximately half that of wild type, similar to *unc-54(0)/+* heterozygotes. Thus, the stability of wild-type MHC B is not affected by the presence of mutant MHC B in dominant heterozygotes.

We examined *unc-54(d)* mutants for accumulation of MHC B-related polypeptides that might be shorter than full length. Actomyosin proteins were immunoprecipitated from *unc-54(d);sup-3* mutants using a polyclonal serum that recognizes all MHC isoforms (R. Francis and R.H. Waterston, unpubl.). The immunoprecipitates were

Table 2. *unc-54(d)* mutations are remarkably frequent following EMS mutagenesis

Genotype	Number isolated	Mutation frequency (mutations/gamete)
<i>unc-54(d)</i>		
+	6	1.0×10^{-3}
<i>unc-54(0)</i>		
+	8	1.3×10^{-3}

Wild-type (N2) animals were mutagenized with EMS. Among 3000 F1 offspring, we isolated six *unc-54(d)/+* heterozygotes and eight *unc-54(0)/+* heterozygotes. We identified *unc-54(d)/+* heterozygotes by their muscle-defective, paralyzed phenotype and *unc-54(0)/+* heterozygotes by scoring among the F2 offspring for the presence of *unc-54(0)* paralyzed homozygotes.

Figure 2. Myosin heavy-chain proteins accumulated by *unc-54(d)* mutants. The 3.75% SDS-PAGE of total nematode protein resolves the myosin heavy chains. Only the high molecular weight region of the gel is shown. MHC A is present in all strains, and is elevated approximately twofold in strains that carry a *sup-3(e1407)* mutation. MHC B is absent in the recessive, null mutant *unc-54(e190)* [cf. lanes 1 (wild type) and 2]. *unc-54(r371)* and *unc-54(r383)* accumulate small amounts of full-length MHC B (e.g., lanes 5 and 7). The MHC B in these strains is indicated with arrows. *unc-54(r342)* accumulates a very low level of MHC B, but it was not resolved from MHC A in this experiment. The mutant MHC B proteins are absent in nondominant derivatives of these alleles [cf. lane 5 with 6, and lane 7 with 8].

then analyzed on SDS–polyacrylamide gels. We tested 8 *unc-54(d)* mutations, and shortened MHC B-related polypeptides were not detected (data not shown). The full-length MHC B proteins described above were immunoprecipitated in each case.

The severity of the dominant phenotype for various *unc-54(d)* alleles correlates inversely with the amount of mutant MHC B. The most strongly dominant mutations (those exhibiting the most severe motility defect as heterozygotes) accumulate little or no detectable MHC B [for example, *unc-54(r342)*]. Mildly dominant alleles accumulate substantial amounts of MHC B [for example, *unc-54(r383)*]. Several *unc-54(d)* alleles have been previously described. Alleles that are viable when homozygous accumulate normal amounts of a mutant MHC B

(MacLeod et al. 1977). Compared to the alleles discussed here, however, they are among the least dominant *unc-54(d)* mutations. The protein levels of other alleles that are inviable when homozygous (Waterston et al. 1984) had not been previously determined (R.H. Waterston, pers. comm.).

If the dominance of *unc-54(d)* mutations is mediated by a mutant MHC B polypeptide, then *unc-54* loss-of-function mutations located in *cis* should eliminate the disruptive product; such double mutants should no longer be dominant. We have shown that this is true for three *unc-54(d)* alleles. We mutagenized balanced heterozygotes [genotypes *unc-54(d) +/+* Let(e2000)] and isolated wild-type F1 offspring. In each case, these nondominant derivatives proved to harbor recessive, loss-of-

Figure 3. *unc-54(d)* mutants show allele-specific differences in the amount of accumulated MHC B protein. Proteins were immunoprecipitated with polyclonal anti-actomyosin antibodies (R. Francis and R.H. Waterston, unpubl.) and subjected to 3.75% SDS–PAGE. All mutants are *unc-54(d);sup-3(e1407)* homozygotes. The positions of MHCs A, B, C, and D are shown.

function *unc-54* alleles. These *unc-54* alleles are viable when homozygous and are identical to standard recessive *unc-54* mutations in all respects. We infer that these *unc-54* alleles are double mutants [genotype *unc-54(d)unc-54(0)*], in which an *unc-54* loss-of-function mutation eliminates a disruptive gene product of the *unc-54(d)* mutation. Figure 2 demonstrates that the mutant MHC B proteins discussed above are absent in the nondominant derivatives (Fig. 2, cf. lanes 5 with 6, and 7 with 8). We suspect that the dominance of *unc-54(d)* mutations, in all cases, is mediated by a mutant MHC B polypeptide, even for alleles where the amount of mutant MHC B is below our limits of detection.

unc-54(d) mutants have no abnormalities detectable on genomic Southern blots. We performed genomic Southern blots of 17 *unc-54(d)* mutations using a hybridization probe that covers the entire *unc-54* gene. The pattern of restriction fragments was wild type for all mutants. *unc-54(d)* mutants express near-normal amounts of *unc-54* mRNA. Using an *unc-54* hybridization probe, we performed Northern blots for three *unc-54(d)* mutants. The size and abundance of *unc-54* mRNA was normal for all mutants tested.

We conclude from the above results that *unc-54(d)* mutations are small lesions (affecting less than 50 bp), that most *unc-54(d)* mutants accumulate small amounts of full-length MHC B protein, and that the low levels of MHC B protein in *unc-54(d)* mutants are not due to low levels of *unc-54* mRNA. The most likely EMS-induced mutations having these properties are single amino acid substitution (missense) alleles.

unc-54(d) protein interferes with assembly of wild-type MHC B

The ultrastructure of body-wall muscle in *unc-54(d)/+* heterozygotes demonstrates that the mutant MHC B interferes with assembly into thick filaments of wild-type

MHC B. Before considering these data, we will review briefly the ultrastructure of wild-type body-wall muscle. A more thorough description of *C. elegans* muscle can be found in Waterston et al. (1980) and Francis and Waterston (1985).

Figure 4 shows a transmission electron micrograph of a transverse section of wild-type body-wall muscle. Because nematode muscle is obliquely striated (Rosenbluth 1965), each cross section reveals all elements of a sarcomere. Approximately two and one-half sarcomeres of a single body-wall muscle cell are shown in Figure 4. Each sarcomere is delimited by dense bodies, which extend from the overlying plasma membrane deep into the myofilament lattice. Surrounding each dense body is the I-band, containing only thin filaments. Thin filaments attach to the dense bodies and extend longitudinally into the sarcomere, where they interdigitate with thick filaments in the A-band. An H-zone and M-line are apparent in the center of each A-band. Thick filaments are approximately 10- μ long and 25 nm in diameter, except near their termini where they taper to about 15 nm.

Figure 5 compares the muscle tissue of representative *unc-54(d)/+* and *unc-54(0)/+* heterozygotes. *unc-54(0)/+* heterozygotes are behaviorally wild type and Figure 5B shows that their muscle ultrastructure closely resembles wild type. Thick filaments are fewer in number than wild type because of the reduced content of MHC B. *unc-54(d)/+* heterozygotes (Fig. 5A) contain many fewer thick filaments than *unc-54(0)/+* heterozygotes. Dense bodies are diffuse and irregularly spaced, and the myofilament lattice is generally disorganized. The number and orientation of thin filaments are normal. The sections pictured in Figure 5 were chosen because they are typical examples of *unc-54(d)/+* and *unc-54(0)/+* heterozygotes. We counted the number of thick filaments present in a constant cross-sectional area of 4 representative sections of each strain. *unc-54(r371)/+* contained 305 ± 31 thick filaments (mean $\pm$ standard

Figure 4. Transmission electron micrograph of a transverse section of wild-type body-wall muscle. The bar represents 0.5 μ in this and all subsequent micrographs.

Figure 5. *unc-54(d)/+* heterozygotes contain many fewer thick filaments than *unc-54(0)/+* heterozygotes. (A) A representative section of *unc-54(r371)/+*. (B) A representative section of *eDf10/+*. Both strains express equal amounts of wild-type MHC B. Mutant MHC B of the *unc-54(d)* gene, therefore, interferes with assembly into thick filaments of wild-type MHC B.

deviation); *eDf10/+* (a recessive heterozygote) contained 833 ± 83 thick filaments. Since the animals pictured in Figure 5 contain the same levels of wild-type MHC B, we conclude that mutant MHC B of the *unc-54(d)* gene interferes with assembly of wild-type MHC B into thick filaments. The reduced number of thick filaments seen in cross sections of *unc-54(d)* heterozygotes could be because the filaments are either fewer in number or because they are shorter in length. We cannot distinguish between these possibilities.

unc-54(d) protein interferes with assembly of wild-type MHC A

Figure 6 compares the muscle tissues of representative *unc-54(d);sup-3* and *unc-54(0);sup-3* homozygotes. Since *unc-54(0)* mutations are null alleles, the thick filaments present in *unc-54(0);sup-3* (Fig. 6B) are composed exclusively of MHC A. *unc-54(d);sup-3* homozygotes contain many fewer thick filaments than *unc-54(0);sup-3* homozygotes. The sections pictured in Figure 6 were chosen because they are typical examples of these strains. We counted the number of thick filaments present in a constant cross-sectional area of 3 representative sections for each strain. *unc-54(r371);sup-*

3(e1407) contained 215 ± 39 thick filaments; *unc-54(e190);sup-3(e1407)* contained 320 ± 12 thick filaments. Since the level of MHC A is the same in these two strains, we conclude that the mutant MHC B interferes with assembly of wild-type MHC A. Again, this effect causes the filaments to be either fewer in number or shorter in length.

Figure 7 compares the muscle tissue of a representative *unc-54(d)/eDf10* hemizygote with that of an *unc-54(0)* homozygote. Because *unc-54(0)* mutations are null alleles, the thick filaments present in *unc-54(0)* homozygotes (Fig. 7B) are composed exclusively of MHC A. The number of thick filaments in this strain reflects the wild type level of expression of MHC A. *unc-54(d)/eDf10* hemizygotes (Fig. 7A) contain so few thick filaments that we were unable to accurately determine their number. As the level of MHC A is the same in these two strains, these results confirm our conclusion (above) that mutant MHC B of the *unc-54(d)* gene interferes with assembly of wild-type MHC A.

We have examined mutant muscle ultrastructure for three different *unc-54(d)* alleles (*r342*, *r371*, and *r383*). Heterozygotes, hemizygotes, and suppressed homozygotes all show a striking effect of the *unc-54(d)* mutation on thick filament number and organization. However,

Figure 6. *unc-54(d);sup-3* homozygotes contain many fewer thick filaments than *unc-54(0);sup-3* homozygotes. Panel A shows a representative section of *unc-54(r371);sup-3(e1407)*. Panel B shows a representative section of *unc-54(e190);sup-3(e1407)*. Both strains express equal amounts of wild-type MHC A. Mutant MHC B of the *unc-54(d)* gene, therefore, interferes with assembly into thick filaments of wild-type MHC A.

the three alleles cause different degrees of ultrastructural disruption and this correlates directly with the severity of behavioral phenotype. *r342* mutants consistently have fewer thick filaments than *r371*, which in turn have fewer thick filaments than *r383* mutants. *r342* is the most strongly dominant of these mutations and *r383* the least strongly dominant.

Discussion

We describe here properties of *unc-54* myosin heavy-chain mutations that disrupt the process of thick filament assembly. Myosin heavy-chain B, the product of the *unc-54* gene, is abnormal in these mutants. The mutant MHC B fails to assemble into thick filaments, and its presence in muscle cells interferes with assembly of wild-type MHC A and B. Due to their effects on assembly of wild-type MHC B, these mutants have a dominant muscle-defective phenotype. Due to their effects on assembly of wild-type MHC A, these mutants have a recessive-lethal phenotype. *unc-54(d)* mutations are missense alleles, and their MHC B product is unstable in vivo. *unc-54(d)* protein, when present in quantities as low as 2% of wild-type amounts, is sufficient to cause these disruptive effects.

At what point during thick filament assembly might *unc-54(d)* mutations exert their effects? To produce a wild-type thick filament, myosin heavy chains must fold appropriately after their synthesis, they must coassemble with myosin light and heavy chains to form myosin dimers, and myosin dimers must assemble with each other and with additional proteins in the growing thick filament. In theory, *unc-54* dominant mutations could disrupt any of these steps. We think it likely that the important disruptive effects of *unc-54(d)* mutations occur during assembly of the thick filament itself, after myosin dimer formation. *unc-54(d)* mutants accumulate very little mutant MHC B (as little as 2% of wild-type levels). Yet, this mutant MHC B has drastic effects on the assembly of a vast excess of wild-type myosins A and B. There is simply too little *unc-54(d)* protein for the disruption to occur at any other stage.

We envision two general mechanisms by which *unc-54(d)* protein inhibits assembly of both wild-type myosins A and B. The first involves mutant MHC B acting to disrupt initiation of thick filament assembly. Thick filament assembly initiates in the bare zone, where myosin dimers stack in an antiparallel fashion. Functions of the nonhelical tip of the MHC rod segment are important for antiparallel interactions (Kuznicki et

Figure 7. *unc-54(d)/eDf10* hemizygotes contain very few thick filaments. Panel A shows a representative section of *unc-54(r371)/eDf10*. Panel B shows a representative section of *unc-54(e190)*. Both of these strains express equal amounts of wild-type MHC A. The mutant MHC B of the *unc-54(d)* allele, therefore, interferes with assembly into thick filaments of wild-type MHC A.

al. 1985; Pagh and Gerisch 1986). In *C. elegans* body-wall thick filaments, myosin A alone is localized to this region (Miller et al. 1983). Myosin A interactions at the bare zone, either with components of the M-line or with nonmyosin components of the thick filament (Epstein et al. 1985), may be important for initiating filament assembly. Perhaps the *unc-54(d)* mutant MHC B localizes inappropriately to this region (either as homodimers or as A/B heterodimers), thereby disrupting the initial steps of thick filament assembly. According to this model, wild-type myosins A and B fail to assemble because there are fewer nucleated centers available for filament elongation.

The second general mechanism involves mutant MHC B acting to inhibit elongation of assembling thick filaments. Myosin, paramyosin, and components of the core structure (Epstein et al. 1985) are added to growing filaments during assembly. Perhaps *unc-54(d)* mutant myosin is able to incorporate into a growing filament, but once incorporated it prevents or interferes with the addition of further thick filament components. The result would be a truncated or 'capped' structure that is incapable of further elongation.

The two general models discussed above make testable predictions. If *unc-54(d)* mutations inhibit filament elongation but not initiation, then thick filaments in *unc-54(d)* mutants should be normal in number but

shorter in length. If *unc-54(d)* mutations inhibit filament initiation but not elongation, then thick filaments should be fewer in number but normal in length. Experiments are in progress to distinguish between these possibilities.

Where within the myosin molecule are typical *unc-54(d)* mutations located? The high frequency of *unc-54(d)* mutations following EMS mutagenesis indicates that *unc-54(d)* mutations are distributed over a substantial portion of the MHC B molecule. The rod segment of myosin is known to be important for both parallel and antiparallel interactions (Kiehart et al. 1984; Kuznicki et al. 1985; Peltz et al. 1985; Pagh and Gerisch 1986). The alpha-helical rod segment, in common with many fibrous proteins, shows a repeating pattern of 7 amino acids (McLachlan and Karn 1982, 1983). The position of hydrophobic and charged amino acids within these repeats is crucial for establishing the coiled-coil structure and for aligning adjacent myosin dimers within the thick filament (McLachlan and Karn 1982, 1983). Perhaps amino acid substitutions that alter the repeat structure disrupt inter-chain or inter-dimer interactions, resulting in the assembly defects that we observe. Alternatively, *unc-54(d)* mutations might lie in the globular head region. Phosphorylation of the regulatory light-chain is important for assembly of smooth muscle and nonmuscle myosin filaments (Suzuki et al.

1978; Kendrick-Jones et al. 1982). Craig et al. (1987) suggest that light chain phosphorylation activates the myosin molecule by altering interactions between adjacent heads or between heads and the filament backbone. Thus, functions of the head may be required for filament assembly and alterations of head structure might lead to assembly defects. Experiments are in progress to determine the locations and exact sequences of typical *unc-54(d)* mutations.

The DNA sequence of one partially dominant *unc-54* mutation has been previously determined (Dibb et al. 1985). *unc-54(e1152)*, causes two adjacent amino acid substitutions within the rod, very near its beginning. This mutation appears to disturb the rod either by affecting its charged surface or by altering its stability (Dibb et al. 1985). *e1152* is atypical of *unc-54(d)* mutations, however, because it is viable when homozygous and accumulates a normal amount of mutant MHC B.

One of the most surprising features of *unc-54(d)* mutants is that such a small amount of mutant MHC B suffices to produce dramatic effects. *unc-54(d)* mutants that accumulate the least amount of MHC B (<2% wild-type levels) are the most strongly dominant; mutants that accumulate greater amounts of mutant MHC B generally show a less severe phenotype. Perhaps the amount of mutant MHC B that accumulates in *unc-54(d)* mutants reflects how much of it is incorporated into mutant thick filaments and, therefore, protected from degradation. Abnormal proteins are proteolyzed rapidly (for review, see Goldberg and St. John 1976; Hershko and Ciechanover 1982), but they may be stabilized when incorporated into a structure (Clark et al. 1986). The less strongly dominant alleles might express a less disruptive MHC B, where more molecules in total, or a greater number adjacent to each other, must be incorporated before assembly is aborted. The degradation of unassembled mutant MHC B, therefore, might be secondary to its primary defect concerning assembly.

In a broader context, the frequent occurrence of *unc-54* dominant mutations may reflect a general property of proteins that are structural components of filaments or similar macromolecular complexes. For example, collagen, keratin, and troponin, among other proteins, exhibit a coiled-coil structure similar to that of the MHC rod segment. The assembly of complexes like muscle or the cytoskeleton may be very sensitive to mutational disruption. The spontaneous mutation rate of several autosomal dominant human disorders is in excess of 10^{-5} mutations per gamete per generation (Leviton 1988). Perhaps this high mutation rate results from the genetic properties of structural proteins like those described here.

Materials and methods

Nematode strains

Most strains used in this work are derived from the wild-type Bristol (N2) stock. The conditions for culturing *C. elegans* are described by Brenner (1974). The *unc-54(d)* mutations discussed here were induced with ethylmethane sulfonate (EMS). Many

of our *unc-54(d)* alleles were isolated using a selection that enriches for muscle-defective uncoordinated mutants (Bejsovec et al. 1984; Park and Horvitz 1986). Others were identified directly by their heterozygous muscle-defective phenotype. *unc-54(e190)* is an EMS-induced deletion that expresses no MHC B (Epstein et al. 1974; MacLeod et al. 1981). *eDf10* is an *unc-54* deletion that eliminates the *unc-54* gene and two nearby lethal complementation groups (Anderson and Brenner 1984).

Frequency of *unc-54(d)* mutations

To directly compare the frequencies of *unc-54(d)* and *unc-54(0)* mutations in a wild-type genetic background, a synchronized population of early L4 larvae was mutagenized with 50 mM EMS for 3.5 hr. Pools of 10 F1 progeny were established from these animals. The F1 progeny were screened, and animals exhibiting the characteristic *unc-54(d)/+* phenotype were retained. Three days later, the F2 populations were screened for the presence of *unc-54(0)* homozygotes. To ensure that all mutations are independent, no more than one mutant was retained from each population of F1 animals. Complementation tests with canonical *unc-54* alleles confirmed the genotypes of these mutants.

Reversion of dominance

The dominance of three *unc-54(d)* alleles was eliminated following EMS mutagenesis. *unc-54(r342)*, *unc-54(r371)*, and *unc-54(r383)* were balanced with *Let(e2000)*. The *Let(e2000)* chromosome is wild-type for *unc-54* and lethal when homozygous. Balanced heterozygotes have a muscle-defective, uncoordinated phenotype due to dominance of the *unc-54(d)* mutation. Heterozygotes were synchronized, and L4 larvae were mutagenized with EMS. Small pools of F1 progeny were established from single parents. These F1 pools were screened for motile, wild-type revertants. Wild-type F1 animals were isolated singly and their progeny scored for segregation of *unc-54(0)* homozygotes. Such animals have a characteristic paralyzed phenotype. Complementation tests with canonical *unc-54* alleles verified the genotypes of these mutants. Several independent nondominant revertants were isolated for each of the *unc-54(d)* strains tested.

Electron microscopy

Thin sections were prepared for electron microscopy as described by Waterston et al. (1980) and Moerman et al. (1982). Animals chosen for sectioning were all young adults, the stage which occurs in the wild type at about 70 hr after hatching. Sections were taken slightly posterior to the second pharyngeal bulb. A Phillips 410 electron microscope was used to view the specimens.

Gel electrophoresis of proteins

Proteins from whole nematode homogenates were solubilized in sodium dodecylsulfate (SDS) sample buffer. Myosin immunoprecipitates were obtained by sonicating nematodes in lysis buffer [10 mM NaH_2PO_4 (pH 7.5), 100 mM NaCl, 12 mM deoxycholate, 0.01% BSA, 1% Triton X-100, 0.1% SDS, 250 mM iodoacetamide] and then precipitating with polyclonal anti-actomyosin antibodies (R. Francis and R.H. Waterston, unpubl.) and Staph A (Kessler 1975). Samples were electrophoresed on 3.75% SDS-polyacrylamide gels using the buffer system of Neville (1971). Proteins were visualized by silver staining (Wray et al. 1981). A Bio-Rad GS-300 scanning densitometer was used to

estimate the MHC B levels. Individual lanes were compared to each other by normalizing the MHC B band intensity to that of the pharyngeal MHC C plus D, which are constant (Waterston et al. 1982). Several observations indicate that immunoprecipitation of the MHC was quantitative: (1) the relative proportions of wild-type MHC A, B, C, and D agreed well with published values (Garcea et al. 1978; Waterston et al. 1982); (2) the approximately twofold increase in MHC A level caused by *sup-3* mutations (Waterston et al. 1982) was evident in all cases; and (3) repetitions of the experiments yielded reproducible results. The MHC B band of *unc-54(r342)* is barely detectable, even by eye. Based on its densitometry, we estimate that our limits of detection are ~2% of wild-type MHC B levels.

Northern blot analysis

Total RNAs were extracted from mixed populations of worms by the method of Ross (1976) in the presence of vanadylribonucleosides. These RNAs were glyoxylated, separated on a 0.8% agarose gel, and transferred to a Nytran filter. Hybridizations were done according to Maniatis et al. (1982), with only minor modifications. The hybridization probes were radiolabeled by nick-translation (Rigby et al. 1977). We used hybridization probes SG23/1 and SG25/3 for *unc-54* (MacLeod et al. 1981) and pCeA7 for *act-1,2,3,4* controls (Landel et al. 1984).

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