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Coordinate Synthesis of Two Myosins in Wild-Type and Mutant Nematode Muscle during Larval Development

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Summary

In this paper we examine the role of two myosins in body-wall muscle cells of the nematode Caenorhabditis elegans. Large populations of nematodes are synchronized, and the synthesis and accumulation of myosin heavy chains and total protein are followed through postmitotic larval development. Growth is exponential with time for both the wild-type N2 and the body-wall muscle-defective mutant E675, with a longer doubling time for the mutant. Utilizing the electrophoretic polymorphism of the E675 myosin heavy chains, we show that distinguishable classes of heavy chains accumulate differentially throughout development. Immunochemical measurements confirm a similar result in N2. Total myosin heavy chain accumulation is also quantitatively similar for the two strains. Myosin heavy chain relative synthetic rates as determined by pulse-labeling are constant throughout development and are equivalent for the two strains. The final fraction of accumulated *unc-54* to total heavy chains of approximately 0.63 equals the constant synthetic fraction of approximately 0.62.

Since myosin heavy chain accumulation and relative synthesis are equivalent, we conclude that the turnover of heavy chains is also similar in N2 and E675 despite the extensive structural and functional disruption within body-wall muscle cells of the latter strain. Since the accumulated fraction of *unc-54* myosin heavy chains reaches a plateau at the constant synthetic fraction, myosin accumulation in the body-wall muscle cells may be attributed to a constant ratio of synthetic rates of the two body-wall myosin species. The coordinate synthesis of two myosins in the same body-wall muscle cells is discussed.

Introduction

The assembly of the muscle filament lattice is a complex example of biological structure formation which involves maintaining proper stoichiometry of the components being assembled and directing the specificity of their interaction to achieve a very regular structure. The influence of the final structure on the further synthesis of components is also an issue; in particular, the discovery of two different myosin heavy chains within the same muscle type by Starr and Offer (1973) and Epstein, Waterston and Brenner (1974) raises the specific question of their synthetic control during filament assembly. Larval development in the nematode Caenorhabditis elegans is accompanied by a dramatic increase in the assembly of new body-wall sarcomeres (Mackenzie et al., 1978b), and thus by following the developmental course of myosin synthesis and accumulation, regulatory interactions between the myosins and other components of the assembling filament lattice might be determined. In a similar manner, the synthetic control of contractile proteins has been studied during vertebrate skeletal muscle development in vitro (Paterson and Strohman, 1972; Whalen, Butler-Browne and Gros, 1976; Devlin and Emerson, 1978). The body-wall muscle cells of C. elegans are a model system useful for this study because the larval developmental period is short (Byerly, Cassada and Russell, 1976), the number of muscle cells remains constant (Sulston and Horvitz, 1977), the muscle proteins comprise a significant proportion of total protein (Harris and Epstein, 1977) and mutants are available that affect specific myosin heavy chains and disrupt the filament lattice (Epstein et al., 1974).

C. elegans has several distinct muscle cell types: body-wall, pharynx, vulva, anal sphincter and uterus. The body-wall musculature is by far the largest set in terms of mass and number, comprised of 95 cells arranged in four longitudinal bundles (White et al., 1976). The *unc-54* genetic locus in C. elegans specifies one of the classes of myosin heavy chains present in the body-wall muscle (Epstein et al., 1974). The *unc-54* mutant E675 has a partially deleted heavy chain (MacLeod, Waterston and Brenner, 1977a), reducing its mass from 210,000 daltons (210 kd) to 203 kd, whereas the E190 allele is apparently null for this type of myosin heavy chain (Epstein, Schachat and Wolff, 1977; Schachat, Harris and Epstein, 1977; MacLeod et al., 1977b). Both E675 and E190 have severely disrupted body-wall muscle structure and decreased motility throughout larval development. The sodium dodecylsulfate-polyacrylamide gel electrophoretic (SDS-PAGE) pattern of myosin heavy chains reveals 210, 206 and 203 kd species while the wild-type N2 has only 210 and 206 kd bands. Three types of evidence have defined the anatomic location of the myosin heavy chains species. Dissection of E675 shows that the 203 and 210 kd species are associated with the body-wall (Epstein et al., 1974); residual thick filament-like structures in E190 body-wall muscle suggest that some of the non-*unc-54* 210 kd species may be present (Epstein et al., 1974; MacLeod et al., 1977b); immunocytochemical localization using

specific anti-*unc-54* myosin and anti-myosin antibodies shows that *unc-54* and non-*unc-54* myosins are in all body-wall muscle cells and that *unc-54* myosin is unique to these cells (Mackenzie, Schachat and Epstein, 1978a).

Taking advantage of the synchrous populations of nematodes, and the electrophoretic polymorphism of E675, we have carried out titrations with specific anti-myosin and anti-*unc-54* myosin antibodies and measured the accumulation and synthesis of the distinguishable myosin heavy chains through postmitotic larval development. We have shown that the structural disruption and the alteration of a specific myosin due to mutation do not change the relative synthesis and accumulation of two myosins in nematode body-wall muscle. Furthermore, we suggest that the two body-wall myosins are coordinately synthesized throughout a developmental period in which the assembly of myofilament lattices and construction of sarcomeres occur at a high rate.

Results

Growth Characteristics of Wild-Type N2 and Mutant E675

The life cycle of the wild-type N2 has been described by Byerly et al. (1976); it proceeds through four larval molts to sexual maturity and egg laying at 65 hr after hatching at 20°C. Eggs hatch 10–12 hr after fertilization (Hirsh, Oppenheim and Klass, 1976) and their resistance to a solution of 1.25% NaOCl, 0.5 N NaOH enables synchronization of large populations of nematodes which can be followed through development (R. Hecht and D. Hirsh, personal communication). We have chosen to study larval development after 12 hr post-hatching because Sulston and Horvitz (1977) showed that somatic cell mitosis has ceased and Mackenzie et al. (1978b) demonstrated that sarcomere addition in the body-wall muscle cells is greatest after this time.

The accumulation of total protein during nematode development can easily be followed by growth on ^{35}S-labeled bacteria. The accumulation of total protein and total myosin heavy chain can be fitted by an exponential function (Figure 1), and doubling times determined by such plots are characteristic for each strain. E675 grows more slowly than N2, probably due to its paralysis and consequent relative inability to feed (12.0 hr versus 7.9 hr doubling times, Table 1). Developmental landmarks such as the appearance of eggs are also retarded. A similar lag might be expected when comparing the developmental expression of specific proteins between mutant and wild-type. Indeed, we find that the accumulation of myosin heavy chains in E675 also

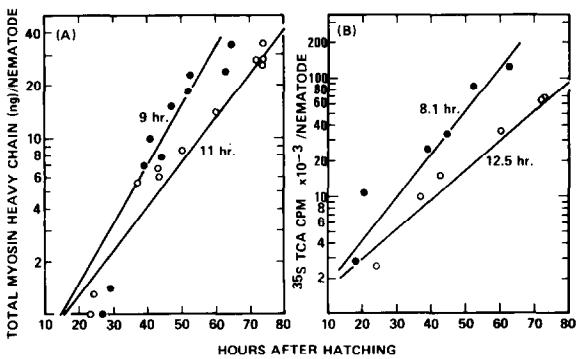

Figure 1. Developmental Accumulation of Total Protein and Myosin Heavy Chains

Semi-log plots of total myosin accumulation (A) and ^{35}S-TCA-precipitable cpm (B) for N2 (●) and E675 (○). Determinations are from single experiments, and doubling times shown are exponential fits for this data.

lags behind the wild-type (Figure 1). The TCA-soluble cpm also change with developmental time. The TCA-soluble cpm may reflect changes in the total pool of nonprotein amino acids (in this case, cysteine and methionine). If so, their fluctuations limit determination of absolute rates of protein synthesis. Moreover, whole nematodes are being analyzed here without separation of different cell types or identification of amino acid pools within them. Thus only relative rates of accumulation and synthesis are determined.

Total Myosin Heavy Chains Accumulate to Equal Extents in N2 and E675

Total myosin heavy chains represent contributions from several muscle types in the nematode. In adults, the body-wall and pharyngeal muscles contribute about 90 and 5%, respectively, of the total population of muscle cells (Sulston and Horvitz, 1977), which agrees with the relative contributions of different myosins (Schachat et al., 1977). The contribution of nonmuscle cells to total myosin is assumed to be quantitatively insignificant in comparison to muscle cells in larvae and adults. Myosin heavy chain accumulation is exponential and follows the growth rate for both N2 and E675 (Figure 1). Total myosin heavy chains accumulate to equal extents by egg-laying time in both the wild-type N2 and myosin mutant E675 (Figure 2), and the ratios of total myosin heavy chain to total protein doubling times are not significantly different for N2 and E675 (Table 1). Myosin therefore represents a similar fraction of total accumulated protein in both strains. There is no absolute decrease in myosin heavy chains associated with the myosin alteration and body-wall muscle disruption of E675, and the decreased rate of accumulation correlates with the decreased rate of growth secondary to body-wall alteration and paralysis in E675.

Table 1. Summary of Doubling Times and Heavy Chain Ratios for N2 and E675

Strain	Accumulation: Doubling Times (Hr)		Ratio: Total Myosin	Pulse Ratios:	
	TCA	Total Myosin	TCA	Total Myosin[a] / TCA (× 10²)	*Unc-54* Myosin[a] / Total Myosin
N2	7.9 ± 0.2	9.5 ± 0.4	0.82 ± 0.07	0.78 ± 0.13	0.615 ± 0.023
E675	12.0 ± 0.6	13.0 ± 0.8	0.93 ± 0.13	0.70 ± 0.18	0.626 ± 0.008

[a] Mean of single time point ratios over entire course of development.

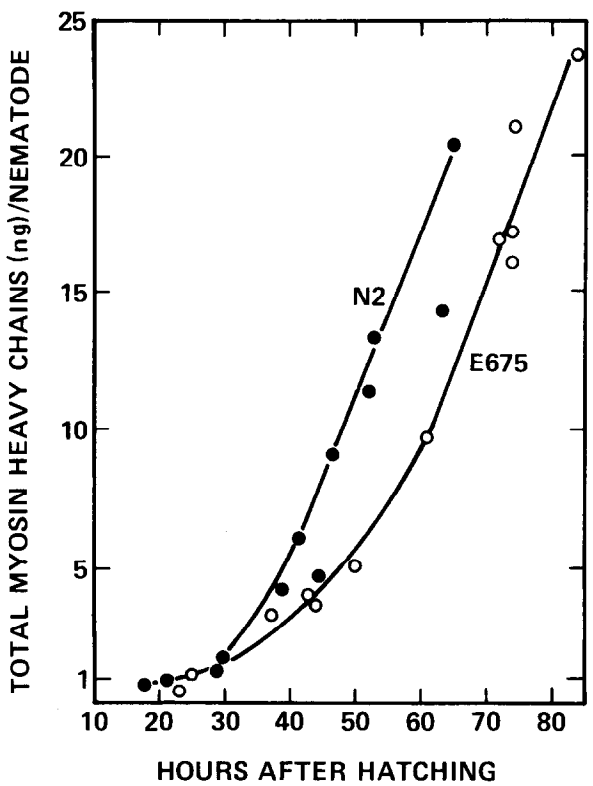

Figure 2. Comparison of Total Myosin Heavy Chain Accumulation through Development

N2 (●) and E675 (○) total myosin heavy chain determinations represent data from single experiments.

Individual Classes of Myosin Heavy Chains Accumulate Differentially

When the accumulation of E675 myosin heavy chains during development is examined, a striking change in the relative amounts of the distinguishable species is apparent (Figure 3). The 203 kd *unc-54* product of body-wall muscle cells increases dramatically relative to the pharyngeal 206 kd species and the 210 kd chains. Figure 4 shows this differential accumulation. Each of the heavy chains increases exponentially (fits of kinetic plots not shown) although the rates of these increases are different from the rates of growth and total heavy chain accumulation (Figure 4). Figure 4 also shows the accumulation of non-*unc-54* 210 kd heavy chains in the *unc-54* null mutant E190 to be similar to that of the corresponding E675 chains. The same comparison holds for the pharyngeal 206 kd heavy chains within both mutants. The lack of *unc-54* gene product, therefore, does not alter the amounts of the remaining myosin heavy chains.

The differential accumulation of heavy chains leads to a changing fraction of *unc-54* relative to total myosin heavy chains during development (Figure 5B). The *unc-54* product, uniquely associated with body-wall muscle cells, increases in proportion to total myosin heavy chains as the body-wall muscle cells undergo dramatic growth to become the predominant muscle cell type of the nematode by mass as well as by cell number. Once adulthood is reached (55–70 hr), the fraction appears to reach a plateau between 0.61 and 0.65. This plateau may represent the end of substantial muscle cell growth in the animal and the subsequent maintenance of fully established fibrillar structures. Immunochemical titration of the *unc-54* myosin fraction in N2 at three developmental times shows changes very similar to those in E675. Thus both the total and relative amounts of distinct myosin heavy chains and their resultant myosins (Schachat, Garcea and Epstein, 1978) are similar for wild-type N2 and mutant E675. Furthermore, the final value of the *unc-54* fraction places an upper limit on the stoichiometry of *unc-54* and non-*unc-54* myosins in the body-wall muscle cells.

Synthetic Rate of *Unc-54* Relative to Total Myosin Heavy Chains Remains Constant during Development

Since the myosin heavy chains accumulate similarly in N2 and E675, determination of either the relative synthetic or degradative rates will allow comparison of myosin turnover. We have attempted to measure degradative rates directly by pulse-chase experiments, but because of either inadequate chase of ^{35}S from cysteine and methionine pools or reutilization of ^{35}S from turnover of other proteins, this approach was not feasible.

Myosin heavy chain synthesis is examined by pulse-labeling nematodes with ^{35}S-labeled E. coli; then total myosin incorporation is compared to

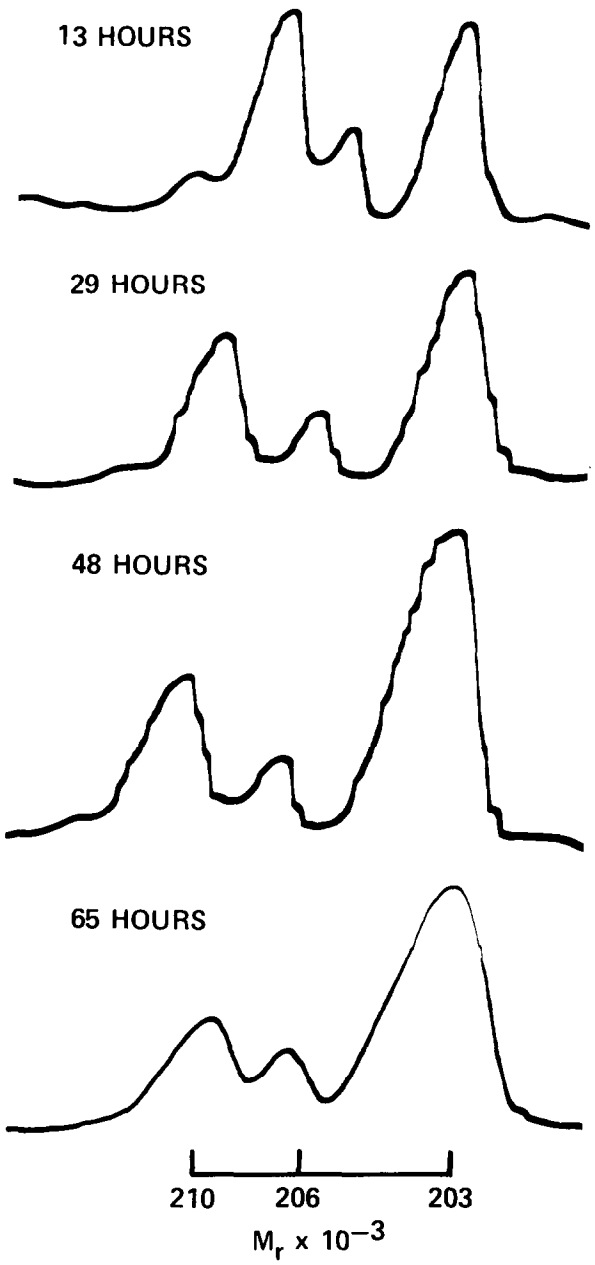

Figure 3. Densitometry of E675 Myosin Heavy Chains through Development

Densitometry tracings are from Coomassie blue-stained 5% PAGE-SDS gels from separate experiments. The M_r bar refers directly to the 65 hr scan.

total protein incorporation and the amount incorporated into *unc-54* myosin is compared to total myosin. In E675, total myosin heavy chain and *unc-54* heavy chain (203 kd) incorporations are determined by densitometry of autoradiograms of SDS-PAGE separation of the heavy chains. In N2, total myosin and *unc-54* myosin incorporation are determined by immunochemical titration. Figure 5A shows that the synthesis of *unc-54* heavy chains relative to total heavy chains does not increase through development, as does the accumulated fraction, but remains constant within experimental error. This constant synthetic fraction of about 0.62 equals the plateau value of about 0.61–0.65 for the accumulated fraction (compare Figures 5A and 5B). Since the body-wall muscle is the type of muscle growing most prominently during these developmental times, the constancy of the synthetic fraction strongly suggests that the two body-wall myosins *unc-54* and non-*unc-54* are synthesized at a fixed ratio. This constant stoichiometry of synthesis of the two body-wall muscle cell myosins eventually determines the accumulation ratio at later developmental times.

Moreover, the mean of the *unc-54* to total myosin synthetic fraction is similar for the body-wall muscle mutant E675 and wild-type N2 throughout development, as is relative synthesis of total myosin heavy chains to total protein (Table 1). Thus we conclude that the relative synthetic rates for total myosin heavy chains and the *unc-54* product are the same for E675 and N2. Since accumulation and relative synthesis are the same we further conclude that the relative turnover of myosin heavy chains is equivalent in E675 and N2.

Discussion

Body-wall muscle cells undergo extensive growth and construction of new sarcomeres in C. elegans during postmitotic larval development (Mackenzie et al., 1978b). The synthesis and accumulation of myosin heavy chains have been measured in large synchronous populations of nematodes undergoing these larval changes as a first step towards understanding the regulation of gene expression during this period of rapid growth and assembly of contractile structures. We have investigated the relative synthesis and accumulation of myosin heavy chains in the highly organized muscle cells of wild-type N2 and the structurally disrupted and paralyzed muscle cells of the body-wall defective mutant E675. In both the wild-type and mutant nematodes, the two major classes of myosin heavy chains are synthesized at the same constant ratio, suggesting that their synthesis may be coordinated and results in a fixed stoichiometry of the myosins with a single cell type. The accumulation and relative synthesis of the different myosin heavy chains is similar for both strains, indicating that the structural disarray of E675 does not influence the relative turnover of these muscle proteins.

Epstein et al. (1974) presented evidence suggesting that two distinct myosin heavy chains coexist within body-wall muscle in C. elegans, and Mackenzie et al. (1978a) have directly verified by antibody localization procedures that two myosins exist in the same body-wall muscle cells. In these

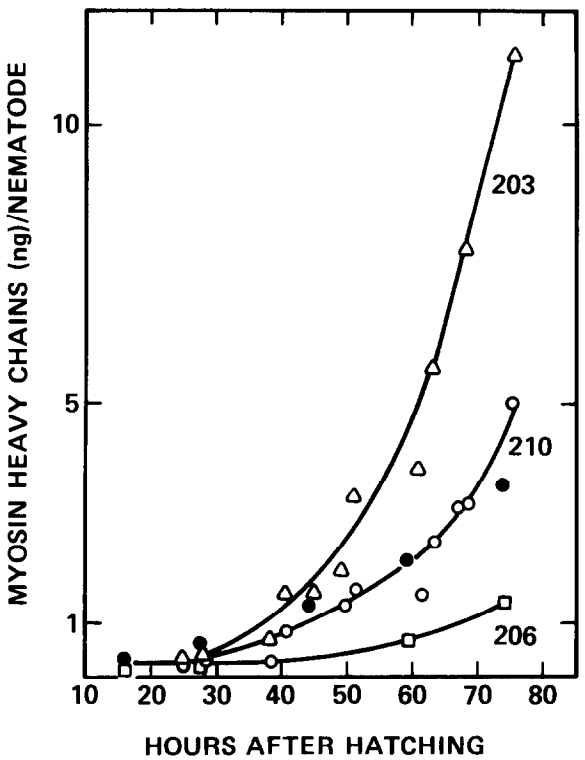

Figure 4. Individual Myosin Heavy Chain Accumulation through Development

203 (△), 210 (○) and 206 kd (□) myosin heavy chains for E675, and 210 kd (●) heavy chain from E190.

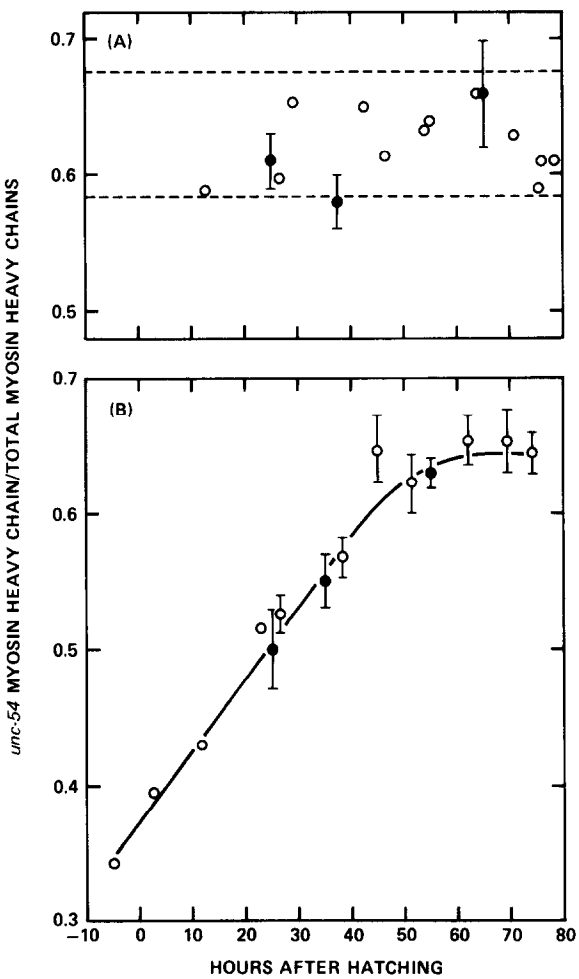

Figure 5. Developmental Relationship of *Unc-54* Myosin to Total Myosin Heavy Chains in Pulsed and Continuously Labeled Nematodes

(A) Pulse-labeled ratios of *unc-54* to total myosin heavy chains in E675 (○) (±3 hr) and N2 (●) (±2 hr). Ratios for E675 were determined by autoradiographic densitometry and represent single determinations. Ratios for N2 were determined by immunoprecipitation and are means of three determinations. Pulse length was 2 hr.

(B) Ratio of *unc-54* to total accumulated myosin heavy chains in E675 (○) (±3 hr) and N2 (●) (±3 hr). Ratios for E675 were determined by Coomassie brilliant blue densitomery and represent the mean of ratios (±3 hr) from three experiments. Ratios without standard deviations are the mean of three determinations in a single experiment. Ratios for N2 were determined by immunoprecipitation and are the mean of three determinations.

cells, one myosin heavy chain is structurally affected by *unc-54* mutants and is designated the *unc-54* heavy chain; the other is then designated the non-*unc-54* heavy chain. In wild-type N2 these two chains have apparent masses of 210 kd, but in E675 the *unc-54* chain has an apparent mass of 203 kd resulting from an internal deletion (MacLeod et al., 1977a). The non-*unc-54* myosin heavy chain in E675 remains at 210 kd. This species, which may represent one or more gene products, is shared between the body-wall and pharyngeal muscles in both E675 and N2. A third and minor species of heavy chain at 206 kd is localized in the pharynx in both wild-type and mutant nematodes. All three myosin heavy chains accumulate differentially during larval development in E675. A similar situation occurs in N2, where the relative amounts of *unc-54* myosin and total myosin are determined by immunochemical titration. Since our results are derived from the whole animal rather than isolated muscle cells, we believe that the changing proportions reflect the differential growth of specific muscle cell types, principally the body-wall and pharyngeal muscles, and that the ratio of different classes of heavy chains is actually constant within a specific type of muscle cell. This conclusion is supported by the high proportion of the pharyngeal heavy chain at 206 kd relative to *unc-54* heavy chain of the body-wall early in larval development, when the pharynx is a comparatively large structure. At later stages, when body-wall muscle growth predominates, the amount of 206 kd chains is small relative to *unc-54* myosin heavy chains.

The *unc-54* and non-*unc-54* classes of myosin heavy chains represent discrete populations of myosin molecules in C. elegans. Nematode myosins are dimers of heavy chains associated with two 18 kd and two 16 kd light chains (Harris and Epstein, 1977). Each myosin contains either two

unc-54 or two non-*unc-54* heavy chains, and no hybrid myosins are detected (Schachat et al., 1977, 1978). We therefore designate these homodimeric myosin species as *unc-54* and non-*unc-54* myosins. Thus our measurements of the synthesis and accumulation of the two major classes of myosin heavy chains directly correspond to the behavior of two populations of homodimeric myosin molecules. This assertion is experimentally supported by the similarity of our immunochemical measurements of native myosins and our electrophoretic determinations of myosin heavy chains during development.

In contrast to the significant developmental change in the accumulated ratio of *unc-54* to total myosin, the synthetic fraction is relatively constant in both N2 and E675. Since this is the time period in which the body-wall muscle undergoes dramatic growth, we believe that this synthetic ratio is dominated by synthesis of myosins in the body-wall muscle cells. This assumption is supported not only by morphologic developmental changes but also by the rapid accumulation of the *unc-54* body-wall-specific heavy chain during this period. The two body-wall myosins therefore appear to be coordinately synthesized; their synthesis establishes a constant stoichiometry reflected by their final accumulated quantities.

Such a constant stoichiometry is consistent with the proposal of Schachat et al. (1977) that the two myosins could act as a vernier mechanism (Huxley, 1963; Huxley and Brown, 1967) for the determination of precise thick filament length in vivo. Alternatively, a constant stoichiometry may be required for the organization of filaments into lattices or for the contractile properties of a particular muscle. The fraction of *unc-54* myosin relative to total myosin, 0.61–0.66, determined either by the plateau of myosin accumulation or by the fixed ratio of myosin synthesis, places a limit on the stoichiometry of the two myosins within body-wall muscle cells. If essentially all the synthesis of *unc-54* and non-*unc-54* myosins is due to body-wall activity as we have suggested, then the stoichiometry of the two myosins within body-wall muscle cells is about 2:1. Although we consider this ratio to be the most probable (Schachat et al. 1977), the possibility that other cell types contribute significantly to the synthesis of non-*unc-54* myosin cannot be excluded. In this case, the stoichiometry would be higher than 2:1 since *unc-54* myosin is rigorously localized in these cells (Mackenzie et al., 1978a), but only a portion of non-*unc-54* myosin can be synthesized in them.

Since the accumulation and relative synthesis of *unc-54* and non-*unc-54* myosins are similar for N2 and E675, the relative turnover of these myosins must also be similar for the wild-type and mutant muscles. This surprising result suggests that structural alteration of the *unc-54* myosin and the disorganization of the muscle filament lattice in E675 do not lead to increased degradation of either myosin. The lack of increased turnover of a mutant protein is in marked contrast to conclusions reached in other systems (Goldberg and St. John, 1976). We further infer that both the synthesis and turnover of *unc-54* myosin are independent of the formation of a normal structure in nematode body-wall muscle cells during larval development and early adulthood. A similar conclusion can also be drawn for the non-*unc-54* myosin. Since the accumulation of non-*unc-54* myosin in the *unc-54* null mutant E190 is similar to E675 and to wild-type, the presence or absence of one myosin does not influence the accumulated quantities of the other. Thus the two myosins appear to be coordinately synthesized, their synthesis determines the final amounts of product accumulated during larval development and the responsible regulatory mechanism seems to be independent of both the structural state of the muscle and the myosins within it.

To explore further the control of specific myosin synthesis the developmental relationships of myosins in C. elegans studied here are being investigated in mutants of the *unc-52* locus that are developmentally retarded in body-wall muscle sarcomere construction (Mackenzie et al., 1978b). The potential regulation of other muscle filament lattice proteins is also being examined in wild-type and mutant nematode strains. The post-mitotic larval stages in C. elegans provide us with a model for studying the mechanisms of structural assembly and control of gene expression during muscle development.

Experimental Procedures

Nematode Strains

N2 and *unc-54* mutants E675 and E190 of C. elegans are described by Brenner (1974) and Epstein et al. (1974).

Nematode Growth

Nematodes were grown as described by Brenner (1974) with the following exceptions: NA22 instead of OP50 Escherichia coli was used, bactopeptone was increased 8 fold in the NG agar of Brenner (1974) and the agar was increased to 2.5% (w/v). All experiments were carried out at 20 ± 0.5°C. Nematodes were always maintained with a large excess of bacteria during developmental study.

^{35}S Labeling

In vivo labeling with ^{35}S-sulfate was carried out by the procedure of Schachat et al. (1977) on plates without peptone (NP). NA22 grown in media having a specific activity of 62.5 mCi/mmole were used for continuous labeling, although continuous labeling up to 312.5 mCi/mmole gave identical results. NA22 grown at a specific activity of 625 mCi/mmole were used for pulse-labeling. Pulse lengths were 2 hr. Nematodes were transferred from cold bacteria by filtering onto 8 μm Millipore filters, washing with M9 salts (1.16 g Na_2HPO_4, 0.6 g KH_2PO_4, 0.1 g NaCl, 0.2 g NH_4Cl in 200 ml),

washing from the filter with M9 salts and then plating with labeled bacteria. Harvesting after the 2 hr pulse was carried out as described below.

Synchronization of Nematodes

Nematodes and eggs were washed off plates under sterile conditions with M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO, 0.12 g $MgSO_4$, 5 g NaCl in 1 l) and sedimented at 1500 rpm in a bench centrifuge. The supernatant was removed and 8–10 vol of 1.25% NaOCl, 0.5 N NaOH were added and allowed to sit for 10 min at 20°C with occasional shaking. The solution was then recentrifuged at 1500 rpm and the supernatant was discarded. The pellet was washed with 10–12 ml of M9 buffer and repelleted. The final pellet was resuspended in 4–5 vol and plated. This procedure was repeated, for example, 8 hr later for a population of nematodes synchronized to 8–12 hr, or ±2 hr. M9 salts were used in the final washes and resuspension if the worms were subsequently to be labeled.

Harvesting of Eggs and Larvae

Nematode eggs were synchronized to within 4–5 hr and plated in M9 salts on 9 cm NP plates with NA22 labeled at 62.5 mCi/mmole. Nematodes were harvested at various times after hatching by filtering onto 8 μm Millipore filters, washing well with M9 buffer, washing the nematodes from the filter with 0.1 M NaCl, 25% sucrose, and sedimenting at 1500 rpm. The supernatant was removed and the pellet was resuspended in 2.0 ml of 0.1 M NaCl, 25% sucrose. Three separate 30 ml aliquots were removed and placed on glass slides for later counting. The remainder was resedimented at 1500 rpm, the supernatant was removed and the pellet was solubilized in SDS sample buffer (10% glycerol, 4% SDS, 5% β-mercaptoethanol, .0625 M Tris (pH 6.8), 0.002% bromphenol blue). The sample was then heated at 95°C for 10–15 min and frozen until electrophoresed. Eggs were assayed by synchronizing to ±3 hr filtering, washing, resuspending in SDS sample buffer and homogenizing in a French pressure cell at 250 psi. The entire resultant homogenate was heated at 95°C for 10–15 min and stored frozen until electrophoresed.

Gel Electrophoresis

SDS-PAGE was performed according to Epstein et al. (1974) with 5% acrylamide to separate myosin bands. Gels were stained with Coomassie brilliant blue, destained with 5% methanol–7.5% acetic acid and densitometered on a Transidyne RFT gel scanner at 580 nm. Dupont Cronex-4 X-ray film was used for autoradiography against dried gels. Both Coomassie staining and autoradiographic density were linear within the ranges used. Each gel contained two purified nematode myosin standards whose concentration was determined against a rabbit myosin standard established by ϵ max. Purified nematode ^{35}S-myosin standards were used to calibrate autoradiograms. Myosin concentration or cpm for an individual sample was the average of 2 or more gel determinations.

Total Protein Determinations

^{35}S trichloroacetic acid (TCA)-insoluble cpm were determined by taking 5 μl of a 1/10 dilution of a sample in SDS sample buffer into 1 ml of cold 10% TCA plus 50 μl of bovine serum albumin as carrier. The solution was heated to 90°C for 20 min, cooled and filtered onto a 0.22 μm Millipore filter, washing both tube and filter with cold 5% TCA. Filters were dried and counted in 3 ml of Aquasol (New England Nuclear).

Immunochemical Titrations for N2 Accumulation and Pulse Determinations

N2 was synchronized to ±2 hr. NA22 grown at 125 mCi/mmole were used for continuous labeling and 1250 mCi/mmole for 2 hr pulse-labeling. Worms were harvested by settling and washing as described in Schachat et al. (1977). For each continuous and pulse-labeled time point, myosin was isolated from 1 g of nematodes by the procedure of Harris and Epstein (1977). The myosin was then titered by indirect immunoprecipitation with antibodies to total myosin and antibodies specific for *unc-54* myosin (Schachat et al., 1978). The ratio of *unc-54* to total myosin was determined by titration at three saturating concentrations of either anti-myosin or anti-*unc-54* IgG.

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