Functions of the *Caenorhabditis elegans* Regulatory Myosin Light Chain Genes *mlc-1* and *mlc-2*

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ABSTRACT

Caenorhabditis elegans contains two muscle regulatory myosin light chain genes, *mlc-1* and *mlc-2*. To determine their *in vivo* roles, we identified deletions that eliminate each gene individually and both genes in combination. Functions of *mlc-1* are redundant to those of *mlc-2* in both body-wall and pharyngeal muscle. *mlc-1*(*0*) mutants are wild type, but *mlc-1*(*0*) *mlc-2*(*0*) double mutants arrest as incompletely elongated L1 larvae, having both pharyngeal and body-wall muscle defects. Transgenic copies of either *mlc-1*(+) or *mlc-2*(+) rescue all defects of *mlc-1*(*0*) *mlc-2*(*0*) double mutants. *mlc-2* is redundant to *mlc-1* in body-wall muscle, but *mlc-2* performs a nearly essential role in the pharynx. Approximately 90% of *mlc-2*(*0*) hermaphrodites arrest as L1 larvae due to pharyngeal muscle defects. Lethality of *mlc-2*(*0*) mutants is sex specific, with *mlc-2*(*0*) males being essentially wild type. Four observations suggest that hermaphrodite-specific lethality of *mlc-2*(*0*) mutants results from insufficient expression of the *X*-linked *mlc-1*(+) gene in the pharynx. First, *mlc-1*(*0*) *mlc-2*(*0*) double mutants are fully penetrant L1 lethals in both hermaphrodites and males. Second, *in situ* localization of *mlc* mRNAs demonstrates that both *mlc-1* and *mlc-2* are expressed in the pharynx. Third, transgenic copies of either *mlc-1*(+) or *mlc-2*(+) rescue the pharyngeal defects of *mlc-1*(*0*) *mlc-2*(*0*) hermaphrodites. Fourth, a mutation of the dosage compensation gene *sdc-3* suppresses hermaphrodite-specific lethality of *mlc-2*(*0*) mutants.

CONVENTIONAL myosins (myosins II) are hexameric, consisting of two myosin heavy chains (MHCs) and four myosin light chains (MLCs). One molecule of regulatory MLC (rMLC) and one of essential MLC are bound noncovalently to the neck region of each globular myosin head. Light chains provide structural support to the neck region of myosin, thereby amplifying conformational changes associated with myosin motor functions (Rayment *et al.* 1993; Holmes 1997). Regulatory MLCs (rMLCs) are named so because they regulate activity of the myosin ATPase. In molluscan muscle, rMLCs inhibit interactions between myosin and actin. Calcium binding to myosin relieves this inhibition, thereby activating the myosin ATPase and provoking muscle contraction (Szent-Gyorgyi *et al.* 1973; Kendrick-Jones *et al.* 1976). In vertebrate smooth muscle and nonmuscle cells, phosphorylation of certain rMLC residues by myosin light chain kinase (MLCK) activates the myosin ATPase, whereas phosphorylation of other residues by protein kinase C (PKC) inhibits the ATPase (Sellers 1991; Tan *et al.* 1992; Gallagher *et al.* 1997). Phosphorylation of rMLCs by $p34^{cdc2}$ inhibits the myosin ATPase in nonmuscle cells and likely regulates the timing of cytokinesis by inhibiting contractile ring activity during mitosis (Satterwhite and Pollard 1992).

In addition to their role in regulating myosin ATPase activity, rMLCs also regulate thick filament assembly in smooth muscle and nonmuscle cells (Trybus 1991; Tan *et al.* 1992). Phosphorylation of the rMLCs by MLCK destabilizes a folded myosin conformation, favoring an extended conformation that is competent for thick filament assembly (Craig *et al.* 1983). Although the extent to which the rMLCs function in thick filament assembly *in vivo* has not been established (Somlyo *et al.* 1981; Horowitz *et al.* 1994), rMLC phosphorylation provides an appealing mechanism for rapid redistribution of myosin filaments in transient cellular functions.

MLCs appear to play a more limited role in vertebrate skeletal and cardiac muscle. Calcium regulation of the myosin ATPase is mediated by the thin filament troponin-tropomyosin complex (Holmes 1995; Gulick and Rayment 1997). Actin-binding and actin-activated ATPase activity of skeletal myosin heavy chains stripped of all light chains is nearly normal (Wagner and Giniger 1981; Sivaramakrishnan and Burke 1982; Lowey *et al.* 1993). Such heavy chains can, however, inhibit myosin movement of actin filaments *in vitro* (Lowey *et al.* 1993). Phosphorylation of rMLCs appears to increase isometric force and the rate of force production in skeletal muscle (Sweeney *et al.* 1993).

Our current understanding of the rMLCs comes primarily from their *in vitro* manipulation. Genetic analysis

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will be important to reveal their *in vivo* functions. For example, the Drosophila muscle regulatory MLC gene, *MLC-2*, is required for normal myogenesis (Warmke *et al.* 1992). *MLC-2* null mutants undergo gastrulation, germ band extension, and germ band shortening but die late in embryogenesis, presumably due to defects in embryonic musculature. Myofibrillar structure of the indirect flight muscles is disrupted in *MLC-2* null mutant heterozygotes, demonstrating that *MLC-2* stoichiometry is important for the ultrastructure of this specialized muscle. *MLC-2* constructs having substitutions at the MLCK phosphorylation sites rescue the lethality of *MLC-2* null mutants (Tohtong *et al.* 1995), consistent with a limited role of the rMLCs in skeletal muscle. Indirect flight muscle is structurally normal in such mutants, but their flight is impaired, and the power output of isolated flight muscle fibers is reduced (Tohtong *et al.* 1995). The Drosophila nonmuscle rMLC gene, *spaghetti-squash* (*squ*), is essential for cytokinesis (Karess *et al.* 1991). squ^{1} mutants die as pupae with a large number of polyploid cells. Thus, this nonmuscle rMLC likely regulates myosin contractile ring functions. Mutants expressing *squ* alleles having altered activating phosphorylation sites are nearly indistinguishable from *squ* null alleles (Jordan and Karess 1997), which demonstrates an essential role of rMLC phosphorylation in nonmuscle cells. Dictyostelium rMLC null mutants ($mlcR^{-}$) have similar cytokinesis defects (Chen *et al.* 1994). Myosin is localized aberrantly in $mlcR^{-}$ cells, and purified $mlcR^{-}$ myosin has abnormal disassembly properties and decreased ATPase activity *in vitro* (Chen *et al.* 1994). Surprisingly, the cytokinesis defects of $mlcR^{-}$ mutants are rescued by a mutant rMLC that cannot be phosphorylated, suggesting that rMLC phosphorylation plays a more limited role in Dictyostelium (Ostrow *et al.* 1994).

The nematode *Caenorhabditis elegans*, like many invertebrates, exhibits both actin-linked and myosin-linked regulatory systems (Lehman and Szent-Gyorgyi 1975; Harris and Epstein 1977). The precise role, however, of these calcium regulatory systems in modulating actomyosin interactions *in vivo* remains unknown. Two *C. elegans* regulatory MLC genes, *mlc-1* and *mlc-2*, were previously described (Cummins and Anderson 1988). MLC-1 and MLC-2 are nearly identical, being distinguished by a single conservative amino acid substitution. Despite this similarity, *mlc-1* and *mlc-2* have striking differences outside of their protein coding regions. For example, *mlc-1* has a very long 3′ untranslated region, whereas *mlc-2* does not. *mlc-2* is trans-spliced, but *mlc-1* is not. Such differences in gene structure might reflect differences in their expression. To determine whether *mlc-1* and *mlc-2* have tissue-specific functions, and to establish their roles in muscle and possibly nonmuscle cells, we isolated mutations that eliminate each gene individually and both genes in combination. We describe here the isolation and phenotypic characterization of such *mlc-1* and *mlc-2* deletion mutants.

MATERIALS AND METHODS

Strains and genetic analysis: Conditions for growth and maintenance of *C. elegans* are described by Brenner (1974). *sup-10*(*n184*) deletes *sup-10*, *mlc-1* (Greenwald and Horvitz 1980) and deletes ~70 kb of DNA between *sup-10* and *mlc-1* (C. White, J. M. Levin, D. Albertson, H. R. Horvitz and P. Anderson, unpublished results). *n184* does not affect *mlc-2* (see Figure 1). *sup-10*(*n245*) deletes most of the region between *mlc-1* and *sup-10* (Greenwald and Horvitz 1980) but does not affect either *mlc-1* or *mlc-2*. The "left" endpoint of *n245* is ~500 bp rightward of the 3′ end of the *mlc-1* transcribed region (see Figure 1). We used *n245* as a control to distinguish *n184* phenotypes caused by deletion of *mlc-1* from those caused by deletion of *sup-10* or genes between *mlc-1* and *sup-10*. Both *n184* and *n245*, which were initially isolated as suppressors of *unc-93*(*e1500*), were outcrossed and segregated as single mutants before analysis. To examine whether dosage compensation mutations suppress the hermaphrodite-specific lethality of *mlc2*(*r1133*), we crossed *r1133*/*0* males with *sdc3*(*y129*); *unc-3*(*e151*) hermaphrodites. *sdc3*(*y129*) *mlc2*(*r1133*) homozygotes were identified among the F2 progeny by their dumpy phenotype (*y129* homozygotes are Dpy), by their failure to segregate Unc-3 offspring (*unc-3* is linked to *mlc-2*), and by a PCR test diagnostic for *r1133* homozygotes. *sdc3*(*y129*) is a weak *sdc-3* allele that is semiviable when homozygous and exhibits a partial dosage compensation defect (DeLong *et al.* 1993).

Isolation and sequencing of *mlc* deletions: We established multiple independent populations of strain TR1690 [genotype *mut2*(*r459*); *dpy-19*(*n1347*); *rP2::Tc1*] and screened them by PCR for spontaneous deletions in the vicinity of *rP2::Tc1* (Zwaal *et al.* 1993). *mut2*(*r459*) activates excision of Tc1 (Collins *et al.* 1987), and *dpy19*(*n1347*) marks the presence of *mut2* (Finney 1987). *rP2::Tc1* is a previously described insertion of the transposable element Tc1 (Plasterk and van Luenen 1997) into noncoding sequences 174 bp downstream of *mlc-2* (Rushforth *et al.* 1993). Amplification primers used to identify deletions flanked *rP2::Tc1* and annealed outside of *mlc-1* and *mlc-2* coding regions. To amplify deletion-containing molecules, reactions were heated to 94° for 2 min and processed through 30 cycles of 94° for 0.5 min, 57° for 1 min, and 72° for 3 min, followed by a 10-min incubation at 72°. We subdivided populations in which deletion molecules were detected and isolated the *mlc-2* or *mlc-1,2* deletions using a sib-selection protocol, as previously described (Rushforth *et al.* 1993). We sequenced the novel *mlc* deletion junctions using standard protocols after PCR amplification and cloning of appropriate genomic fragments. *mlc2*(*r1133* and *r1142*) delete *mlc-2* coordinates 1069 through 2590 and 44 through 2589, respectively. *mlc-1,2*(*r1141*) deletes *mlc-1* nucleotide 1658 through *mlc-2* nucleotide 2588 [coordinates of Cummins and Anderson (1988)]. To reduce unrelated mutations, *mlc* deletions were outcrossed and resegregated eight times after their initial isolation. Lethal and semilethal *mlc* mutations were maintained as balanced heterozygotes using *mnDp1*(*XV*), which carries the right end of linkage group *X* [including *mlc-1*(+) and *mlc-2*(+)] attached to linkage group *V* (Herman *et al.* 1976).

RNA extractions and Northern analysis: We prepared RNA as previously described (Cummins and Anderson 1988). For northern transfers, 16 μg of total RNA was denatured with glyoxal and dimethylsulfoxide and electrophoresed through 1.2% agarose gels in 0.01 m NaH_2PO_4 (pH 7.0). Samples were transferred to Zeta-Probe blotting membrane (Bio-Rad Labo-

ratories, Richmond, CA) and hybridized with plasmids TR#115 and T7/T3-18-103 radiolabeled by primer extension of random hexanucleotides. Plasmid TR#115 contains a 5.8-kb genomic *Eco*RV-*Bgl* II fragment that includes all of *mlc-1* and *mlc-2* (Cummins and Anderson 1988). Plasmid T7/T3-18-103 (a gift of Mike Krause) contains a portion of the *act-1* gene (Krause *et al.* 1989) and was used as a normalization control.

Growth properties of *mlc* mutants: All experiments were performed at 20°, unless otherwise noted. Brood sizes were measured by transferring hermaphrodites daily and counting their offspring. To quantify *r1133* hermaphrodite survivorship, eggs collected during a 12- to 24-hr period were examined daily for a month, and those that did not develop beyond L1 were counted. To quantify *r1133* male survivorship relative to that of hermaphrodites, *r1133* or N2 males (10 to 15 per plate) were mated to groups of five *dpy-11*(*e224*); *mlc-2*(*1133*) hermaphrodites and transferred daily to fresh plates. Male and hermaphrodite offspring developing to at least L4 were counted over a 10-day period. Hatching times were measured by collecting eggs during a brief period and determining the length of time until they hatched as L1 larvae (Byerly *et al.* 1976). The length of hermaphrodite postembryonic development was measured as the time from hatching until the onset of egg laying.

Transformation rescue: Transgenic animals were generated by microinjecting plasmid DNAs into the distal gonadal syncytium of young adult N2 hermaphrodites (Mello and Fire 1995). Plasmid TR#233 contains a 3.7-kb *Eco*RV-*Apa*I genomic fragment that includes only *mlc-2*(+). Plasmid TR#234 contains a 4.5-kb *Sma*I-*Bgl* II genomic fragment that includes only *mlc-1*(+). These clones were coinjected with plasmid pRF4, which carries *rol6*(*su1006*) and provides a dominant marker indicating successful transformation (Kramer *et al.* 1990). Regulatory MLC and pRF4 plasmid DNAs were coinjected into N2 animals at concentrations of 10 and 100 μg/ml, respectively. Multiple transgenic lines carrying each rMLC plasmid were established. In all cases, the transgenes behaved as extrachromosomal arrays. Transgenic males were crossed to *mlc-2*(*r1133*); *mnDp1*(*XV*)/+(*V*) or *mlc-1,2*(*r1141*); *mnDp1*(*XV*)/+(*V*) hermaphrodites, and roller male offspring were crossed again to the same hermaphrodite parent. Homozygous *mlc-2*(*r1133*) or *mlc-1,2*(*r1141*) transgenic lines were established from the progeny of these crosses. For each transgenic line, PCR was used to confirm the presence of the *mlc* deletion, the *mlc* plasmid DNA, and the absence of *mnDp1*.

***In situ* hybridization:** *In situ* hybridization to detect *mlc-1* and *mlc-2* mRNAs was performed as previously described (Albertson *et al.* 1995). Digoxygenin-dUTP-labeled *mlc-1* and *mlc-2* antisense hybridization probes were prepared by asymmetric PCR (Seydoux and Fire 1995). To generate transcript-specific probes, we selected amplification primers from the *mlc-1* and *mlc-2* unique 3′-untranslated regions. For the *mlc-1* probe, we used primer MLC1-2498 (5′TGCACCACAAGTA ACTGCTC3′) to PCR amplify a ~500-nt fragment using plasmid TR#234 cut with *Bsa*HI as a template. For the *mlc-2* probe, we used primer MLC2-2398 (5′GCACTAATCCATTGAAA GAT3′) to PCR amplify an 85-nt fragment using plasmid TR#233 cut with *Hpa*II as a template. PCR reactions (25 μl) included 25 pmol of primer, 0.4 μg of linearized plasmid DNA, and 5.0 μl of DIG DNA labeling mix (Boehringer Mannheim, Indianapolis) containing 1 mm dATP, dCTP, and dGTP; 0.65 mm dTTP; and 0.3 mm digoxygenin-dUTP in a standard PCR-reaction buffer. Samples were heated to 100° for 5 min, 1.25 units of Taq polymerase was added, and samples were processed through 35 cycles of 94° for 0.5 min, 55° (*mlc-1* probe) or 50° (*mlc-2* probe) for 1 min, and 72° for 1 min, and completed by a 10-min incubation at 72°. Samples were precipitated and washed with ethanol, and resuspended in 300 μl of hybridization solution.

RESULTS

***sup-10*(*n184*) deletes *mlc-1*:** *mlc-1* and *mlc-2* are located immediately adjacent to each other and are transcribed divergently, with their 5′ ends separated by 2.6 kb (see Figure 1) (Cummins and Anderson 1988). We mapped *mlc-1* and *mlc-2* to the right end of the *X* chromosome by *in situ* hybridization to embryonic metaphase chromosomes and by Southern blot analysis of a series of *X* chromosome deficiencies and duplications (C. White, J. M. Levin, D. Albertson, H. R. Horvitz and P. Anderson, unpublished results). We identified a deletion of *mlc-1* as part of our molecular analysis of this chromosomal region. The muscle-affecting gene *sup-10* is located ~70 kb rightward of *mlc-1* (see Figure 1). Among 46 spontaneous and mutagen-induced *sup-10* mutations that we analyzed, one of them, *sup-10*(*n184*), proved to be a deletion that removes both *sup-10* and *mlc-1* (see Figure 1). The left endpoint of *sup-10*(*n184*) breaks within the *mlc-1* second exon, and most of the MLC-1 coding sequences are deleted by *n184*. As discussed below, *sup-10*(*n184*) exhibits only minor growth and reproductive abnormalities. As a control for these

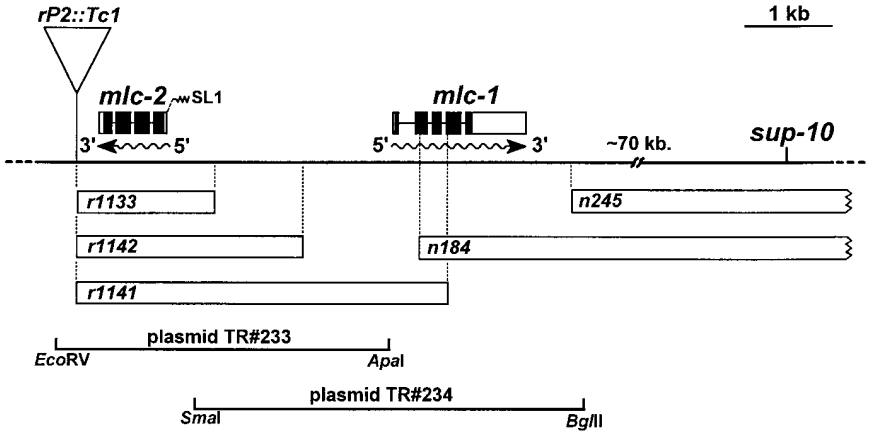

Figure 1.—Map of the *mlc-1* and *mlc-2* genomic region. Boxes represent *mlc-1* and *mlc-2* exons, with unfilled regions indicating 5′- and 3′-untranslated regions. Arrows indicate the direction of *mlc-1* and *mlc-2* transcription. Like many *C. elegans* mRNAs (Blumenthal and Steward 1997), the 5′ end of *mlc-2* mRNA is *trans*-spliced to SL1. *rP2::Tc1* is an insertion of the transposable element Tc1 near *mlc-2* (Rushforth *et al.* 1993). The endpoints of deletions *sup-10*(*n184*), *sup-10*(*n245*), *mlc-2*(*r1133*), *mlc-2*(*r1142*), and *mlc-1,2*(*r1141*) are shown. Plasmids TR#234, which contains only *mlc-1*(+), and TR#233, which contains only *mlc-2*(+), were used for transformation rescue experiments.

phenotypes, we analyzed *sup-10*(*n245*) (Greenwald and Horvitz 1980). *sup-10*(*n245*) deletes most of the region between *mlc-1* and *sup-10*, but it does not affect either *mlc-1* or *mlc-2* (see Figure 1). The left endpoint of *n245* is ~500 bp rightward of the *mlc-1* transcribed region. *sup-10*(*n245*) provides a control to distinguish *n184* phenotypes caused by deletion of *mlc-1* from those caused by deletion of *sup-10* or other genes located between *mlc-1* and *sup-10*. The right endpoints of both *n184* and *n245* are located an unknown distance rightward of *sup-10*.

Isolation of *mlc2* and *mlc-1,2* deletions: Since none of the characterized *sup-10* mutations deleted both *mlc-1* and *mlc-2*, and because we wanted to identify unambiguous *mlc-2* null alleles, we isolated several *mlc-2* and *mlc-1 mlc-2* deletions by site-selected deletion of *rP2::Tc1*. *rP2::Tc1* (*P* is polymorphism) is a Tc1 insertion 175 bp downstream of the *mlc-2* transcribed region (Rushforth *et al.* 1993). It was isolated as one of several Tc1 insertions within or near *mlc-2*, all of which proved to be non-null alleles of *mlc-2* (see discussion). We adapted a site-selected deletion protocol (Zwaal *et al.* 1993) in combination with sib-selection to identify deletions emanating from *rP2::Tc1*. We isolated three *mlc* deletions using this procedure (see Figure 1). *mlc-2*(*r1133*) and *mlc-2*(*r1142*) delete only *mlc-2*, whereas *mlc-1,2*(*r1141*) deletes both *mlc-1* and *mlc-2*. To determine the precise deletion endpoints, we sequenced the novel junction of each mutation. *mlc-2*(*r1133*) and *mlc-2*(*r1142*) delete 1523 and 2546 bp, respectively (see materials and methods). The deleted material includes all of *mlc-2* and either 555 bp (*r1133*) or 1580 bp (*r1142*) of the intergenic region between *mlc-2* and *mlc-1*. *mlc-1,2*(*r1141*) deletes 4245 bp that remove all of *mlc-2* and the 5′ half of *mlc-1* (see Figure 1). Because *mlc-1,2*(*r1141*) is lethal when homozygous (see below), it was initially isolated and subsequently maintained as a heterozygote.

Northern analysis of *mlc-1* and *mlc-2* deletions: Because *mlc-1* and *mlc-2* are transcribed divergently and because their 5′ ends are separated by only 2.6 kb, regulatory elements important for their expression likely reside within the intergenic region. To establish that *r1133*, which deletes a portion of the intergenic region, does not affect expression of *mlc-1*(+) and that *n184* does not affect expression of *mlc-2*(+), we measured by Northern blots the abundance of *mlc* mRNAs in *mlc-2*(*r1133*) and *sup-10*(*n184*) (see Figure 2). As expected, *mlc-1* mRNA is absent in *sup-10*(*n184*), whereas the size and abundance of *mlc-2* mRNA is normal (lane 3). Similarly, *mlc-2* mRNA is absent in *mlc-2*(*r1133*), whereas the size and abundance of *mlc-1* mRNA is normal (lane 2). *sup-10*(*n245*) affects neither *mlc-1* nor *mlc-2* mRNAs (lane 4). We conclude that *sup-10*(*n184*) and *mlc-2*(*r1133*) are true null alleles of their respective genes.

Phenotype of *mlc-1*(*0*) mutants: *sup-10*(*n184*) homozygotes [abbreviated below as *mlc-1*(*0*), where 0 indicates a null allele] are essentially wild type in phenotype. They exhibit normal motility (both larval and adult), egg laying, and pharyngeal pumping, which are phenotypes indicative of normal body-wall, vulval, and pharyngeal muscle function. As judged by polarized light microscopy, *mlc-1*(*0*) body-wall muscle ultrastructure is normal (see Figure 3). *mlc-1*(*0*) males exhibit abnormal mating behavior and only rarely succeed in cross-fertilizing hermaphrodites. However, the control deletion *sup-10*(*n245*), which is *mlc-1*(+) *mlc-2*(+) (see above), is similarly affected. Thus, the male mating defect of *n184* is not due to deletion of *mlc-1*. Most aspects of *mlc-1*(*0*) growth, development, and reproduction are normal (see Table 1). The brood size of *mlc-1*(*0*) is about two-thirds that of N2, but *sup-10*(*n245*) is similarly affected. A small, but probably insignificant, proportion of *mlc-1*(*0*) offspring arrest development as either embryos or young larvae. The lengths of embryonic and postembryonic development of *mlc-1*(*0*) mutants are not significantly different from wild type at either 20° or 25°. Numerous indicators of embryonic and postembryonic nonmuscle actomyosin function, such as the multiple cell migrations required for normal gonadal morphogenesis, egg laying, and muscle cell positioning (Antebi *et al.* 1997), are normal in *mlc-1*(*0*) mutants.

Phenotype of *mlc-2*(*0*) mutants: Although *mlc-1*(*0*)

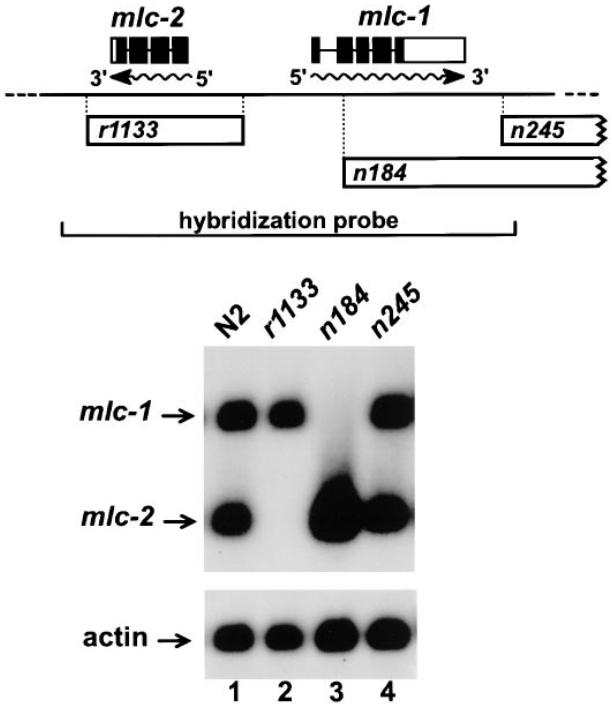

Figure 2.—Northern analysis wild-type (N2) and *mlc* deletions. The *mlc-1 mlc-2* genomic region is diagrammed above an autoradiogram of a Northern blot hybridized with probes that detect *mlc-1*, *mlc-2*, and actin (control) mRNAs. Solid boxes of *mlc-1* and *mlc-2* correspond to protein-coding regions; open boxes correspond to 5′ and 3′ untranslated regions. The arrows indicate the direction of transcription.

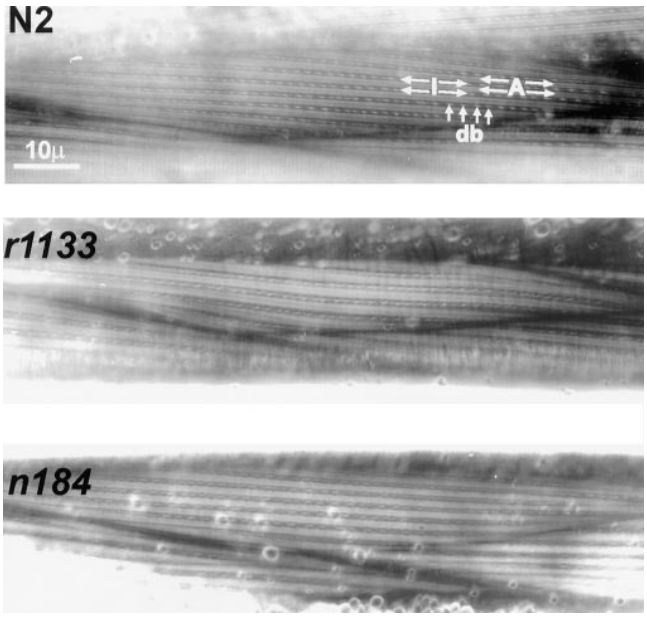

Figure 3.—Wild-type (N2), *mlc-2*(*r1133*), and *sup-10*(*n184*) body-wall muscle. Each photograph is a polarized light micrograph of a single, adult, body-wall muscle cell. Representative A bands (A), I bands (I), and dense bodies (db) of wild type are shown.

mutants are essentially wild type, deletion of *mlc-2* is semilethal. *mlc-2*(*r1133*) homozygotes exhibit an Eat (eating abnormal) phenotype, and about 90% of *r1133* hermaphrodites die as L1 or L2 larvae. Newly hatched *r1133* larvae are fully elongated and morphologically normal (see Figure 4). They exhibit vigorous motility, indicative of near-normal body-wall muscle function, with occasional animals appearing slightly sluggish. Pharyngeal pumping, however, is irregular and feeble. The morphology and polarized light phenotype of *r1133* pharynxes are normal, but *r1133* larvae pump less frequently, their pumps are generally longer than normal, and the pharyngeal lumen frequently fails to open completely. Pumping in *r1133* larvae is, however, somewhat variable. Feeble pumps are often interspersed with brief strong pumps, and pharyngeal pumping is nearly normal in a small number of animals. Arrested *r1133* L1 larvae can remain alive and motile without significant growth for up to 4 wk. These animals gradually deteriorate, become less motile, and eventually die in a starved, sickly state. We conclude that *mlc-2* performs an important and nearly essential function in pharyngeal muscle.

Embryonic development of *mlc-2*(*r1133*) is approximately normal. A small but probably insignificant percentage (4.8%) of *r1133* zygotes arrest as dead embryos. We did not investigate the terminal phenotypes of the few *r1133* embryonic lethals. For those that hatch, the length of embryonic development is indistinguishable from wild type (see Table 1). Embryonic twitching of *r1133* body-wall muscle is normal. *r1133* homozygotes segregating from *r1133*/+ (heterozygous) or *r1133*/*r1133* (homozygous) mothers are indistinguishable. Thus, maternally inherited MLC-2, if such exists, does not contribute significantly to the phenotype of *mlc-2*(*0*) mutants.

Remarkably, about 10% of *mlc-2*(*r1133*) homozygotes grow to be nearly normal adults (see Table 1). Such escapers exhibit normal motility and egg laying. Pharyngeal pumping in *r1133* adults may be less frequent than that of wild type but only slightly so. As judged by polarized light microscopy, *r1133* adult body-wall muscle ultrastructure is normal (see Figure 3), as is pharyngeal muscle, vulval muscle, and diagonal muscles of the male tail (data not shown). Although the rare *r1133* adults are nearly normal, certain parameters of their growth and reproduction are abnormal (see Table 1). The brood size of *r1133* adults is about two-thirds that of wild type. Postembryonic development of *r1133* is pro-

TABLE 1

Growth, development, and reproduction of *mlc-1(0)* and *mlc-2(0)* mutants

Genotype	Brood size	Hatching time[a] (hr)	Dead eggs[b] (%)	Larval arrest (%)	Length of postembryonic development (hr)
N2	347 ± 30 (n = 4)	12.6 ± 0.8 (n = 94)	0.8[c]	ND	73 ± 1[c]
mlc-2(*r1133*) [*mlc-1*(+) *mlc-2*(*0*)]	193 ± 28 (n = 7)	13.7 ± 1.4 (n = 112)	4.8 (181/3763)	88.6 (1148/1296)	197 ± 109 (n = 57)
sup-10(*n184*) [*mlc-1*(*0*) *mlc-2*(+)]	212 ± 37 (n = 6)	12.2 ± 1.4 (n = 132)	1.5 (38/2480)	2.2 (18/836)	78 ± 9 (n = 123)
sup-10(*n245*) [*mlc-1*(+) *mlc-2*(+)]	201 ± 23 (n = 11)	ND	ND	ND	70 ± 6 (n = 310)

All measurements are averages ± standard deviations. ND, not determined.

[a] Hatching time, the length of time from when an egg is laid until it hatches as an L1 larva, represents the majority of embryonic development.

[b] Dead eggs are zygotes that arrest development as unhatched embryos.

[c] Taken from Hodgkin *et al.* (1979) and Rushforth *et al.* (1993).

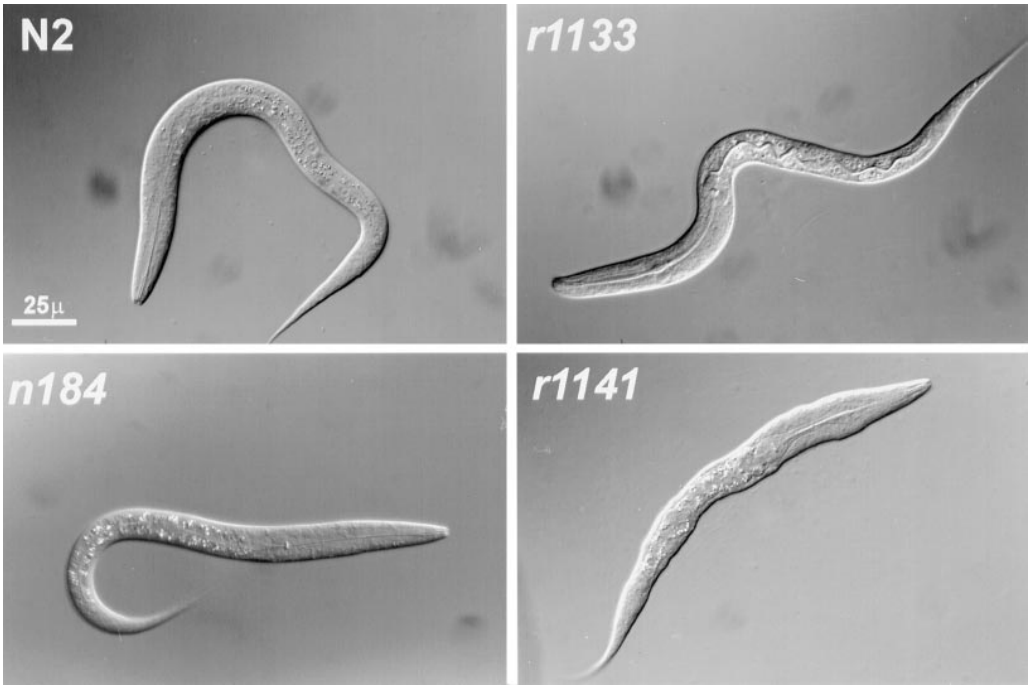

Figure 4. — Nomarski photomicrographs of wild-type (N2), *mlc-2*(*r1133*), *sup-10*(*n184*), and *mlc-1,2* (*r1141*) L1 larvae.

tracted, requiring an average of 8 days from hatching to sexual maturity. The length of postembryonic development of *r1133* is highly variable, with some individuals requiring 3 wk or more to reach sexual maturity. For most of this time, *r1133* animals appear as thin, pale, and somewhat starved L1 larvae. Once animals grow to a size comparable to L2 or L3 larvae of wild type, however, they usually develop into healthy-looking adults within a day or two. As described above for *mlc-1*(*0*) mutants, several indicators of embryonic and postembryonic nonmuscle actomyosin function are apparently normal in *r1133* escapers.

The semilethal phenotype of *mlc-2*(*r1133*) is sex specific. Whereas only 11% of *r1133* hermaphrodites grow to adulthood, about 90% of *r1133* males develop into normal adults. The high survivorship of *r1133* males was evident as an unexpected sex ratio of adult crossprogeny when we crossed *r1133* males with *dpy-11*(*e224*); *mlc-2*(*r1133*) hermaphrodites. Ninety percent of cross-progeny adults (279/310, recognized by their non-Dpy phenotype) were male. This deviation from an expected 1:1 sex ratio is due to frequent L1 arrest of cross-progeny hermaphrodites relative to cross-progeny males. When N2 control males were mated with *dpy-11*(*e224*); *mlc-2*(*r1133*) hermaphrodites, 41% (177/432) of cross-progeny adults were males, reflecting a high survival (but <100% survival) of *r1133* males. *r1133* males mate normally and cannot be distinguished from wild type.

The phenotype of *mlc-2*(*r1133*) is more severe at 25°. Indeed, *r1133* is effectively lethal at 25°. Over two-thirds (21/30) of *r1133* raised at 25° were sterile as adults. The remaining animals had few (<10) progeny each. *r1133* adults raised at 25° have abnormal gonads and are sickly and lethargic compared to N2 raised at 25° or *r1133* raised at 20°. Stocks of *r1133* homozygotes cannot be propagated at 25°.

Dosage compensation contributes to hermaphrodite-specific lethality of *mlc-2(0)* mutants: We investigated whether the hermaphrodite-specific lethality of *mlc-2*(*r1133*) results from sex-specific differences in *mlc1*(+) expression. One possible source of sex-specific differences in *mlc-1* expression is dosage compensation. Dosage compensation reduces the rate at which individual *X*-linked genes are transcribed in hermaphrodites relative to males, thereby compensating for the fact that hermaphrodites contain two *X* chromosomes whereas males contain only one (Meyer 1997). We reasoned that perhaps dosage compensation reduces *mlc-1*(+) expression in hermaphrodites below the level normally found in males. In wild-type animals, which are *mlc-1*(+) *mlc-2*(+), sex-specific differences in *mlc-1*(+) expression may be of little consequence. In *mlc-2*(*0*) mutants, however, perhaps *mlc-1*(+) expression in the hermaphrodite pharynx is insufficient, resulting in the Eat phenotype that we observe.

To test this, we constructed a *mlc-2*(*0*); *sdc-3* double mutant. Although *sdc-3* is an essential gene, the weak (and therefore viable) allele *sdc-3*(*y129*) exhibits a partial defect in dosage compensation and increased transcription of *X*-linked genes in hermaphrodites (DeLong *et al.* 1993). Whereas *mlc-2*(*r1133*) cannot be propagated at 25°, the *sdc-3*(*y129*); *mlc-2*(*r1133*) double mutant can be maintained as a self-fertile stock at 25°. Although *mlc-2*(*r1133*); *sdc-3*(*y129*) grows slowly at 25°, its growth is not substantially different than that of *sdc-3*(*y129*) single mutants. Thus, *sdc-3*(*y129*) suppresses the hermaphro-

dite-specific lethality of *mlc-2*(*r1133*). This result does not establish that *sdc-3* suppression of *mlc-2*(*0*) hermaphrodite lethality is due to elevated *mlc-1*(+) expression, but data presented below demonstrate that elevated expression of *mlc-1*(+) is sufficient to suppress *r1133* lethality.

Phenotype of *mlc-1,2*(*0*) mutants: *mlc-1,2*(*r1141*), which deletes both *mlc-1* and *mlc-2*, is unconditionally lethal, exhibiting defects of both pharyngeal and body-wall muscle. *r1141* homozygotes arrest as nearly paralyzed L1 larvae. Such animals are severely Unc, moving poorly or not at all. Motility of the anterior of *r1141* larvae is slightly better than that of the posterior. Pharyngeal pumping of *r1141* is irregular and feeble, similar to that described above for *mlc-2*(*r1131*). Arrested larvae persist without significant growth for as long as 4 wk. Embryonic development of *r1141* is slightly protracted (15.9 hr ± 2.3, $n = 9$ compared to 13.2 hr ± 0.6, $n = 19$ for *r1141*/+ and +/+ scored together), but a high proportion of *r1141* homozygotes hatch. Embryonic twitching of *r1141* commences on schedule at about 1.5-fold elongation but is somewhat less vigorous than that of wild type. The motility defects of *r1141* embryos become progressively more severe, with late embryos, which normally roll vigorously within the egg, being nearly paralyzed. *r1141* homozygotes exhibit mild elongation defects. Hatched L1 larvae are not fully elongated and have morphological irregularities along their body length ("lumpy dumpy" phenotype; see Figure 4). The elongation defects of *r1141*, however, are relatively mild compared to the twofold arrest of severely muscle-defective mutants (Williams and Waterston 1994; Moerman and Fire 1997). *r1141* is fully recessive, and homozygotes exhibit approximately the same phenotype at both 20° and 25°. *r1141*-arrested larvae are slightly larger at 25° compared to 20°.

From the above results, we conclude that: (1) *mlc-1* and *mlc-2* perform redundant functions in body-wall muscles, (2) functions of *mlc-1* are redundant to those of *mlc-2* in pharyngeal muscle, but *mlc-2* performs an important and nearly essential role in the pharynx, and (3) *mlc-1* and *mlc-2* likely perform redundant functions in vulval muscles. *mlc-1*(*0*) and *mlc-2*(*0*) single mutants do not exhibit egg-laying defects, and our expectation is that a *mlc-1,2*(*0*) deletion would be egg-laying defective. This cannot be directly demonstrated, however, due to the larval lethality of the *mlc-1,2*(*r1141*).

Transformation rescue of *mlc-2*(*0*) and *mlc-1,2*(*0*) mutants: To confirm that the phenotypes described above are due to rMLC defects, and to investigate rMLC rescue of the *mlc-2*(*0*) and *mlc-1,2*(*0*) mutant phenotypes, we transformed genomic copies of either *mlc-1*(+) or *mlc-2*(+) (plasmids TR#233 and TR#234; see Figure 1) into N2 and established extrachromosomal arrays. Genomic *mlc-1*(+) or *mlc-2(+)* clones were coinjected with a plasmid that marks extrachromosomal arrays with a dominant allele of *rol-6* (Kramer *et al.* 1990). Extrachromosomal arrays were then crossed into *mlc-2*(*r1133*) and *mlc-1,2*(*r1141*) using the roller marker to identify array-containing cross-progeny, which were then scored for their *mlc* phenotypes. Both *mlc-1*(+) and *mlc-2*(+) individually rescued all phenotypes of both *mlc-2*(*0*) and *mlc-1,2*(*0*) mutants. Array-containing transformants have large brood sizes, high rates of hatching, and apparently normal postembryonic development. Motility, pharyngeal pumping, and egg laying of transgenic adults is normal at both 20° and 25°, as is the ultrastructure of body-wall muscle when viewed by polarized light microscopy (data not shown). Because of the high copy number of typical extrachromosomal arrays (Stinchcomb *et al.* 1985), levels of expression of transgenic *mlc-1*(+) and *mlc-2*(+) are likely elevated relative to that of the endogenous genes. We conclude that either MLC-1 or MLC-2 is sufficient for all aspects of *mlc-1* and *mlc-2* function. The striking difference between the *mlc-1*(*0*) and *mlc-2*(*0*) mutant phenotypes, therefore, likely reflects differing levels of MLC-1 *vs.* MLC-2 expression in the hermaphrodite pharynx.

***In situ* localization of *mlc-1* and *mlc-2* mRNAs:** The results described above predict that both *mlc-1* and *mlc-2* are expressed in body-wall, pharyngeal, and possibly vulval muscles. To confirm this localization, we performed *in situ* hybridization to *mlc-1* and *mlc-2* mRNAs using gene-specific antisense probes. We prepared single-stranded probes from the unique 3′ untranslated region of each gene. Hybridization of the *mlc-2* probe to *mlc-2*(*r1133*) and the *mlc-1* probe to *sup-10*(*n184*) yielded no signal (Figure 5A and 5D, respectively), confirming that these probes are mRNA specific. Hybridization of *mlc* probes to wild type demonstrated that both *mlc-1* and *mlc-2* are expressed in body-wall muscles (Figure 5B and 5E), pharyngeal muscles (Figure 5C and 5F), and vulval muscles (Figure 5B and 5G). Both *mlc-1* and *mlc-2* mRNAs are present in all regions of the pharynx (procorpus, metacorpus, isthmus, and terminal bulb). We did not detect *mlc-1* or *mlc-2* mRNA in several of the minor muscle groups, such as the intestinal, uterine, anal depressor, and sphincter muscles, but these tissues were not well-preserved by our fixation methods. Similarly, we did not detect *mlc* mRNAs in nonmuscle cells, although such expression might be below detectable levels.

DISCUSSION

mlc-1 and *mlc-2* encode nearly identical regulatory myosin light chains (Cummins and Anderson 1988). To investigate the *in vivo* functions of these rMLCs, we identified or isolated deletions that remove *mlc-1* and *mlc-2* individually and both genes in combination. A deletion that removes both *mlc-1* and *mlc-2* is lethal when homozygous and exhibits both body-wall and pharyngeal muscle defects. We conclude that rMLC function is required in both of these muscle types for normal

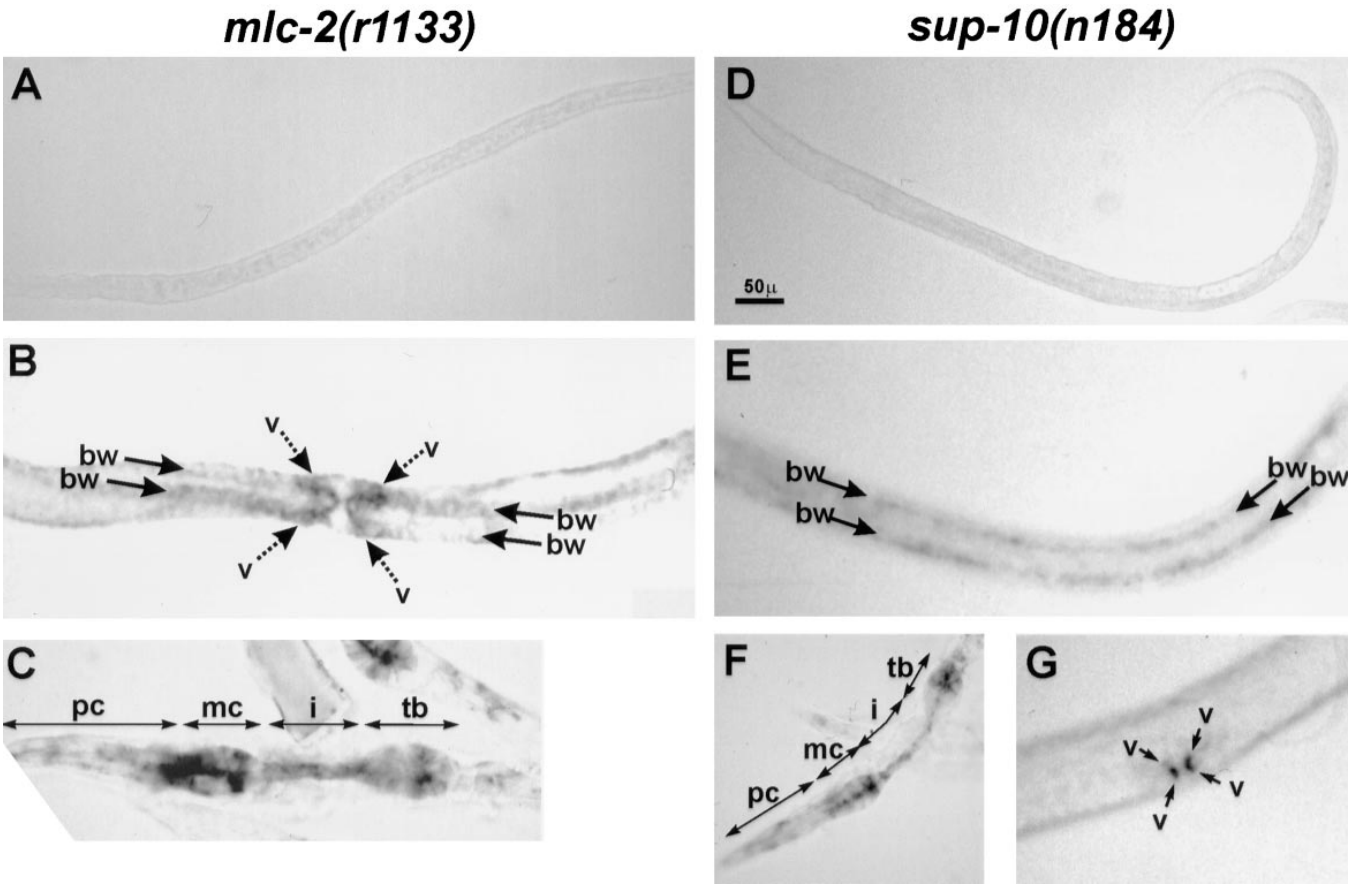

Figure 5.—Localization of *mlc-1* and *mlc-2* mRNAs in *mlc-1*(*0*) and *mlc-2*(*0*) mutants. *mlc-2*(*r1133*) (A–C) and *sup-10*(*n184*) (D–G) were hybridized with antisense *mlc-1*-specific probes (B, C, and D) or *mlc-2*-specific probes (A, E, F, and G). Body-wall muscles (bw) are indicated with solid arrows in B and E. The procorpus (pc), metacorpus (mc), isthmus (i), and terminal bulb (tb) regions of the pharynx are labeled in C and F. Vulval muscles (v) are indicated with dotted arrows in B and with solid arrows in G.

contraction. Three observations demonstrate that *mlc-1* and *mlc-2* are functionally redundant in body-wall muscle. First, *in situ* hybridization shows that both *mlc-1* and *mlc-2* are expressed in body-wall muscle. Second, both *mlc-1*(*0*) and *mlc-2*(*0*) single mutants have normal body-wall muscle. The unaffected *mlc* gene in each mutant is sufficient for normal rMLC function. Third, transgenic copies of either *mlc-1*(+) or *mlc-2*(+) rescue the body-wall muscle defects of a deletion that removes both *mlc-1* and *mlc-2*.

mlc-2 performs an important and nearly essential role in the pharynx. *mlc-2*(*0*) mutants have an Eat (eating abnormal) phenotype. Pharyngeal morphology and muscle birefringence of *mlc-2*(*0*) mutants is normal, but pharyngeal contractions are weak and attenuated. Motility of *mlc-2*(*0*) larvae is normal, but arrested larvae do not increase in size and eventually die of apparent starvation after several weeks. The phenotype of *mlc-2*(*0*) mutants is much more severe than that of our previously isolated *mlc2::Tc1* insertions (Rushforth *et al.* 1993). Despite having Tc1 insertions within *mlc-2* exons, these previously isolated mutations are non-null alleles. They express 10–40% of the wild-type quantity of *mlc-2* mRNA, with Tc1 having been removed by splicing (Rushforth *et al.* 1993). Such splicing leaves small insertions and deletions within mature *mlc-2* mRNAs, but their weak phenotype indicates that the altered MLC-2 proteins are functional.

Remarkably, about 10% of *mlc-2*(*0*) homozygotes grow into nearly normal adults. Such escapers grow slowly and appear starved as larvae, but adults are essentially indistinguishable from wild type. Other eating abnormal mutants also have phenotypes more severe in larvae than in adults (Avery 1993). Presumably, ingesting, concentrating, and grinding food are more difficult for young larvae, whose pharynxes are smaller than those of adults. As the pharynx grows larger during larval development, *mlc-2*(*0*) mutants likely eat more effectively. The critical differences that cause 10% of *mlc-2*(*0*) mutants to survive are unknown. Perhaps escapers are simply larvae that express slightly more *mlc-1*(+) than their siblings.

Although function of *mlc-2* is required in the pharynx, two observations suggest that *mlc-1* and *mlc-2* are partially

redundant in the pharynx and that *mlc-2*(*0*) larvae arrest development due only to an insufficient quantity of MLC-1. First, *in situ* hybridization demonstrates that both *mlc-1* and *mlc-2* mRNAs are expressed in the pharynx. Second, expression of either MLC-1 or MLC-2 from transgenes is sufficient to rescue the pharyngeal defects of *mlc-2*(*0*) mutants. As judged by transformation rescue of *mlc-2*(*0*) and *mlc-1,2*(*0*) mutants, we detect no functional differences between *mlc-1*(+) and *mlc-2*(+). This result suggests that the single, conservative, amino acid substitution that distinguishes MLC-1 and MLC-2 is not significant with regard to MLC function. If it were significant, and if MLC2 performed a unique qualitative function, *mlc-2*(*0*) mutants should not be rescued by elevated expression of *mlc-1*(+). We believe, therefore, that the *mlc-2*(*0*) pharyngeal defects result from lowering, but not eliminating, expression of rMLCs in the pharynx. Similar quantitative effects have been observed in Drosophila, where null alleles of *MLC-2*, a muscle rMLC, exhibit a dominant flightless phenotype due to abnormalities of indirect flight muscles in heterozygotes (Warmke *et al.* 1992). Perhaps the *C. elegans* pharynx is similarly sensitive to modest perturbations of rMLC levels.

The hermaphrodite-specific lethality of *mlc-2*(*0*) mutants may result from quantitative differences in *mlc-1*(+) expression in males *vs.* hermaphrodites. Whereas only about 10% of *mlc-2*(*0*) hermaphrodites survive to adulthood, nearly all *mlc-2*(*0*) males develop as normal adults. Elevated *mlc-1*(+) expression, such as likely occurs in the presence of *mlc-1*(+) transgenes or in a *sdc-3*(*y129*) genetic background, suppresses the hermaphrodite-specific lethality of *mlc-2*(*0*) mutants. Dosage compensation in *C. elegans* reduces the per-gene rate of *X*-linked transcription in hermaphrodites relative to males, such that the overall level of expression is approximately equal in the two sexes (Meyer 1997). Dosage compensation, however, may not be precise for every locus, and expression of certain *X*-linked genes may be different in hermaphrodites and males. *mlc-1* may be one such gene. Pharyngeal expression of *mlc-1*(+) may be lower in hermaphrodites than in males. In wild-type animals, which are *mlc-1*(+) *mlc-2*(+), such sex-specific differences in gene expression would be of little consequence. In *mlc-2*(*0*) mutants, however, *mlc-1*(+) levels in the hermaphrodite pharynx may be insufficient. *sdc-3*(*y129*) is a weak (and therefore viable) allele that partially disrupts dosage compensation and increases expression of *X*-linked genes in hermaphrodites (DeLong *et al.* 1993). *sdc-3*(*y129*) suppresses the hermaphrodite-specific lethality of *mlc-2*(*0*) mutants at 25°. We presume that elevated *mlc-1*(+) expression is the basis of this suppression, but elevated expression of other *X*-linked genes might contribute to the suppression phenotype.

We find no compelling evidence that *mlc-1* or *mlc-2* functions in nonmuscle cells. Indeed, all *mlc* deletion phenotypes can be explained by their muscle defects. Actomyosins are known to be important for *C. elegans* embryogenesis (Strome and Wood 1983; Priess and Hirsh 1986; Hill and Strome 1988; Hird and White 1993), but none of our mutants exhibit significant embryonic defects. A high proportion of *mlc-2*(*0*) and *mlc-1*(*0*) *mlc-2*(*0*) zygotes hatch as viable, albeit arrested, L1 larvae. The larval arrest of these mutants appears to be due exclusively to their pharyngeal defects. Although *mlc-1*(*0*) *mlc-2*(*0*) L1 larvae are incompletely elongated, this may be a secondary consequence of their body-wall muscle defects. Embryonic elongation occurs by the coordinated contraction of circumferential bundles of actomyosin filaments in hypodermal cells (Priess and Hirsh 1986). Such contractions squeeze the ovoid embryo into a long vermiform shape. Although the hypodermis is responsible for elongation, interactions between the hypodermis and the developing body-wall muscle (which underlies the hypodermis) are important for this process (Moerman and Fire 1997). Indeed, severe defects of body-wall muscle cause embryonic elongation to arrest at the twofold stage (Waterston 1989; Barstead and Waterston 1991; Chen *et al.* 1994; Williams and Waterston 1994). The modest elongation defects of *mlc-1*(*0*) *mlc-2*(*0*) homozygotes, which are not as severe as those of the twofold arrest muscle mutants, may well be a secondary consequence of their muscle defects.

Numerous indicators of embryonic and postembryonic nonmuscle actomyosin function, such as the multiple cell migrations required for normal gonadal morphogenesis, egg laying, and muscle cell positioning (Antebi *et al.* 1997), are normal in *mlc-1*(*0*) mutants and in *mlc-2*(*0*) escapers. Unfortunately, such phenotypes cannot be scored in *mlc-1*(*0*) *mlc-2*(*0*) mutants due to their larval lethality. Thus, a role for MLC-1 and MLC-2 in postembryonic nonmuscle cells cannot be excluded. We note, however, that the *C. elegans* genome sequencing effort (Hodgkin *et al.* 1995) identifies a third probable rMLC (Swiss-Prot accession no. Q09510) with very high amino acid identity (73%) to Drosophila *spaghetti-squash*, a nonmuscle rMLC gene (Karess *et al.* 1991). This probable rMLC is 46% identical to *mlc-1* or *mlc-2* and is, therefore, an excellent candidate for performing nonmuscle rMLC functions in *C. elegans*. Genetic analysis of this locus will be important for understanding the *in vivo* functions of regulatory myosin light chains in both muscle and nonmuscle cells.

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LITERATURE CITED

Albertson, D. G., R. M. Fishpool and P. S. Birchall, 1995 Fluorescence *in situ* hybridization for the detection of DNA and RNA, pp. 339–364 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Antebi, A., C. R. Norris, E. M. Hedgecock and G. Garriga, 1997 Cell and growth cone migrations, pp. 583–609 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Avery, L., 1993 The genetics of feeding in *Caenorhabditis elegans*. Genetics **133:** 897–917.

Barstead, R. J., and R. H. Waterston, 1991 Vinculin is essential for muscle function in the nematode. J. Cell Biol. **114:** 715–724.

Blumenthal, T., and K. Steward, 1997 RNA processing and gene structure, pp. 117–145 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Byerly, L., R. Cassada and R. Russell, 1976 The life cycle of the nematode *C. elegans* I. Wild type growth and reproduction. Dev. Biol. **51:** 23–33.

Chen, L., M. Krause, M. Sepanski and A. Fire, 1994 The *Caenorhabditis elegans* MYOD homologue HLH-1 is essential for proper muscle function and complete morphogenesis. Development **120:** 1631–1641.

Chen, P., B. D. Ostrow, S. R. Tafuri and R. L. Chisholm, 1994 Targeted disruption of the *Dictyostelium* RMLC gene produces cells defective in cytokinesis and development. J. Cell Biol. **127:** 1933–1944.

Collins, J., B. Saari and P. Anderson, 1987 Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. Nature **328:** 726–728.

Craig, R., R. Smith and J. Kendrick-Jones, 1983 Light-chain phosphorylation controls the conformation of vertebrate non-muscle and smooth muscle myosin molecules. Nature **302:** 436–439.

Cummins, C., and P. Anderson, 1988 Regulatory myosin light chain genes of *Caenorhabditis elegans*. Mol. Cell. Biol. **8:** 5339–5349.

DeLong, L., J. D. Plenefisch, R. D. Klein and B. J. Meyer, 1993 Feedback control of sex determination by dosage compensation revealed through *Caenorhabditis elegans sdc-3* mutations. Genetics **133:** 875–896.

Finney, M., 1987 The genetics and molecular biology of *unc-86*, a *C. elegans* cell lineage gene. Ph.D. Thesis, Massachusetts Institute of Technology, Boston.

Gallagher, P. J., B. P. Herring and J. T. Stull, 1997 Myosin light chain kinases. J. Muscle Res. Cell Motil. **18:** 1–16.

Greenwald, I., and H. Horvitz, 1980 *unc-93*(*e1500*): a behavior mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

Gulick, A. M., and I. Rayment, 1997 Structural studies on myosin II: communication between distant protein domains. Bioessays **19:** 561–569.

Harris, H., and H. Epstein, 1977 Myosin and paramyosin of *Caenorhabditis elegans*: biochemical and structural properties of wild-type and mutant proteins. Cell **10:** 709–719.

Herman, R. K., D. G. Albertson and S. Brenner, 1976 Chromosome rearrangements in *Caenorhabditis elegans*. Genetics **83:** 91–105.

Hill, D. P., and S. Strome, 1988 An analysis of the role of microfilaments in the establishment and maintenance of asymmetry in *Caenorhabditis elegans* zygotes. Dev. Biol. **125:** 75–84.

Hird, S. N., and J. G. White, 1993 Cortical and cytoplasmic flow polarity in early embryonic cells of *Caenorhabditis elegans*. J. Cell Biol. **121:** 1343–1355.

Hodgkin, J., H. Horvitz and S. Brenner, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

Hodgkin, J., R. H. Plasterk and R. H. Waterston, 1995 The nematode *Caenorhabditis elegans* and its genome. Science **270:** 410–414.

Holmes, K. C., 1995 The actomyosin interaction and its control by tropomyosin. Biophys. J. **68:** 2–5.

Holmes, K. C., 1997 The swinging lever-arm hypothesis of muscle contraction. Curr. Biol. **7:** 112–118.

Horowitz, A., K. M. Trybus, D. S. Bowman and F. S. Fay, 1994 Antibodies probe for folded monomeric myosin in relaxed and contracted smooth muscle. J. Cell Biol. **126:** 1195–1200.

Jordan, P., and R. Karess, 1997 Myosin light chain-activating phosphorylation sites are required for oogenesis in Drosophila. J. Cell Biol. **139:** 1805–1819.

Karess, R. E., X. J. Chang, K. A. Edwards, S. Kulkarni, I. Aguilera *et al.*, 1991 The regulatory light chain of nonmuscle myosin is encoded by *spaghetti-squash*, a gene required for cytokinesis in *Drosophila*. Cell **65:** 1177–1189.

Kendrick-Jones, J., E. Szentkiralyi and A. Szent-Gyorgyi, 1976 Regulatory light chains in myosins. J. Mol. Biol. **104:** 747–775.

Kramer, J. M., R. P. French, E. Park and J. J. Johnson, 1990 The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encode a collagen. Mol. Biol. Cell **10:** 2081–2089.

Krause, M., M. Wild, B. Rosenzweig and D. Hirsh, 1989 Wild-type and mutant actin genes in *Caenorhabditis elegans*. J. Mol. Biol. **208:** 381–392.

Lehman, W., and A. Szent-Gyorgyi, 1975 Regulation of muscular contraction: distribution of actin control and myosin control in the animal kingdom. J. Gen. Physiol. **66:** 1–30.

Lowey, S., G. S. Waller and K. M. Trybus, 1993 Skeletal muscle myosin light chains are essential for physiological speeds of shortening. Nature **365:** 454–456.

Mello, C., and A. Fire, 1995 DNA transformation, pp. 451–482 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Meyer, B. J., 1997 Sex determination and *X* chromosome dosage compensation, pp. 209–240 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Moerman, D. G., and A. Fire, 1997 Muscle: structure, function, and development, pp. 417–470 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ostrow, B. D., P. Chen and R. L. Chisholm, 1994 Expression of a myosin regulatory light chain phosphorylation site mutant complements the cytokinesis and developmental defects of *Dictyostelium* RMLC null cells. J. Cell Biol. **127:** 1945–1955.

Plasterk, R. H. A., and H. G. A. M. van Luenen, 1997 Transposons, pp. 97–116 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Priess, J. R., and D. I. Hirsh, 1986 *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. Dev. Biol. **117:** 156–173.

Rayment, I., W. R. Rypniewski, K. Schmidt-Base, R. Smith, D. R. Tomchick *et al.*, 1993 Three-dimensional structure of myosin subfragment-1: a molecular motor. Science **261:** 50–58.

Rushforth, A. M., B. Saari and P. Anderson, 1993 Site-selected insertion of the transposon Tc1 into a *Caenorhabditis elegans* myosin light chain gene. Mol. Cell. Biol. **13:** 902–910.

Satterwhite, L. L., and T. D. Pollard, 1992 Cytokinesis. Curr. Opin. Cell Biol. **4:** 43–52.

Sellers, J. R., 1991 Regulation of cytoplasmic and smooth muscle myosin. Curr. Opin. Cell Biol. **3:** 98–104.

Seydoux, G., and A. Fire, 1995 Whole-mount *in situ* hybridization for the detection of RNA in *Caenorhabditis elegans* embryos, pp. 323–337 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Sivaramakrishnan, M., and M. Burke, 1982 The free heavy chain of vertebrate skeletal myosin subfragment 1 shows full enzymatic activity. J. Biol. Chem. **257:** 1102–1105.

Somlyo, A. V., T. M. Butler, M. Bond and A. P. Somlyo, 1981 Myosin filaments have non-phosphorylated light chains in relaxed smooth muscle. Nature **294:** 567–569.

Stinchcomb, D., J. Shaw, S. Carr and D. Hirsh, 1985 Extrachromosomal DNA transformation of *C. elegans*. Mol. Cell. Biol. **5:** 3484–3496.

Strome, S., and W. Wood, 1983 Generation of asymmetry and segre-

gation of germ-line granules in early *C. elegans* embryos. Cell **35:** 15–25.

Sweeney, H. L., B. F. Bowman and J. T. Stull, 1993 Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. Am. J. Physiol. **264:** 1085–1095.

Szent-Gyorgyi, A. G., E. M. Szentkiralyi and J. Kendrick-Jones, 1973 The light chains of scallop as regulatory subunits. J. Mol. Biol. **74:** 179–203.

Tan, J. L., S. Ravid and J. A. Spudich, 1992 Control of nonmuscle myosins by phosphorylation. Annu. Rev. Biochem. **61:** 721–759.

Tohtong, R., H. Yamashita, M. Graham, J. Haeberle, A. Simcox *et al.*, 1995 Impairment of muscle function caused by mutations of the phosphorylation sites in myosin regulatory light chain. Nature **374:** 650–653.

Trybus, K. M., 1991 Assembly of cytoplasmic and smooth muscle myosins. Curr. Opin. Cell Biol. **3:** 105–111.

Wagner, P., and E. Giniger, 1981 Hydrolysis of ATP and reversible binding to F-actin by myosin heavy chains free of all light chains. Nature **292:** 560–562.

Warmke, J., M. Yamakawa, J. Molloy, S. Falkenthal and D. Maughan, 1992 Myosin light chain-2 mutation affects flight, wing beat frequency, and indirect flight muscle contraction kinetics in *Drosophila*. J. Cell Biol. **119:** 1523–1539.

Waterston, R. H., 1989 The minor myosin heavy chain, mhcA, of *Caenorhabditis elegans* is necessary for the initiation of thick filament assembly. EMBO J. **8:** 3429–3436.

Williams, B. D., and R. H. Waterston, 1994 Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. J. Cell Biol. **124:** 475–490.

Zwaal, R. R., A. Broeks, J. van Meurs, J. T. Groenen and R. H. Plasterk, 1993 Target-selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. Proc. Natl. Acad. Sci. USA **90:** 7431–7435.

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