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The *unc-45* Gene of *Caenorhabditis elegans* Is an Essential Muscle-Affecting Gene With Maternal Expression

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ABSTRACT

We have isolated three novel alleles of the *unc-45* locus in *C. elegans*, that are recessive lethals. Two of these alleles, when homozygous, result in a nearly total loss of muscle contraction with a concomitant arrest of development and a displacement of muscle cells. The third allele is similar, but showed maternal rescue by a wild-type allele. All previously identified *unc-45* alleles were temperature sensitive and, although they produced paralysis of adult animals, all were homozygous viable. Prior genetic studies with these temperature sensitive alleles had suggested that at least one function of the *unc-45* gene product was to interact with the major myosin heavy chain isoform, MHC B, of body wall muscles. Our observations of the lethal alleles suggest that the *unc-45* product normally interacts with additional muscle components in both the body wall and pharyngeal muscles. In particular, we suggest that the *unc-45* product might interact with all four myosin heavy chains: MHC B; MHC A; and the pharyngeal isoforms, MHC C and MHC D. Maternal rescue of the lethality of the third allele shows that the *unc-45* gene product is present in the oocytes, although it may not be necessary until late in development when myofilaments begin to assemble.

THE advantages of the nematode *Caenorhabditis elegans* as a system for the study of muscle function and development are well documented (WATERSTON, THOMSON and BRENNER 1980; WATERSTON, 1988). Knowledge gained in this system should apply to vertebrate muscles, as they are similar in composition to invertebrate muscles. The ease of manipulating nematodes genetically has yielded numerous muscle-affecting mutations, which have led to the identification of several genes important for thick filaments: *unc-15* = paramyosin (WATERSTON, FISHPOOL and BRENNER 1977; RIDDLE and BRENNER 1978); *unc-54* = myosin heavy chain B (MHC B) (EPSTEIN, WATERSTON and BRENNER 1974; MACLEOD *et al.* 1977); *myo-3* = MHC A (MILLER, STOCKDALE and KARN 1986); *myo-1* = MHC D (MILLER, STOCKDALE and KARN 1986); and *myo-2* = MHC C (MILLER, STOCKDALE and KARN 1986). In the body wall muscles, thick filaments contain only MHC A in their central 2 μm, a region that includes the portion of the filament where polarity reverses (MILLER *et al.* 1983). The MHC B isoform makes up the remainder of the 10-μm long filament, with a small region of overlap between the two forms. MHC C and MHC D are restricted to the pharynx (ARDIZZI and EPSTEIN 1987).

Another thick filament-affecting gene, *unc-45*, whose protein product is unknown, was initially identified through a temperature-sensitive (*ts*) mutation (BRENNER 1974), and all subsequently isolated alleles had also been *ts*. The *unc-45*(*ts*) animals, when grown at the restrictive temperature (25°), are nearly fully paralyzed as young adults and have a reduced number of body wall muscle thick filaments in a disorganized myofilament array (EPSTEIN and THOMSON 1974). At the permissive temperature (15°), *unc-45*(*ts*) animals move essentially as well as wild type and have approximately normal numbers of thick filaments in a substantially improved, but not fully normal organization (EPSTEIN and THOMSON 1974; R. H. WATERSTON and J. N. THOMSON, unpublished results). A temperature shift during any of the larval stages of development, when the bulk of the body wall muscle is being assembled, results in the loss, or gain, of the Unc phenotype over several hours. From this reversibility it was hypothesized that the *unc-45* product might catalyze thick filament assembly (EPSTEIN and THOMSON 1974).

Recovery of viable, *ts* alleles of *unc-45* is rare compared to the recovery of *unc-54* alleles, though their phenotypes are similar (ZENGEL and EPSTEIN 1980; BRENNER 1974). This discrepancy could in part be attributed to differences in gene size, but an alternative explanation is that the *unc-45* gene product is essential. Null alleles of an essential, single-copy gene would obviously be missed in screens for viable, uncoordinated (Unc) worms. Some muscle function is known to be necessary for development and viability (WATERSTON 1989; R. J. BARSTEAD, B. D. WILLIAMS and R. H. WATERSTON, unpublished). The temperature sensitive *unc-45* alleles would then represent mu-

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tations only partially inactivating the *unc-45* activity even at restrictive temperatures. Thus, to search for null alleles at the *unc-45* locus, lethality was assumed. This paper reports the results of such a search, using a noncomplementation screen.

MATERIALS AND METHODS

General methods: General methods for the handling, culture, and ethyl methane-sulfonate (EMS) mutagenesis of *C. elegans* have been described (BRENNER 1974). Under these conditions, the average frequency for EMS-induced mutation is 5×10^{-4}/gene.

Strains and genetic nomenclature: The wild-type strain of *C. elegans* used is var. Bristol strain N2. Alleles generated in the course of this study are *unc-45(st601)*, *unc-45(st603)*, and *unc-45(st604)*. The mutations used in this study were as follows:

LGI: *unc-54(e190)* = *unc-54(0)*, *unc-54(st21)* = *unc-54(sd)*.
LGIII: *dpy-1(e1)*, *vab-6(e697)*, *emb-13(g6)*, *unc-45(e286)*, *unc-45(m94)*, *unc-45(r450)*, *unc-45(su2002)*, *unc-45(b131)*, *dpy-18(e364)*.
LGIV: *dpy-13(e184)*
LGV: *myo-3(st378)* = *myo-3(0)*, *sup-3(e1407 st90 st92)* = *myo-3(gf)*
LGX: *sup-7(st5)*

This paper adheres to the standardized nomenclature for *C. elegans* (HORVITZ *et al.* 1979). The mutations *dpy-1(e1)*, *unc-54(e190)*, *unc-45(e286)*, *dpy-18(e364)*, and *dpy-13 (e184)* were described by BRENNER (1974), *vab-6(e697)* by HODGKIN (1983), *emb-13(g6)* by CASSADA *et al.* (1981), *sup-7(st5)* by WATERSTON (1981), and *unc-54(st21)* is an EMS-induced isolate of this laboratory, and is a semidominant Unc, recessive lethal. The remaining *unc-45(ts)* alleles were isolated in the following laboratories: *unc-45(m94)* [D. L. RIDDLE], *unc-45(r450)* [R. P. ANDERSON], *unc-45 (su2002)* [H. E. EPSTEIN], *unc-45(b131)* [D. HIRSH]. The embryos lacking both MHC A and B are the progeny of the heterozygote: *unc-54(e190)*; *myo-3(st378)/eDf1*, where *eDf1* is a homozygous lethal chromosomal rearrangement carrying multiple copies of *myo-3* (MARUYAMA, MILLER and BRENNER 1989). The *sup-3* mutant was isolated as an enhanced suppressor of *unc-54(e190)* in three steps as follows: *e190;sup-3(e1407)* (allele in RIDDLE and BRENNER 1978), *e190;sup-3(e1407 st90)*, *e190;sup-3(e1407 st90 st92)*. The suppression is the result of an amplification of the *myo-3* gene region (MARUYAMA, MILLER and BRENNER 1989). The amplified region will simply be referred to as *myo-3(gf)* or *st92*. Genetic constructions involving the *unc-45(st604)* allele are diagrammed in Figure 1. These constructions started with the RW2329 strain of *unc-54(e190);myo-3(gf)*.

A two-factor map distance of 6.6 map units (m.u.) ± 0.9 has been reported between *unc-45* and *dpy-1* (SWANSON, EDGLEY and RIDDLE 1984). Additional *unc-45(ts)* twofactor map data were collected in the course of this study. *unc-45(e286)* was mapped in a *cis* double heterozygote with *dpy-1(e1)*, at both 20° and 25° (results were comparable), yielding 276 recombinants in 3857 worms screened, which is a map distance of 7.4 m.u. *unc-45(m94)* was similarly mapped at 20° (viability is significantly reduced at 25°) and gave 145 recombinants with *dpy-1(e1)* in 2301 worms screened, indicating a separation of 6.5 m.u. The inability to maintain *m94* at 25° highlights differences observed between the five *unc-45(ts)* alleles. By the criteria of mobility

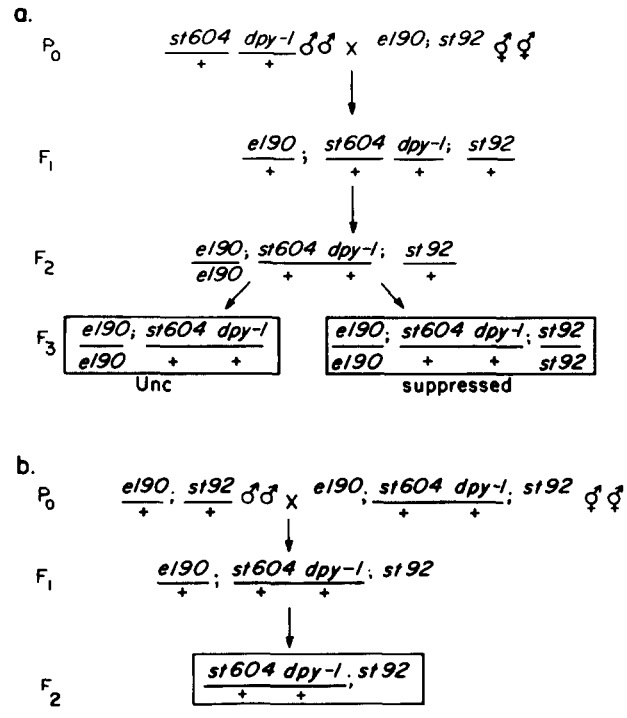

FIGURE 1.—Genetic constructions with *st604*. These constructions take advantage of the fact that one allele of *st92* partially suppresses *e190*, while two alleles provide a scoreable increase in suppression. *dpy-1* serves as a marker for *st604* and the presence of *st604* in the final product was confirmed by its failure to complement *e286*. In (a), the F_2 has a semi-Unc phenotype, while the selected phenotypes in the F_3 are Unc and Suppressed. In (b), the F_1 were picked as wild-type, and screened for the presence of *dpy-1* and the absence of severe Uncs in the F_2. The desired F_2 is also wild type, and is identified by having no slow progeny in the F_3. The phenotypes of the *st604* homozygous progeny of the three constructs are described in Table 1.

and fecundity at 25° the alleles can be ranked in descending order: *e286*, *r450*, *su2002*, *b131*, *m94*. These alleles all fail to complement each other. Despite their differences there was no difference in their interactions with the lethal alleles.

The *emb-13(g6)* mutation may be closely linked to *unc-45*, and so was shown to complement *unc-45(e286)*.

Noncomplementation screen: An *unc-45(e286)* male strain was isolated by random screening and maintained by mating at 15°. These males were mated with *dpy-1(e1)* hermaphrodites that had been mutagenized with EMS about 24 hr earlier. Matings and F_1 screens were performed at 20°, as in Figure 2. New *ts* alleles were indicated by an Unc F_2 with 25% Dpy progeny, and new lethal alleles by an Unc F_2 with a few percent Dpys, presumably resulting from recombination between *unc-45* and *dpy-1*. In 7000 F_1 screened, 4 new alleles were isolated: 1 ts (discarded) and 3 lethal (*st601*, *st603* and *st604*). New *unc-45* isolates were outcrossed several times to N2 and were tested for complementation with the nearby marker, *vab-6(e697)*, by mating with *vab-6/+* males. The *unc-45* lethal alleles could be maintained partially balanced by *vab-6*. For the maintenance of unbalanced *ts* or + heterozygotes, it was sufficient to pick six worms in each generation, and screen their progeny for the presence of the lethal allele.

Microscopy: Examination of the terminal phenotypes of various lethal mutants was made using Nomarski differential interference contrast optics. Fertilized eggs were released

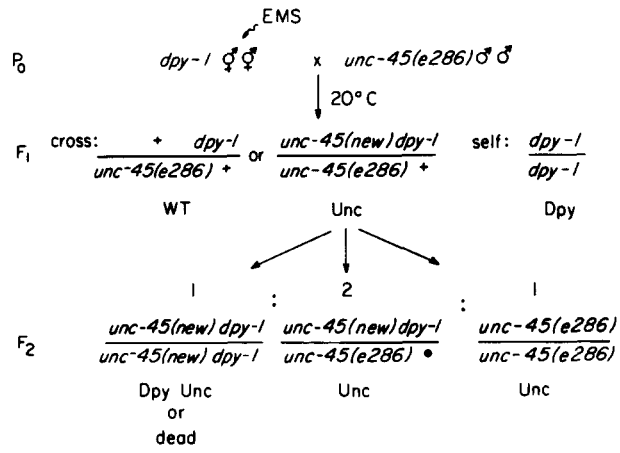

FIGURE 2.—Generation of *unc-45* alleles by noncomplementation screening. In the case of new *unc-45* lethal alleles, about 7% Dpys are seen due to recombination.

from hermaphrodites into a pool of M9 buffer (WOOD 1988), by cutting the adult midsections with a razor blade. Released eggs were transferred to a 5% agar pad, which was kept moist, allowing periodic observation throughout embryonic development and hatching.

Embryos were prepared for immunofluorescent staining, either by rinsing free embryos from the surface of four, well populated 60mm plates with M9 buffer, or by releasing them from adults with 4 min of alkaline hypochlorite treatment. The eggs were collected in small glass tubes and frozen at −70°. Alternate fixing procedures were either 95% ethanol at 0° for 5 min, or a combination of 4% paraformaldehyde at 0° for 15 min followed by acetone at 0° for 5 min. Fixed worms were rinsed in phosphate buffered saline (PBS) (WOOD 1988), and treated with a mouse monoclonal antibody in PBS at 37° for 1 hr. We used either anti-MHC A (designated DM 5-6 used at 1/1500 dilution of ascites fluid) or anti-MHC B (DM 5-8 used at 1/800 dilution of ascites fluid) generously provided by MILLER *et al.* (1983). The samples were then rinsed extensively with PBS and treated with a goat anti-mouse IgG conjugated with fluorescein or rhodamine. Phalloidin (0.07–0.55 μM) conjugated with the opposite fluorescent label was often included at this stage, and treatment was at 37° for 1 hr (WULF *et al.* 1979). Phalloidin binds to actin filaments, which accumulate during the first folding of the worm. Thus, younger embryos should not stain as intensely as older embryos. Samples were viewed on a Zeiss Universal microscope (Carl Zeiss, Inc., Thornwood, New York) equipped with epifluorescent illumination and selective filters. They were viewed either in a 1:1 solution of PBS and glycerol, or in an antiquenching solution of 1 mg/ml *p*-phenylenediamine in 10% PBS, 90% glycerol (pH 8) (JONSON *et al.* 1982). Ilford HP-5 films were exposed 20 sec to 1 min and developed in Diafine (Acufine, Inc., Chicago, Illinois) to an exposure index of 1200.

RESULTS

Recovery of new *unc-45* alleles: The goal of the noncomplementation screen was to isolate potentially lethal alleles of *unc-45* (Figure 2). It was assumed that an *unc-45(ts)* allele would provide enough *unc-45* function to allow full development even when heterozygous with a null allele, but that the double heterozygote would result in an Unc phenotype. Using EMS as the mutagen, from 7000 F_1 progeny, three recessive lethal alleles (*st601*, *st603* and *st604*) were recovered along with one additional temperature-sensitive allele, which was discarded. All three lethal alleles fail to complement the motility defect of the *unc-45(ts)* allele but do complement the closely linked *vab-6* mutations. This complementation, together with the mutation frequency and the similarity of the terminal phenotypes associated with the three mutations argue strongly that the lethal phenotype is the result of alterations of *unc-45* activity and not the result of double mutations or deletions affecting a closely linked essential gene.

The new *unc-45* mutations may represent loss-of-function (*lf*) alleles. The frequency of recovery, 2.8×10^{-4}, is similar to the mutation frequency for the average gene under these conditions (BRENNER 1974), and the alleles are fully recessive, to both the wild-type allele and *unc-45(ts)* alleles. They are obviously more severe in their phenotypes than existing temperature sensitive alleles and the two more severe alleles (see below), *st601* and *st603*, yield identical phenotypes. However, neither of the more severe alleles is suppressed by an amber tRNA nonsense suppressor (WILLS *et al.* 1983) and no deficiency exists for the region for purposes of comparison. None of the three new mutations in trans to an *unc-45(ts)* allele produces a phenotype more severe than the homozygous *unc-45(ts)* animal.

Mutant phenotypes: The development of the three new homozygous *unc-45* lethal mutants was observed using Nomarski differential interference contrast microscopy of the live eggs. Because of the lethality of the mutations, eggs were obtained from heterozygous parents. For all three mutations regardless of the genotype of the parent, the homozygous animals could not be distinguished from their siblings until after most cell division had taken place and elongation had begun (about 400 min) (SULSTON *et al.* 1983). Thereafter, alterations in movement and elongation were detected, but surprisingly the phenotype was influenced by the maternal *unc-45* genotype, as detailed below.

The *st601* and *st603* homozygotes from an *unc-45(ts)/(st601* or *st603)* mother have an identical phenotype, whether grown at 15° or 20°. As the embryos elongate to the twofold stage, they lack all body movements. In contrast wild type embryos begin to twitch by the one-and-a-half fold stage (SULSTON *et al.* 1983) and by the twofold stage show coordinated movements, such as rolling. The mutant embryos fail to elongate beyond the twofold length (Figure 3), whereas wild type embryos extend to more than threefold (Figures 4 and 5). The pharynges of the mutant embryos fail to pump and hatching is very much delayed or does not occur at all (wild type embryos

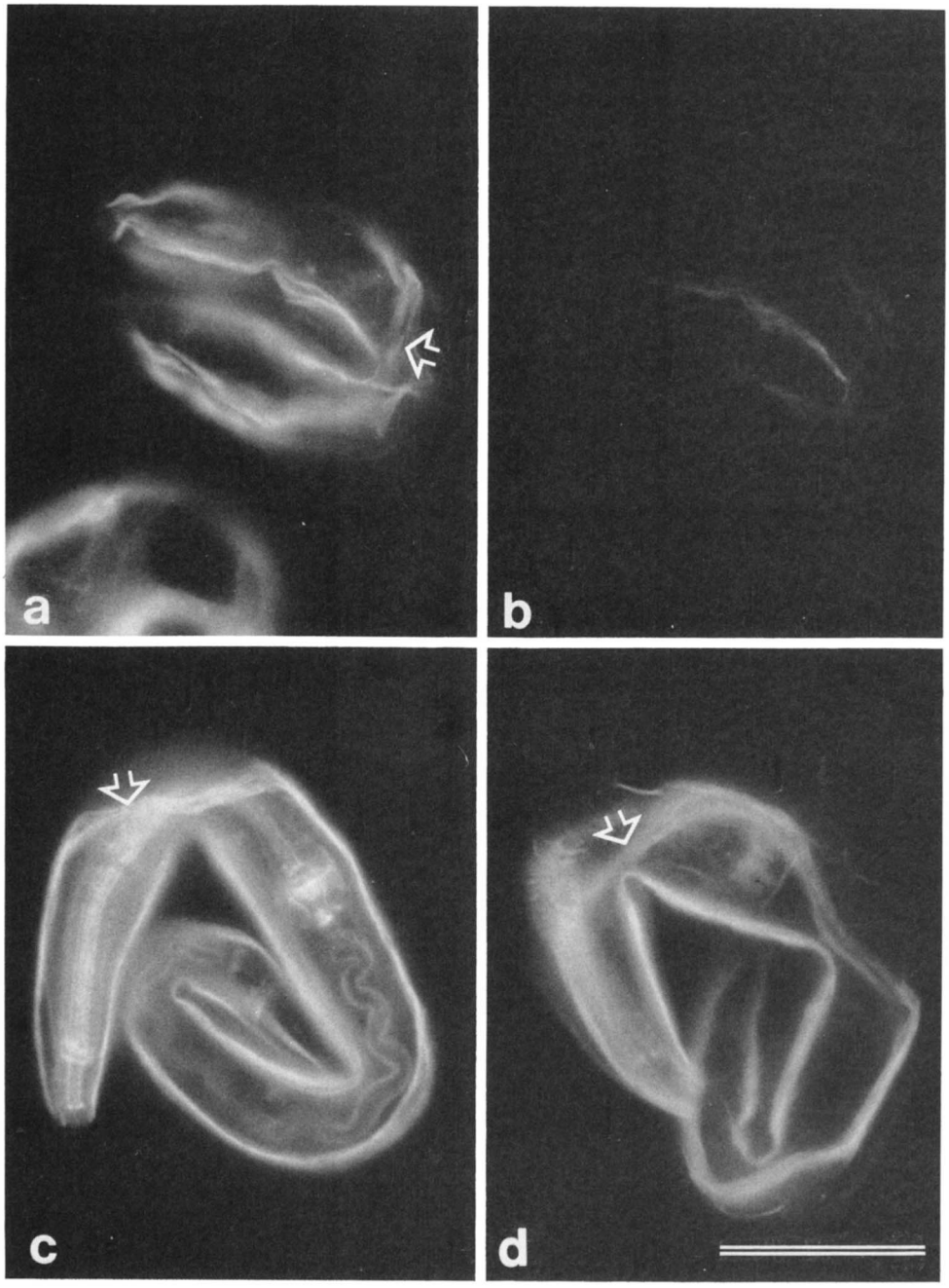

FIGURE 3.—Two- and threefold *unc-45(st603)* and *unc-54(st21)* embryos. Arrows in a, c and d identify displaced muscles. (a) A twofold *unc-45(st603)* embryo stained with fluorescently labeled phalloidin, which binds to actin filaments. (b) The embryo in (a) stained with antibody to MHC A, which probably had limited access to the fibers, due to cuticle formation. (c) A threefold *unc-45(st603)* embryo from *st603/+* parent, stained with phalloidin. (d) A threefold *unc-54(st21)* embryo stained with phalloidin. Note the muscle fibers in the outer curve of the head, collapsed to touch the inner curve, in (c) and (d). Bar = 20 μm.

pump vigorously for about 30 min prior to hatching at 800 min (SULSTON *et al.* 1983)). Other developmental events such as cuticle deposition continue to occur in the mutants (as evidenced by increased refractility of the lumen of the pharynx) suggesting that all aspects of development are not arrested at the twofold stage.

The *st601* and *st603* homozygotes from an *unc-45(+)/st601* or *st603* mother showed a slightly milder phenotype. While some of the embryos arrested at the twofold stage, most achieved the threefold elongation and feeble movements were seen. About 20% of the embryos showed pharyngeal pumping and hatched. These animals then arrested in the first larval stage, often without unfolding (Figure 3c).

The *st604* homozygotes from a *st604/unc-45(ts)* parent at 15° or 25° also arrested elongation at the twofold stage but their pharynges pumped and the animals hatched (Figure 6). Some body wall muscle contraction was also apparent, but the hatched larvae generally failed to unfold and move across a surface. There was considerable variation in the size these animals reached before they arrested, but no larval molting was observed.

Remarkably, *st604* homozygotes from a *st604/unc-45(+)* parent were viable (Figure 7). They attained the adult stage and were fertile. Though they were slow and had disorganized muscle, especially when raised at higher temperatures, they were not as uncoordinated as the *unc-45(ts)* mutants at the restrictive

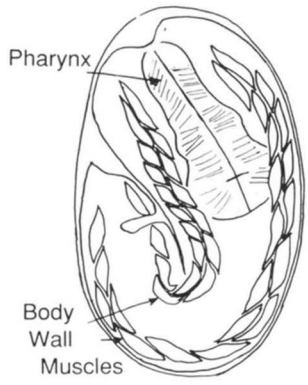

FIGURE 4.—Diagram of the wild-type embryo. The location of the body wall muscle cells in strips in the subdorsal and subventral quadrant is shown in this diagram of a 1 $\frac{1}{2}$-fold embryo. This corresponds roughly in age to the embryo shown in Figure 5b. This diagram shows a lateral view, whereas the embryo in Figure 5b is rotated slightly. The position of the pharynx is also indicated.

temperature. The progeny of most such *st604* homozygotes failed to develop, arresting at the twofold stage. However, occasional self-progeny survived to adulthood, with a phenotype similar to the maternally rescued animals. For example, in 102 progeny of maternally rescued animals, 50 failed to hatch, 50 hatched but arrested as twofold embryos, and 2 survived to adulthood. When the maternally rescued *st604* homozygotes were mated with wild-type or *unc-45(ts)* males at 20°, the heterozygous progeny were viable, demonstrating that zygotic rescue can also occur.

Interactions of the *unc-45(lf)* alleles with other thick filament genes: Previous work on the interactions among *unc-54*, *myo-3* and *unc-45* mutations has suggested that *unc-45(ts)* alleles result in the production of an abnormally functioning MHC B protein, with the consequence that *unc-54* is epistatic to *unc-45(ts)* (WATERSTON, THOMSON and BRENNER 1980; R. H. WATERSTON and A. M. CURRY, cited in WATERSTON 1988). The underlying molecular events are unknown, but might involve a partial failure of the *unc-45(ts)* product to modify or otherwise interact with the MHC B protein. It was thus possible that the new *unc-45(lf)* alleles resulted in the accumulation of a severely defective or toxic MHC B, which, like severely abnormal MHC B produced by missense mutations within the *unc-54*(MHC B) gene, might result in lethality (DIBB *et al.* 1985; BESJOVEC and ANDERSON 1988). To test this possibility, all MHC B was removed by introducing an *unc-54(0)* mutation. Animals of the genotype *unc-54(0);st601 dpy-1/++* were constructed and their progeny examined. The *unc-54(0);st601 dpy-1* embryos had the same phenotype as the control *st601* embryos. Also, *unc-54(0)*; *unc-45(st604)/+* showed the expected maternal rescue of their *unc-54(0)*; *unc-45(st604)* progeny, but no increased viability in the following generation. Thus a defective

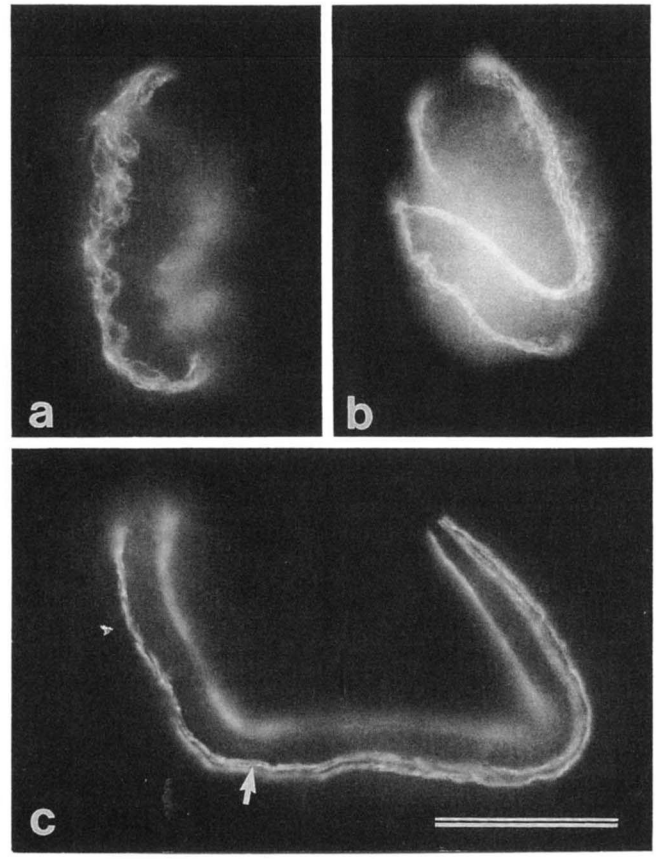

FIGURE 5.—Three stages of wild-type development, all stained with an antibody specific to myosin. (a) Comma stage. Note cytoplasmic staining. (b) Twofold stage. (c) Threefold stage. The arrow denotes a region showing two A-bands within a single cell. Bar = 20 μm.

MHC B cannot be a significant cause of *unc-45(lf)* lethality.

The effects of alterations of MHC A levels on the *unc-45(st604)* allele were also examined. Since the *myo-3(lf)* alleles produce a phenotype very similar to the *unc-45(lf)* alleles, they were not studied. But amplifications of the *myo-3* gene, here designated *myo-3 (gain-of-function, gf)*, have been identified and result in an increased accumulation of MHC A (WATERSON *et al.* 1982). They suppress *unc-54(0)* alleles well, but suppress *unc-54* semidominant missense alleles only poorly. We chose the *unc-45(st604)* allele to test for interaction with a *myo-3(gf)* allele, because occasional viable *st604* animals had been found, and suppression might result in improved viability. Surprisingly, the *myo-3(gf)* mutation appeared to eliminate maternal rescue: that is, the *unc-45(st604)*; *myo-3(gf)* progeny of *unc-45(st604)/+*; *myo-3(gf)* mothers did not survive. In one brood for example, only 1 of 14 animals that were observed closely, hatched and this animal then arrested. Inclusion of *unc-54(0)* in the same background did not alleviate the effects of the *myo-3(gf)* allele. Thus increased amounts of MHC A enhance the *unc-45* mutant phenotype rather than suppress it.

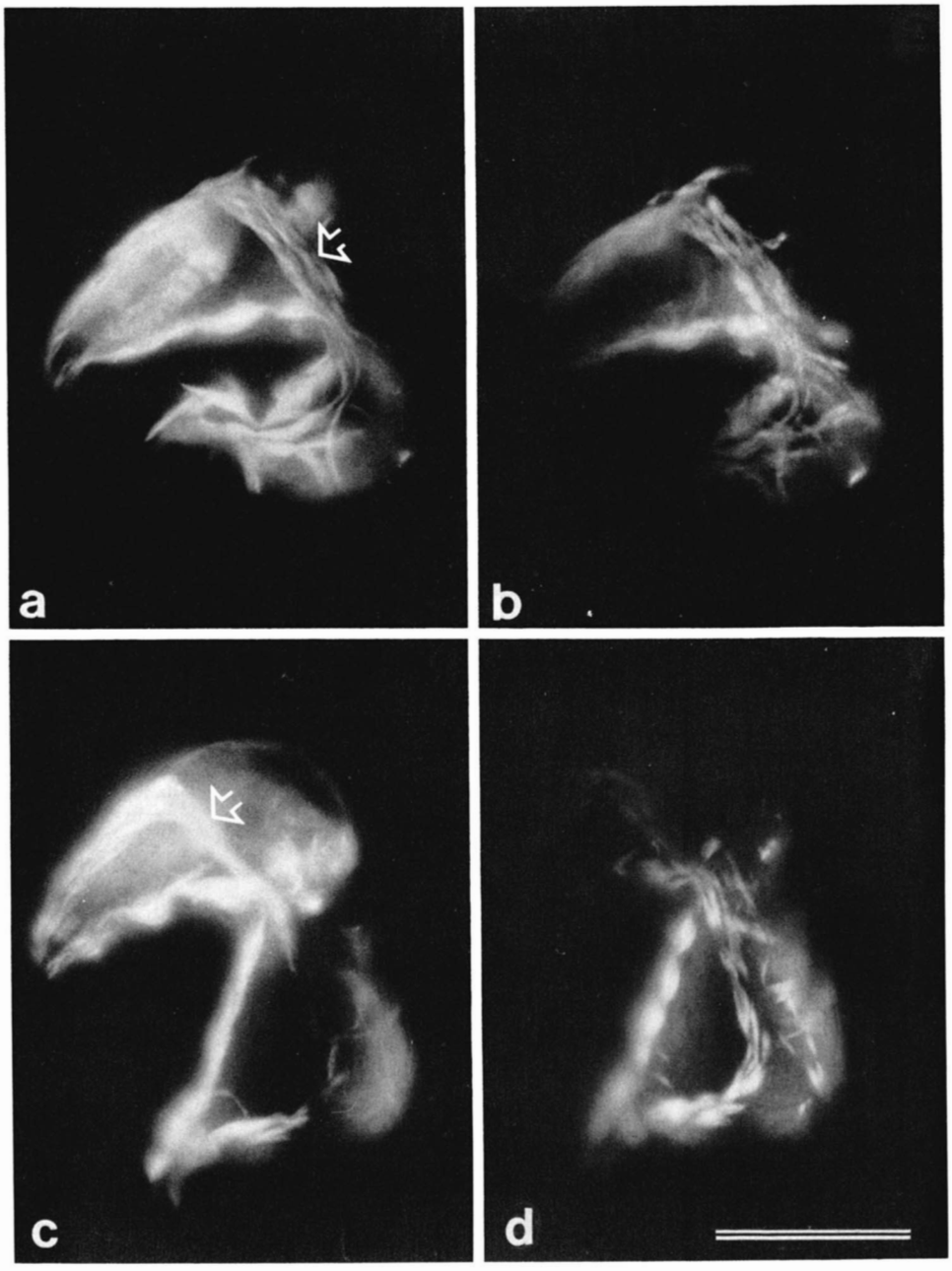

FIGURE 6.—The twofold *unc-45(st604)* embryos (non-rescued). (a) and (b) are the same embryo stained with phalloidin and an antibody to MHC B respectively. (c) and (d) are another embryo, stained with phalloidin and an antibody to MHC A, respectively. Antibody staining in the head is decreased, probably because cuticle has begun to form, excluding antibody. The muscle is displaced from its normal dorsal position, as indicated by arrows in (a) and (c). Bar = 20 μm.

Table 1 provides a summary of the observations on *unc-45*, *unc-54* and *myo-3* mutations.

Muscle organization of *unc-54(lf)* animals: In order to examine the effects of these *unc-45* mutations on myosin organization and muscle structure, fixed wild-type and mutant embryos were incubated with mouse monoclonal antibodies specific for either MHC A or MHC B, and counterstained with fluorescently labeled phalloidin, which binds to thin filaments (F-actin).

When myosin is first synthesized in wild-type embryos at about the 400-cell stage, it appears diffusely throughout the muscle cell cytoplasm, leaving the nucleus clearly defined as a negative image (Figure 5a). Just prior to the twofold stage, it begins to be localized in nascent sarcomeres adjacent to the hypodermis, and nuclei are no longer outlined (Figure 5b). This organization becomes sharper as elongation proceeds, until two clear A-bands are visible in favorable views in the threefold embryos (Figure 5c). In both *st603* (Figure 3b) and *st604* (Figure 6d) twofold embryos, the MHC A organization clearly progresses beyond the diffuse stage, but the two A-bands fail to form. The same is true for MHC B in *st604* (Figure 6b) embryos, but in some *st603* embryos, MHC B remains diffusely distributed in the cytoplasm even in late embryos (not shown).

An obvious feature apparent in all of the twofold lethal embryos is the frequent appearance of a displaced body wall muscle (Figures 3 and 6). The muscle, which in wild type, is attached to the hypodermis along the dorsal curve of the body wall, instead

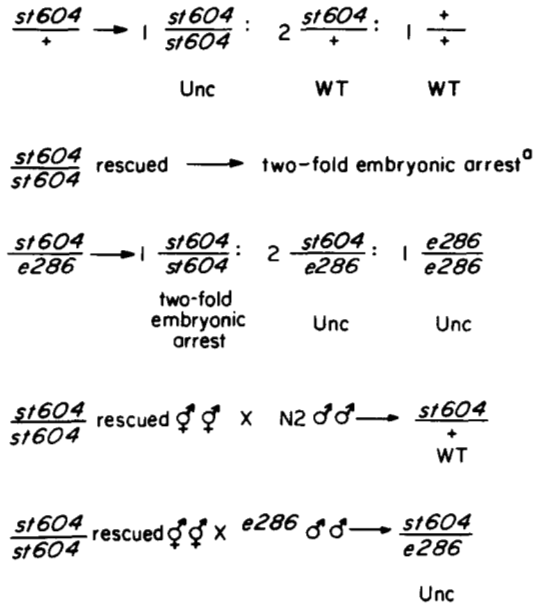

FIGURE 7.—Crosses demonstrating maternal and zygotic rescue of *st604*. [a] About 2% of these homozygotes survive to adulthood.

stretches across to contact the ventral wall along the inner curvature of the embryo. This phenomenon has also been observed in mutants of at least two other genes. Very similar muscle displacement is seen in *myo-3(0)* twofold arrested embryos (WATERSTON 1989). And an *unc-54* lethal mutation, *st21*, which ends development of homozygous animals at the threefold hatched stage, often without unfolding has muscles which can also be found to be displaced inward at the folded corners (Figure 3d).

DISCUSSION

We have isolated novel, recessive lethal alleles of the *unc-45* locus which demonstrate that normal *unc-45* gene function is essential for muscle function, and for the development of the worm. Two of these alleles, *st601* and *st603* seem likely to be loss-of-function mutations and may be null alleles. They were recovered at a frequency typical of loss-of-function mutations, are fully recessive, and result in the same phenotype, which is the most severe of all *unc-45* alleles.

The target of the *unc-45* activity is unknown, but its interactions with *unc-54(0)* mutations have previously suggested that MHC B is one target (WATERSTON, THOMSON and BRENNER 1980; R. H. WATERSTON and X. CURRY, cited in WATERSTON 1988). The lethal phenotypes associated with the *unc-45(lf)* alleles described here demonstrate that the *unc-45* protein interacts with additional components. The *unc-45(lf)* phenotype is more severe than that associated with simple *unc-54(0)* alleles which yield paralyzed, but fertile adults. The *unc-45(lf)* lethality cannot be due solely to an altered, toxic MHC B as the *unc-45(st601)*; *unc-54(0)* double mutant, which can have no MHC B, had the same terminal phenotype as *unc-45(st601)*. Absence of MHC A leads to a failure of effective

TABLE 1

Observations on *unc-45*, *unc-54* and *myo-3* mutations

Genotype	Myosin state	Phenotype
unc-54(0) is epistatic to *unc-45(ts)*		
unc-54(0)[a]	No MHC B	Viable, paralyzed adult
unc-45(ts)[b]	MHC A and MHC B present	Viable, paralyzed adult
unc-54(0); *unc-45(ts)*[c]	No MHC B	Viable, paralyzed adult
unc-54(0); *myo-3(gf)*[d]	No MHC B, increased MHC A	Significant suppression of paralysis
unc-45(ts); *myo-3(gf)*	Increased MHC A	No significant suppression
unc-54(0); *unc-45(ts)*; *myo-3(gf)*	No MHC B, increased MHC A	Significant suppression of paralysis
Disruptive MHC B does not account for *unc-45(lf)* lethality		
unc-54(sd)[e]	Toxic MHC B	Threefold, arrested L1 larva
unc-45(lf)[f]	MHC A and MHC B present	Twofold, paralyzed embryo
unc-54(0); *unc-45(lf)*	No MHC B	Twofold, paralyzed embryo
Maternal rescue of *unc-45(mr)* is independent of MHC B, and antagonized by increased MHC A		
unc-45(mr)[g]	MHC A and MHC B present	Twofold, paralyzed L1 larva from mutant mother; viable, Unc adult from (+) heterozygous mother=maternal rescue
unc-54(0); *unc-45(mr)*	No MHC B	Same as *unc-45(mr)*
unc-45(mr); *myo-3(gf)*	Increased MHC A	Loss of maternal rescue
unc-54(0); *unc-45(mr)*; *myo-3(gf)*	No MHC B, increased MHC A	Loss of maternal rescue

[a] *(0)* signifies a null mutation, or absence of protein; the *e190* allele was used in these cases (EPSTEIN, WATERSTON and BRENNER 1974; MACLEOD *et al.* 1977).
[b] *(ts)* signifies a temperature-sensitive mutation (BRENNER 1974). Most often the *e286* allele was used.
[c] WATERSTON, THOMSON and BRENNER (1980).
[d] *(gf)* signifies a gain-of-function mutation; the allele used was *st92*. See MATERIALS AND METHODS (RIDDLE and BRENNER 1978).
[e] *(sd)* signifies a semidominant mutation, with altered protein. See MATERIALS AND METHODS.
[f] *(lf)* signifies a loss-of-function mutation, and putative null, in this case, *unc-45(st601)* and *(st603)*.
[g] *(mr)* signifies a maternally rescueable mutation, *unc-45(st604)* in this case.

embryonic muscle contractions and an arrest of morphogenesis very similar to that seen in the *unc-45(lf)* embryos. The previous evidence for an *unc-45*/ MHC B interaction, combined with the latter observation, suggests that it is plausible that loss of *unc-45* activity could produce an inactive or inappropriately active MHC A protein, a possibility consistent with the enhancement of *st604* lethality by the *myo-3(gf)* allele. However, the following observations suggest additional interactions for the *unc-45* product. A double mutant, lacking both MHC A and B has normal pharyngeal pumping (our unpublished observations), yet pharyngeal pumping fails in the *unc-45(lf)* mutants, even though there is no MHC B or A expressed in the pharynx (ARDIZZI and EPSTEIN 1987). This suggests that the *unc-45* product is required for muscular function in this organ, presumably through interactions with MHC C and/or MHC D.

The nature of this proposed interaction with myosin heavy chains is unknown. The immunofluorescence observations show clearly that MHC A and MHC B are present, probably in near normal amounts, which makes it unlikely that *unc-45* plays a role in regulation of gene expression. The myosins fail to form recognizable striations, but they do aggregate and are located in the same regions as the thin filaments within the cell. Thus the *unc-45* protein might modify myosin to directly control its assembly or the assembly of the filaments into organized A-bands. Alternatively, the *unc-45* protein may be necessary for normal contractile activity, which in turn could be required for proper organization of the myofilament array.

The effects of the *unc-45(lf)* mutations on elongation and muscle location might suggest that the *unc-45* protein is involved in tissues other than muscle. However, several other muscle-affecting mutations, including those in *myo-3* lead to similar defects in morphogenesis (WATERSTON 1989; B. O. WILLIAMS, R. H. WATERSTON and R. J. BARSTEAD, unpublished results). The mechanisms by which a failure in body wall muscle contraction might lead to a failure to elongate and to the displacement of dorsal muscles is unclear, but the presence of these defects in *unc-45(lf)* alleles need not imply function in other tissues.

The maternal rescue of the *st604* lethality, and the partial rescue of both *st601* and *st603*, shows that the *unc-45* product, either as mRNA or protein, is present in the oocytes, making it unique among the muscle genes identified so far in *C. elegans*. The fact that a *st604/st604* worm can be rescued by the presence of one *unc-45*(+) allele in its mother, but not by an *unc-45(ts)* allele shows the rescue is specific to the *unc-45* locus and dependent on the presence of *unc-45*(+) product in the oocyte. The function of this maternal product in embryogenesis is uncertain, but progeny of the *st604* homozygous mother can develop as far as the twofold stage. Since this is the same stage at which several mutations affecting body wall muscle arrest, including the *unc-45(lf)* alleles, the *unc-45* product need not have a function other than in muscle development. This is a surprisingly late stage for a maternal product to act, as by this point most cell divisions and early steps in morphogenesis are complete. These embryos however, may have a small amount of residual *unc-45* activity as the *st604* allele is probably not null. It remains possible that this residual activity is necessary for normal early development. However, the fact that the *st604* animals can be rescued by a zygotic copy of the wild-type gene argues against a major role for maternal *unc-45* gene activity in early development, and with the failure of maternal *unc-45* activity to rescue *st601* or *st603* animals, it is clear that zygotic expression of *unc-45* is essential.

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