C. ELEGANS UNC-105 MUTATIONS AFFECT MUSCLE AND ARE SUPPRESSED BY OTHER MUTATIONS THAT AFFECT MUSCLE

EUN-CHUNG PARK 1 AND H. ROBERT HORVITZ

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT

Certain mutations in the unc-105 gene of the nematode Caenorhabditis elegans have dominant effects on morphology and behavior: animals become small, severely hypercontracted and paralyzed. These unc-105 mutants revert both spontaneously and with mutagens at high frequencies to a wild-type phenotype. Most of the reversion events are intragenic, apparently because the null (loss-of-function) phenotype of unc-105 is wild type. One revertant defined an extragenic suppressor locus, sup-20 X. Such suppressor alleles of sup-20 are rare, and the apparent null phenotype of sup-20 is embryonic lethality. By constructing animals genetically mosaic for sup-20, we have shown that the primary effect of sup-20 is in muscle cells. In addition to mutations in sup-20, other mutations causing muscle defects, such as unc-54 and unc-22 mutations, suppress the hypercontracted phenotype of unc-105. The ease of identifying nonhypercontracted revertants of unc-105 mutants greatly facilitates the isolation of new mutants defective in muscle structure and function.

The free-living nematode Caenorhabditis elegans is a favorable organism for a genetic analysis of behavior. C. elegans is anatomically simple (SULSTON and HORVITZ 1977) and well characterized genetically (BRENNER 1974; HERMAN and HORVITZ 1980). Its cell lineage from the single-celled zygote to the adult is invariant and completely known (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON et al. 1983). Its nervous system (WARD 1973; WARD et al. 1975; WARE et al. 1975; WHITE et al. 1976, 1983) and musculature (e.g., see WATERSTON, FISHPOOL and BRENNER 1977; WATERSTON, THOMSON and BRENNER 1980; ZENGEL and EPSTEIN 1980) have been studied extensively, and many mutants defective in simple behaviors, such as locomotion (BRENNER 1974), mecanosensitivity (CHALFIE and SULSTON 1981) and egg-laying (TRENT, TSUNG and HORVITZ 1983), have been isolated and characterized.

We have recently studied a set of mutations with dominant effects on the behavior of C. elegans (PARK and HORVITZ 1986). Of the ten genes we studied in detail, four appeared to have wild-type null phenotypes, i.e., the apparent loss of gene function did not result in any obvious phenotypic abnormality.

1 Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114.
unc-105 is one of these four genes with an apparently wild-type null phenotype. In this paper, we present further phenotypic and genetic characterizations of unc-105 mutants.

MATERIALS AND METHODS

Strains and genetic nomenclature: Standard conditions for the culture, maintenance and ethyl methanesulfonate (EMS) mutagenesis of C. elegans have been described by Brenner (1974). C. elegans strain N2 (Brenner 1974) was the parent wild-type strain from which all mutants used in this study were derived. Most of the mutations have been described by Brenner (1974) and Swanson, Edgley and Riddle (1984): unc-54(e190) I, rol-6(e187) II, unc-22(e66) IV, him-5(e1490) V, dpy-11(e224) V, dpy-21(e428) V, lon-2(e678) X, unc-84(e1410) X, unc-3(e151) X; chromosomal aberrations, mnDp1, mnDp2, mnDp8, mnDp9, mnDp26, mnDp27, C1 dpy-10(e128) unc-52(e444) II. Other mutations we have used include: unc-105(n490, n506) (PARK and HORVITZ 1986), unc-105(n490 r58) (P. ANDERSON, personal communication); sup-20(n821, n1168, n1169, n1279) (this study).

This paper conforms to the standardized nomenclature for C. elegans genetics (Horvitz et al. 1979).

Reversion studies: The isolation of phenotypic revertants of unc-105 mutants was described in the accompanying paper (Park and Horvitz 1986). Briefly, unc-105 mutants with dominant phenotypic defects were reverted as homozygotes or as balanced heterozygotes (in trans to the chromosomal balancer C1 dpy-10 unc-52) and phenotypic revertant hermaphrodites were picked in the F2 or F1 generations, respectively. To determine whether a reversion event was extragenic or intragenic to unc-105, hermaphrodite progeny of each revertant were crossed with lon-2 X hemizygous males. Cross-progeny, as well as their progeny (identified on the basis of their segregating Lon animals), were scored for the Unc-105 phenotype.

Construction of double mutants: The double mutants unc-54(e190); unc-105(n490), unc-54(e190); unc-105(n506), unc-105(n490); unc-22(e66) and unc-105(n506); unc-22(e66) were constructed by crossing heterozygous males (genotype either unc-22/+ or unc-54/+ to hermaphrodites homozygous for unc-105(n490) or unc-105(n506). Individual cross-progeny were placed onto separate Petri plates, one per plate. From among the progeny of those animals that segregated Unc-54 or Unc-22 progeny, hermaphrodites homozygous for unc-105 were picked and placed onto new Petri plates, one per plate. unc-54 or unc-22 segregants from these plates were picked and their genotypes were verified by the Unc-105 cross-progeny (identified on the basis of their non-Unc-54 or non-Unc-22 phenotype) in the F1 generation when the putative double mutant hermaphrodites were crossed with wild-type males.

Animals of genotype unc-105(n506); sup-20(n821) were constructed by crossing sup-20(n821)/0 males to unc-105(n506) hermaphrodites. (The sup-20(n821) mutation was isolated from among self-progeny of unc-105(n490)/+; sup-20(n821)/+ heterozygous hermaphrodites that were generated by crossing unc-105(n490); sup-20(n821) hermaphrodites with N2 males.) Cross-progeny (unc-105(n506)/+; sup-20(n821)/+) were picked and placed onto Petri plates, one per plate. Hermaphrodites homozygous for unc-105(n506) were picked from the progeny of these animals and were placed onto Petri plates, one per plate. Wild-type segregants were picked, and the genotypes of the putative double mutants were verified by the presence of Unc hermaphrodite cross-progeny when the putative double mutants were crossed with N2 males.

RESULTS

The phenotypes of unc-105 mutants: We have described three unc-105 mutants that are grossly abnormal in morphology and behavior (Park and Horvitz 1986). These three mutants show qualitatively similar phenotypes, al-
though animals carrying the alleles \( n490 \) or \( n1274 \) are more severely defective than are animals carrying the allele \( n506 \). All three of these mutations have semidominant effects: heterozygotes (e.g., \( n490/+ \)) are less severely affected than homozygotes (e.g., \( n490/n490 \)). All three \( unc-105 \) mutations cause dramatic phenotypic abnormalities. Homozygotes are extremely small (Figure 1) and severely impaired in movement; morphologically and behaviorally, homozygotes resemble wild-type animals grown in the presence of levamisole, a drug that induces the hypercontraction of \( C. \ waves \) muscle cells (Lewis et al. 1980). When the body wall musculature of \( unc-105 \) mutants is examined using polarized optics (Epstein, Waterston and Brenner 1974), the muscle cells can be seen to be irregular, disorganized and reduced in birefringency. However, the vulval and uterine muscles that are required for egg laying (Trent, Tsung and Horvitz 1983) do not appear to be impaired in \( unc-105 \) mutants, because these animals lay eggs and the birefringency of the sex muscles as observed with polarized optics appears normal (data not shown).

**Isolation of extragenic suppressors of \( unc-105 \) mutations:** \( unc-105 \) mutant homozygotes were mutagenized with EMS, and phenotypic revertant animals were isolated from among the \( F_2 \) progeny. We found 27 phenotypically wild-type revertants and 23 Unc revertants (21 Unc animals that twitched and two that were paralyzed; all were phenotypically distinct from Unc-105 animals) among approximately 40,000 \( F_1 \) progeny from mutagenized parents. These Unc revertants are much longer and/or move better than \( unc-105 \) mutants and, thus, were easily detected among the many small and paralyzed \( unc-105 \) animals. Progeny of each revertant were crossed with \( lon-2/0 \) males to determine whether the reversion event was intragenic (see Materials and Methods and Park and Horvitz 1986). All but one of the phenotypically wild-type

**Figure 1.—**Bright-field photomicrographs of N2 (top) and \( unc-105(n490) \) (bottom) adult hermaphrodites, lateral view. Bar = 0.1 mm.
revertants appeared to be intragenic (Park and Horvitz 1986). All male progeny from the cross with the remaining one phenotypically wild-type revertant were non-Unc-105 in phenotype, whereas most hermaphrodites (presumably cross-progeny) were Unc-105, showing that the suppressor mutation is recessive, extragenic and sex-linked. This suppressor mutation, named “n821,” was genetically mapped (see below) and assigned to a new gene sup-20 X.

All cross-progeny from crosses between the Unc revertants (both twitchers and paralyzed) and wild-type males were Unc-105, showing that the suppressor mutations were all recessive and likely to be extragenic. The twitcher Unc mutations were assigned to unc-22 IV and the paralyzed Unc mutations to unc-54 I by complementation tests: the twitcher Unc revertants were crossed with unc-22/+ males and the paralyzed Unc revertants with unc-54/+ males, generating twitcher male and paralyzed male progeny, respectively. Spontaneous revertants of unc-105 were also found; they included four phenotypically wild-type, one unc-54 and five unc-22 revertants. We tested 35 other phenotypically wild-type unc-105(n490) revertants isolated by P. Anderson (personal communication) and found that all 35 were intragenic, as indicated by their tight linkage to n490 (for example, no Unc-105 progeny were found among 6000 progeny of n490 r58/+; lon-2/+).

To test the allele specificity of the suppression of unc-105 by unc-22 and unc-54 mutations, we constructed a set of double mutant animals: unc-54(e190); unc-105(n490), unc-54(e190); unc-105(n506), unc-105(n490); unc-22(e66), and unc-105(n506); unc-22(e66). These double mutants were phenotypically indistinguishable from the unc-54; unc-105(n490) or unc-105(n490); unc-22 revertants, showing that both alleles of unc-105 tested are suppressed by different unc-54 or unc-22 alleles. These results suggest that the interactions between these genes are gene-specific rather than allele-specific.

sup-20 mapping: As described above, we observed that sup-20(n821) was recessive and sex-linked. sup-20 was further mapped using duplications of linkage group X (mnDp1 and mnDp9) that carried unc-3(+) X and differing extents of the X chromosome (see Figure 2) (Herman, Albertson and Brenner 1976; Herman, Madl and Kari 1979). Phenotypically wild-type males of genotype unc-3/0; Dp were crossed to unc-105; sup-20 hermaphrodites, and male progeny were scored for the Unc-105 phenotype. Unc-105 male cross-progeny were found when males carrying mnDp9 were crossed to unc-105(n490); sup-20(n821) hermaphrodites, but no Unc-105 males were found when a similar cross was done using mnDp1, indicating that mnDp9 carried the sup-20 locus but mnDp1 did not. sup-20 was then mapped to the right of unc-84 by a three-factor cross. Specifically, unc-105(n490); lon-2 sup-20(n821)/0 males were crossed to unc-105(n490)/+; unc-84 hermaphrodites at 20°. unc-105 homozygote cross-progeny (unc-105(n490); lon-2 + sup-20/+ unc-84 +) from the above cross were picked onto Petri plates, one per plate. Two out of five non-Unc-105 non-Lon segregants from these animals carried unc-84. Additional mapping with mnDp2, mnDp8, mnDp26 and mnDp27 (Herman, Albertson and Brenner 1976; Herman, Madl and Kari 1979) placed sup-20 between unc-
unc-105 mutations affect muscle

Figure 2.—Partial map of LG X. All duplications shown have been characterized previously (Herman, Albertson and Brenner 1976; Herman, Madl and Kar 1979). The localization of sup-20 with respect to these duplications is described in the text. Bars indicate extent of duplications, with dotted lines indicating that the corresponding markers have not been tested with respect to that duplication. let-4 has not been tested for complementation by mnDp26 or mnDp27.

84 and let-4. Specifically, Unc-105 male cross-progeny were found when males carrying mnDp26 or mnDp27 (unc-3/0; mnDp) were crossed to unc-105; sup-20 hermaphrodites, but no Unc-105 male cross-progeny were found when similar crosses were done using males carrying mnDp2 or mnDp8. The map position of sup-20 is shown in Figure 2.

The null phenotype of sup-20 appears to be embryonic lethality: We attempted to determine the null phenotype of sup-20 by isolating mutations that fail to complement sup-20(n821). Hermaphrodites of genotype rol-6 ZI; unc-3 X (rol-6 and unc-3 are closely linked to unc-105 and sup-20, respectively) were mutagenized with EMS and were crossed with males of genotype unc-105; lon-2 sup-20/0. Rare phenotypically wild-type hermaphrodite progeny were isolated. [We did not seek to identify rare non-Unc-105 male cross-progeny, because the Unc-3 phenotype of these animals would prevent their mating (Hodgkin 1983)]. We isolated 36 phenotypically wild-type hermaphrodites from among approximately 116,000 non-Rol non-Unc-3 hermaphrodite cross-progeny. None of the phenotypically wild-type revertants segregated Unc-105 progeny, suggesting that none carried a dominant suppressor mutation unlinked to sup-20(n821). Two revertants (n1168 and n1169) segregated Unc-3 progeny, whereas the other 34 segregated approximately one-quarter inviable eggs but neither Unc-3 nor Rol-6 Unc-3 progeny, suggesting that in each case unc-3 had become linked to a recessive lethal mutation. We interpret these results to indicate that most sup-20 alleles result in lethality and that lethal alleles (and not nonlethal suppressor alleles) most likely represent loss-of-function mutations. The lethal phenotype resulting from these new sup-20 alleles was independent of the unc-105 genotype since neither Unc-3 Rol-6 nor Unc-3 progeny were segregated in the F1 generation. Animals carrying nonlethal
suppressor mutations \( (n821, n1168 \text{ and } n1169) \) of \( sup-20 \) do not have obvious phenotypic defects either in the presence or absence of \( unc-105 \) mutations, as determined using a dissecting microscope.

**The suppression of \( unc-105 \) by \( sup-20 \) appears to be gene-specific:** The specificity of the suppression of two \( unc-105 \) mutations \( (n490, n506) \) by the three independently isolated suppressor mutations \( sup-20(n821, n1168, n1169) \) was tested by constructing appropriate double mutants. These three suppressor alleles of \( sup-20 \) were obtained on the basis of their abilities to suppress the phenotype of \( unc-105(n490) \). As mentioned above, \( unc-105(n506) \) animals are much less severely affected than \( unc-105(n490) \) animals. These phenotypic differences are apparently not a result of other mutations unrelated to \( unc-105 \), because apparent intragenic revertants of each are indistinguishable both from each other and from the wild type (Park and Horvitz 1986). A strain of genotype \( unc-105(n506); sup-20(n821) \) was constructed (see MATERIALS AND METHODS) and found to be phenotypically wild type, showing that the \( sup-20(n821) \) mutation also suppresses the defects caused by the \( unc-105(n506) \) mutation. \( unc-105(n506) \) is also suppressed by the other two \( sup-20 \) alleles tested: hermaphrodites of genotype \( unc-105(n506); sup-20(n821) \) were crossed with \( rol-6/C1 \ dpy-10 unc-52 \) males. The phenotypically wild-type male cross-progeny of genotypes \( unc-105(n506)/rol-6; sup-20(n821)/0 \) and \( unc-105(n506)/C1 \ dpy-10 unc-52; sup-20(n821)/0 \) were crossed to \( unc-105(n490); sup-20(n1168 \text{ or } n1169) \ unc-3 \) hermaphrodites. All non-Unc-3 hermaphrodites were phenotypically wild type. These wild-type cross-progeny were picked onto Petri plates, one per plate. Those animals that did not segregate either Rol or Dpy \( Unc-52 \) progeny (i.e., of genotype \( unc-105(n490)/unc-105(n506); sup-20(n821)/sup-20(n1168 \text{ or } n1169) \ unc-3 \) were scored for \( Unc-105 \) segregants. None segregated \( Unc-105 \) progeny, and all segregated Unc-3 and wild-type progeny at a ratio of 1:3. Since one-quarter of the Unc-3 animals should have been homozygous for \( unc-105(n506) \), the \( unc-105(n506) \) mutation must have been suppressed by the other two \( sup-20 \) alleles tested.

The suppression of two dominant alleles of \( unc-105 \), which are phenotypically distinct from each other, by three suppressor alleles of \( sup-20 \) suggests that the interactions between \( unc-105 \) and \( sup-20 \) mutations are gene-specific and allele-nonspecific.

**The suppression of the Unc-105 phenotype by \( sup-20 \) alleles is completely recessive:** To determine whether the interactions between \( sup-20 \) and \( unc-105 \) mutant alleles depend on gene dosage, we constructed strains carrying different numbers of copies of \( unc-105 \) and \( sup-20 \) mutant alleles. We constructed animals of genotype \( unc-105(n490)/+; sup-20(n821)/+ \) by crossing wild-type males to \( unc-105(n490); sup-20(n821) \) hermaphrodites. Animals of genotype \( unc-105(n490); sup-20(n821)/+ \) were constructed by crossing males of genotype \( unc-105(n490); sup-20(n821) \) to \( unc-105(n490) \) hermaphrodites. The phenotypes of these animals were indistinguishable from those of \( unc-105(n490)/+ \) and \( unc-105(n490) \), respectively. We also constructed a strain of genotype \( unc-105(n490)/C1 \ dpy-10 unc-52; lon-2 sup-20(n821) unc-3; mnDp26(sup-20(+)) \) (see below for construction). The phenotype of this strain was like that of \( unc-
105(n490)/C1 dpy-10 unc-52. These results suggest that the absence of the wildtype sup-20 allele is necessary for suppression; in the presence of sup-20(+), two copies of sup-20(n821) do not have any detectable effect on the phenotype of unc-105/+ animals.

We constructed strains carrying three copies (or what we believe to be the equivalent of three copies) of sup-20(n821) X and two copies of unc-105(n506). Both a triplo-X strain and a strain carrying the dpy-21(e428) mutation were constructed. [dpy-21(e428) diplo-X animals phenotypically resemble triplo-X animals, presumably as a result of a defect in dosage compensation (HODGKIN 1983; MENEELY and WOOD 1984; B. MEYER, personal communication)]. Triplo-X hermaphrodites were obtained from among the progeny of him-5; lon-2 hermaphrodites; animals of genotype lon-2/lon-2/lon-2 are non-Lon. [him-5(e1490) results in a high incidence of meiotic nondisjunction of the X chromosome, generating a high frequency of both XO male and triplo-X Dpy hermaphrodite progeny in an otherwise wild-type genetic background (HODGKIN, HORVITZ and BRENNER 1979)]. Animals of genotype dpy-21; lon-2 are similarly non-Lon. Triplo-X animals of genotype unc-105(n506); him-5; lon-2 sup-20(n821) were constructed by crossing males of genotype unc-105(n490)/+; him-5 to unc-105(n506); lon-2 sup-20(n821) hermaphrodites. Hermaphrodites homozygous for unc-105(n506) from the cross (unc-105(n506); him-5/+; lon-2 sup-20(n821)/++) were allowed to self and their Lon non-Unc-105 segregants were isolated. From those that segregated males (unc-105; him-5; lon-2 sup-20), non-Lon (XXX) hermaphrodite progeny were scored for the Unc-105 phenotype. Animals of genotype unc-105(n506); dpy-21(e428); lon-2(e678) sup-20(n821) were constructed using a similar protocol. In both cases, non-Lon animals were non-Unc-105. These results show that the increase of sup-20(n821) to three copies does not counteract the suppression of unc-105.

We tested the apparent null alleles (lethal alleles) of sup-20 for possible dominant effects on the suppression of unc-105. We constructed a strain of genotype unc-105(n490)+; sup-20(n1279) unc-3/+ by crossing unc-3/0; mnDp26 males to unc-105(n490); sup-20(n1279) unc-3/sup-20(n821) hemaphrodites. All Unc-3 cross-progeny were Unc-105, showing that the sup-20(n1279) mutation does not have a dominant effect on the suppression of unc-105. This result is consistent with the hypothesis that the suppression of the Unc-105 mutant phenotype by sup-20 nonlethal suppressor mutations is not caused by a reduced level of sup-20 activity. However, it remains possible that sup-20(n821) homozygotes have a level of sup-20 activity that is reduced beyond that of sup-20(n1279)/+ heterozygotes.

Mosaic analyses: To determine whether the primary effect of sup-20 mutations is in muscle cells (as suggested by the anatomical abnormalities in body wall musculature of unc-105 mutants), we constructed strains carrying unc-105(n490), sup-20(n821 or n1279) and the mitotically unstable free duplication mnDp26, which carries wild-type alleles of genes on LG X including sup-20 and unc-3. We identified and analyzed animals that had lost this duplication in some but not in other cells using a technique for mosaic analysis recently developed by HERMAN (1984). Briefly, rare sup-20 mosaic animals (which were
composed of some cells with the *sup-20(+) allele and some cells lacking the *sup-20(+) allele as a consequence of the somatic loss of a free duplication carrying the *sup-20(+) allele) were isolated. Appropriate markers were used to define the genotypes of certain cells, which when examined in conjunction with the known cell lineage of *C. elegans allowed the point in the lineage of the duplication loss to be inferred. By determining what cells had to be genotypically *sup-20(n821) for Unc-105 suppression to occur, the site of action of *sup-20 was revealed.

For the first mosaic analysis, a strain of genotype *unc-105(n490)/CI dpy-10 *unc-52; lon-2 *sup-20(n821) *unc-3; mnDp26 was constructed as shown in Figure 3. The relevant genetic markers for the mosaic experiment are *unc-105, *sup-20, *unc-3 and *mnDp26, and the remaining markers (CI dpy-10 *unc-52 and lon-2) were used to facilitate the experiment: *unc-3 is recessive to the *unc-3(+) allele and results in an Unc phenotype easily distinguished from the Unc-105 mutant phenotype. HERMAN (1984) has mapped the site of *unc-3(+) action to the embryonic ABp lineage by mosaic analysis (Figure 4): animals in which the descendants of AB or ABp are *unc-3 in genotype are Unc-3 in phenotype, regardless of the genotype of the cells in the rest of the animal; animals composed of cells carrying an *unc-3(+) allele in the descendants of AB or ABp are non-Unc-3. Thus, the Unc-3 phenotype can be used as a marker for the genotype of the ABp descendants (HERMAN 1984). *mnDp26, which carries wild-type alleles of *sup-20 and *unc-3, but not of *lon-2, which is linked to *sup-20 but is not suppressed by *mnDp26, was used to facilitate the identification of mosaic candidates since Lon animals are more easily distinguished from *unc-105/+; *lon-2 animals than

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**Figure 3.**—Schemes used to construct strains for mosaic analysis. A, *unc-105(n490)/CI dpy-10 *unc-52; lon-2 *sup-20(n821) *unc-3; mnDp26 strain construction. In this construction, the alleles *unc-105(n490) and *sup-20(n821) were used. *sup-20(n821) is a nonlethal suppressor allele of *sup-20. CI indicates CI dpy-10 *unc-52. Both rol-6 and CI dpy-10 *unc-52 balance *unc-105. *mnDp26 carried wild-type alleles of *sup-20 and *unc-3, but not of *lon-2. The genotypes of strains are shown in italics, and the phenotypes are indicated in parentheses below genotypes. WT indicates wild type in phenotype; Unc-105/+ indicates the phenotype of animals heterozygous for *unc-105 (i.e., n490/+). Unc-105/+ non-Unc-3 hermaphrodites picked from the last cross could be one of the three genotypes indicated, and the strain desired is shown enclosed in a box. B, *sup-20(n1279) *unc-3; *mnDp26 strain construction. In this construction, the alleles *unc-105(n490) and *sup-20(n1279) were used except for the first cross, which also used *sup-20(n821). n1279 is a lethal allele of *sup-20, and n821 is a nonlethal suppressor allele. *mnT11, which is a translocation between LG II and LG X and has a dominant Him phenotype (i.e., generation of a high frequency of males), was used for balancing *sup-20. *mnT11 behaves like a deficiency of some markers on LG II and LG X; those markers deficient in *mnT11 are present on the free duplication *mnDp11 [for a detailed description of the reciprocal translocation chromosomes *mnT11 and *mnDp11, see HERMAN, KARI and HARTMAN (1982)]. Designation of phenotypes is as described in part A. Phenotypically wild-type hermaphrodites picked in the second cross could be of the four genotypes shown, and hermaphrodites of genotype *sup-20 *unc-3/*mnT11 (identified based on their progeny phenotypes) were used for the third cross. Likewise, *sup-20 *unc-3/+ *unc-3; *mnDp26 and *sup-20 *unc-3; *mnDp26 hermaphrodites were identified in the subsequent stages of strain construction, and the final strain derived is shown enclosed in a box.
unc-105 MUTATIONS AFFECT MUSCLE

A

unc-3: mnDp26 σ \( \times \) lon-2 sup-20 unc-3 \( \delta \)
\( \text{VT} \)
\downarrow pick Lon non-Unc-3 σ
lon-2 sup-20 unc-3/0: mnDp26 σ
\( \text{Lon non-Unc-3} \)
\downarrow pick Lon non-Unc-3 σ
rol-6/C11 σ
\( \text{VT} \)
\downarrow pick Lon non-Unc-3 σ
rol-6/C11: lon-2 sup-20 unc-3: mnDp26
and
C1/+: lon-2 sup-20 unc-3: mnDp26
\( \text{Lon non-Unc-3} \)
\downarrow pick Lon non-Unc-3 σ
unc-105; lon-2 sup-20 unc-3 \( \delta \)
\( \text{Lon Unc-3 non-Unc-105} \)
\downarrow pick Unc-105/+ non-Unc-3 \( \delta \)

1) unc-105/rol-6; lon-2 sup-20 unc-3; mnDp26 \( \rightarrow \) segregate Rol progeny
\( \text{Unc-105/+ non-Unc-3} \)

2) unc-105/+; lon-2 sup-20 unc-3; mnDp26 \( \rightarrow \) no Rol or C1 progeny
\( \text{Unc-105/+ non-Unc-3} \)

or 3) unc-105/C1; lon-2 sup-20 unc-3: mnDp26 \( \rightarrow \) segregate C1
\( \text{Unc-105/+ non-Unc-3} \)

B

unc-3/0: mnDp26 σ \( \times \) unc-105/rol-6; sup-20 unc-3/sup-20(n821) \( \delta \)
\( \text{VT} \)
\downarrow pick Unc-105/+ Unc-3 \( \delta \)

1) unc-3/mmT11; mmDp11
\( \text{VT} \)

2) unc-3/mmT11
\( \text{VT} \)

3) sup-20 unc-3/mmT11; mmDp11 \( \rightarrow \) segregate VT males and hermaphrodites
\( \text{VT} \)

or 4) sup-20 unc-3/mmT11 \( \rightarrow \) segregate only VT hermaphrodites
\( \text{VT} \)

unc-3/0: mnDp26 σ \( \times \) sup-20 unc-3/mmT11 \( \delta \)
\( \text{VT} \)
\downarrow pick VT \( \delta \)

1) sup-20 unc-3/mmT11
\( \text{VT} \)

2) unc-3/mmT11
\( \text{VT} \)

3) unc-3/mmT11; mmDp26 \( \rightarrow \) segregate males
\( \text{VT} \)

or 4) sup-20 unc-3/+ unc-3; mnDp26 \( \rightarrow \) segregate Unc-3, no males
\( \text{VT} \)

\downarrow pick VT \( \delta \)

1) sup-20 unc-3/+ unc-3; mnDp26 \( \rightarrow \) segregate Unc-3
\( \text{VT} \)

or 2) sup-20 unc-3; mnDp26
\( \text{VT} \)

\downarrow self

segregate only VT progeny
E.-C. PARK AND H. R. HORVITZ

It uoc-3 germ line

Duplication in
Abp P4 Mosaic animal Progeny of mosaic animal

From HERMAN (1984)
unc-3: Dp(uoc-3(+))
- + non-unc-3 Unc-3
- + Unc-3 Unc-3 and WT

Mosaic Exp. #1
cunc-105; sup-20(n821) unc-3; mnDp26
+ - non-unc-3 non-unc-105 Unc-3 non-Unc-105
- + Unc-3 WT

Mosaic Exp. #2
cunc-105; sup-20(n829) unc-3; mnDp26
- + Unc-3 WT

FIGURE 4.—Schematic representation showing the generation of the founder cells of C. elegans. The six cells AB, MS, E, C and D predominantly give rise to primary ectoderm, primary mesoderm, endoderm, secondary ectoderm and mesoderm and tertiary mesoderm, respectively. The site of action of unc-3 has been mapped to the descendants of the ABp cell (HERMAN 1984), and the P4 cell gives rise to germ cells (SULSTON et al. 1983). + indicates the presence and - indicates the absence of the duplication in the cell ABp or P4 as determined by the phenotypes of mosaic animals and their progeny.

are wild-type animals from unc-105/+ animals. We used a balanced heterozygote of unc-105 because unc-105 homozygotes have a smaller brood size, and mnDp26 mosaics are rare, requiring many individuals to be examined. C1 dpy-10 unc-52 is an appropriate balancer for this purpose because it results in a distinct, easily scored phenotype and is also sterile (HERMAN 1978).

The self-progeny from this strain of genotype unc-105/C1 dpy-10 unc-52; lon-2 sup-20(n821) unc-3; mnDp26 are primarily of three distinct phenotypes, Lon Unc-105 (of both unc-105 homozygous and unc-105/+ heterozygous phenotypes), Lon Unc-3 or Dpy Unc-52. Specifically, animals carrying the duplication are Lon Unc-105 non-Unc-3 or Dpy Unc-52 (since the duplication suppresses both the Unc-3 and the Sup phenotypes), and animals not carrying the duplication are Lon Unc-3 non-Unc-105 or Dpy Unc-52. We found six rare Lon non-Unc-3 non-Unc-105 animals from among approximately 50,000 non-(Dpy Unc-52) non-Unc-3 progeny of this strain. All six of these Lon animals segregated Lon Unc-3 and Dpy Unc-52 progeny (and no non-Unc-3 non-Unc-105), showing that these animals did not carry the duplication in the germ-line precursor cell and that these animals were mosaic animals rather than recombinants between sup-20(n821) on the X chromosome and unc-3(+)) on the duplication. That these animals were phenotypically non-Unc-3 indicated that they carried the duplication in the descendants of ABp. Since the duplication is absent in the germ-line precursor P4 but present in ABp, the duplication loss presumably occurred at the division that generated the cells P1, P2, P3 or P4 (see Figure 4).

As shown in Figure 4, P4 is the germ-line precursor cell and generates only germ-line cells. P3 generates P4 and D, which is the progenitor of 20 body
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muscle cells. P2 generates P3 and C, which is the progenitor of 13 hypodermal, two neuronal and 32 body muscle cells. P1 generates P2 and MSE, which divides to produce the MS and E cells. The E cell generates the intestine. MS generates mainly 42 body muscle cells, 18 pharyngeal muscle cells, six pharyngeal neurons and six specialized cells named “coelomocytes.” The first division of the zygote generates P1 and AB. AB generates 282 neurons, 200 hypodermal nuclei and one body muscle cell. For details of the C. elegans cell lineage, see SULSTON et al. (1983).

In our mosaic animals, duplication loss probably did not occur in the division that generated P4, since it seems reasonable to assume that mutant germ cells would not affect movement. The loss could have occurred in the division that generated P3 or P2; if so, a mutant genotype in only a fraction of the total muscle or hypodermal cells would have to be responsible for the generally hypercontracted-like phenotype of unc-105 animals. It seems more likely that the duplication was lost in the division that generates the P1 cell (Figure 4). Since body muscle cells are the only cell type generated almost entirely from the P1 cell (94 of 95), this mosaic experiment suggests that the loss of sup-20(+) from muscle cells is responsible for the non-Unc-105 phenotype of the mosaic animals. Thus, the unmasking of sup-20(n821) function in the body muscle cells is sufficient to generate a non-Unc-105 phenotype. The results of this mosaic experiment suggest that the primary effect of sup-20(n821) is in muscle cells.

This mosaic experiment suggests that sup-20(+) activity in the descendants of P1 masks the sup-20(n821) suppressor activity. In a second mosaic experiment, we tested whether sup-20(+) activity in the descendants of the P1 cell is sufficient for the survival of animals homozygous for a recessive lethal allele of sup-20, n1279. We constructed a strain of genotype sup-20(n1279) unc-3(e151); mnDp26, which is wild type in phenotype and segregates almost entirely progeny that are wild type in phenotype (Figure 3B). We found five Unc-3 progeny among approximately 250,000 phenotypically wild-type progeny. All five Unc-3 animals segregated only wild-type progeny, indicating that the duplication must have been present in P4 (which generates the germ line) and establishing that these Unc-3 animals were mosaics rather than recombinants between the sup-20(+) allele carried on the duplication and the unc-3 allele carried on the X chromosome. The Unc-3 phenotype of these mosaic animals indicates that the duplication must be absent in the descendants of ABp. For the duplication to be present in P4 but absent in ABp, the loss of the duplication must have occurred in the division that generated either AB or ABp. Thus, sup-20(+) must be present in all descendants of P1 in these mosaic animals. This result is consistent with the hypothesis that the expression of sup-20(+) in the descendants of P1 in an embryo otherwise homozygous for sup-20(n1279) allows the survival of these embryos. However, we cannot rule out the possibility that the activity of sup-20(+) in the descendants of ABa (either alone or in conjunction with the descendants of P1) is responsible for the viability of these embryos.
DISCUSSION

Certain mutations in the \textit{unc-105} II gene result in a dominant hypercontracted phenotype. We have isolated both intragenic and extragenic suppressors of these mutations. The extragenic suppressors have defined three genes: \textit{unc-54} I, \textit{unc-22} IV and \textit{sup-20} X. \textit{unc-54} and \textit{unc-22} mutations are epistatic to \textit{unc-105} mutations: animals carrying an \textit{unc-105} mutation and either an \textit{unc-54} or an \textit{unc-22} mutation are phenotypically like \textit{unc-54} or \textit{unc-22} single mutants, respectively. The suppressor alleles of \textit{sup-20} suppress all known phenotypic abnormalities of \textit{unc-105} mutants; these \textit{sup-20} alleles result in a wild-type phenotype either in the presence or in the absence of \textit{unc-105} mutations. We have also identified 34 recessive lethal alleles of \textit{sup-20}. These lethal alleles seem likely to be null (loss-of-function) alleles, since they fail to complement nonlethal suppressor alleles of \textit{sup-20} and were obtained at a frequency \((3 \times 10^{-4}\) per haploid genome per generation) comparable to that typical of EMS-induced gene disruption frequencies in \textit{C. elegans} (Brenner 1974; Greenwald and Horvitz 1980), and 15-fold higher than that of obtaining \textit{sup-20} suppressor alleles \((2 \times 10^{-5}\).

The suppression of \textit{unc-105} by \textit{sup-20} suppressor mutations appears to require the absence of the \textit{sup-20}(+) allele. Thus, \textit{sup-20}(n821) is completely recessive to \textit{sup-20}(+). All three suppressor mutations of \textit{sup-20} suppress both mutant alleles of \textit{unc-105} tested, suggesting that interactions between \textit{unc-105} and \textit{sup-20} may be gene-specific rather than allele-specific. There are at least two models that can explain the suppression of \textit{unc-105} by \textit{sup-20} suppressor alleles, the wild-type null phenotype of \textit{unc-105} and the observation that the \textit{sup-20}(n821) allele is recessive to the \textit{sup-20}(+) allele. (1) The \textit{sup-20} wild-type gene product is required for \textit{unc-105} activity. In this model, wild-type \textit{sup-20} would have additional functions, such as the activation of other genes in addition to \textit{unc-105} (since the null phenotype of \textit{sup-20} appears to be lethal), and \textit{sup-20} suppressor mutations would specifically impair the ability of \textit{sup-20} to allow \textit{unc-105} activity. (2) The mutant gene products of \textit{unc-105} and \textit{sup-20} interact, although the wild-type gene products need not do so. The \textit{sup-20} suppressor mutations generate new sites that allow interaction with \textit{unc-105} mutant products, and this physical interaction blocks the function of the \textit{unc-105} mutant product.

\textit{unc-105} mutants show a variety of abnormalities, including shortened body length, small brood size and defective muscle structure. Hypercontraction could well be a consequence of a primary defect in muscle; alternatively, the muscle defect could result from a hypercontraction induced as a result of a neuronal or hypodermal abnormality. We examined these alternatives by mosaic analysis using \textit{sup-20}. Our studies of animals carrying \textit{unc-105} and mosaic for \textit{sup-20}(n821) have shown that \textit{sup-20} suppresses \textit{unc-105}(n490) by acting in descendants of the cell P1, most likely in muscle cells. This experiment indicates that muscle cells are the sites of \textit{sup-20} function, but does not establish that muscle cells are also the sites of \textit{unc-105} function. For example, \textit{sup-20} mutations could alter the response of muscles to an \textit{unc-105}-dependent neuronal stimulus. Nonetheless, the mosaic analyses do establish that \textit{unc-105}-
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dependent defects in muscle cells are required to express all known phenotypes of unc-105, including shortened body length and reduced brood size.

The interactions of unc-105 mutations with mutations that lead to defects in muscle contraction suggest a genetic selection scheme for the isolation of new mutants defective in muscle functioning. Specifically, we have shown that mutations in the unc-22 and unc-54 genes suppress the apparent hypercontracted phenotype of unc-105 mutants. Mutations in other genes necessary for muscle contraction, such as unc-15, have also been found to be epistatic to unc-105 mutations (P. ANDERSON, personal communication). Since unc-22 and unc-54 mutants move better than and are much longer than unc-105 mutants, it is easy to select mutants defective in these genes as partial phenotypic suppressors of unc-105 mutations.

Using our genetic selection scheme, EIDE and ANDERSON (1985a,b) have isolated numerous spontaneous unc-54 mutations. The C. elegans Bergerac strain contains many copies of the repeated element Tcl, which has molecular features characteristic of transposable elements (EMMONS et al. 1983; LIAO, ROSENZWEIG and HIRSH 1983). By crossing unc-105(n490) into a Bergerac genetic background and isolating spontaneous unc-54 mutations as unc-105 revertants, EIDE and ANDERSON (1985b) have directly demonstrated the transposition of Tcl.

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